

**PENGARUH RASIO KEDELAI (*Glycine max*) DAN LUPIN (*Lupinus angustifolius*)  
TIPE STARTER, DAN INTERAKSI KEDUANYA TERHADAP  
KARAKTERISTIK TEMPE SUBSTITUSI**

**EFFECT OF SOYBEAN (*Glycine max*) AND LUPIN (*Lupinus angustifolius*) RATIOS  
STARTER TYPES, AND BOTH INTERACTIONS ON CHARACTERISTICS  
OF SUBSTITUTED TEMPEH**

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**ABSTRACT**

Indonesia's high dependence on soybean imports as a raw material for tempeh has brought various efforts to find a substitute of this raw material. One of the most potential substitute is lupin (*Lupinus angustifolius*). However, the mass adoption of these beans as raw material for tempe production is still low. In this study, we test effects of several ratios between soybeans and lupine, by using mix starters on the characteristics and quality of a substituted tempeh. The results indicated that the best treatment for the substituted tempeh was a<sub>2</sub>b<sub>6</sub> treatment with a 1: 2 ratio of soybeans and lupin by using the starter produced by Indonesian Institute of Sciences (LIPI). Moreover, the substituted tempeh has a low water and fiber content as well as sensory acceptable.

*Keywords:* soybean, lupin, tempeh, substituted tempeh, tempeh starter, LIPI starter.

**ABSTRAK**

Ketergantungan Indonesia yang tinggi terhadap impor kedelai sebagai bahan baku tempe telah membawa berbagai upaya untuk mencari substitusi bahan baku ini. Salah satu alternatif yang paling potensial adalah lupin (*Lupinus angustifolius*). Namun, adopsi massal kacang ini sebagai bahan baku untuk produksi tempe masih rendah. Dalam penelitian ini, kami menguji efek beberapa rasio antara kedelai dan lupin, dengan menggunakan campuran starter pada karakteristik dan kualitas tempe substitusi. Hasil penelitian menunjukkan bahwa perlakuan terbaik adalah perlakuan a<sub>2</sub>b<sub>6</sub> dengan rasio 1: 2 pada kedelai dan lupin dengan menggunakan starter yang diproduksi oleh Lembaga Ilmu Pengetahuan Indonesia (LIPI). Selain itu, tempe substitusi memiliki kadar air dan serat yang rendah serta dapat diterima secara sensorik.

Kata kunci: kedelai, lupin, tempe, tempe substitusi, tempe starter, starter LIPI.

## INTRODUCTION

The Indonesian Ministry of Health (2017) reported that the average protein consumption of Indonesian from legumes occupies the third position after fish, shrimp, squid, and shellfish (8.18 grams) as well as meat (5.70 grams), but still higher than eggs and milk (3.12 grams). The number has increased year-on-year. Soybeans are one of the high contributing sources of vegetable protein derived which are largely used as raw materials for tempeh, tofu, and soy sauce (Aldillah, 2015). Tempeh and tofu are popular sources of protein for Indonesian, due to its highly nutritional content and relatively cheaper price compared to fish, meat, eggs, and milk. However, the high demand for soybeans still cannot be satisfied from domestic production. The average number of soybean imports reaches 2 million tons/year or as much as almost 60 % of domestic demand is realized from imported supplies. Of these, 70 % are supplied from the United States (Idris, 2016). As a result, Indonesia has a very high dependence on exporting countries. Price changes in exporting countries will greatly affect the continuity of domestic production since the majority of domestic tempeh and tofu producers are home industry scales.

The above prevalence has raised an attempt to utilize other beans as material to substitute soy-based products. The substitute is expected to reduce the high consumption of imported soybeans in Indonesia. One of the potential substitutes is lupin. Besides containing higher fiber, non cholesterol, lower fat and oil, as well as non gluten product, imported lupin also provides relatively more stable prices than imported soybeans (Lupinfood, 2018). However, the mass utilization of these beans for tempeh production in Indonesia is still low. Besides the low availability in the market, another factor which contributes to the lack consumption is due to the lack of knowledge in terms of the processing and quality of final tempeh produced.

Previous study, such as Jayasena, (Chih, & Nasar-Abbas 2011) has confirmed that lupin as a highly potential substitute for tempeh. In addition, the use of *Rhizopus oligosporus* starter is suitable and also helps a lupin detoxification process. Furthermore, Priatni, Devi, Kardono, & Jayasena (2013) listed the types of lupin that are suitable for tempeh production, and analyzed the quality of the produced lupin tempeh. The results indicated that lupin tempeh has a bright yellow color that is more attractive than soybean

tempeh. In addition, sensory testing verified an acceptable level as a substitute for soybean tempeh. Further, (Wickramasinghe, 2017) tested the fermentation time for lupin tempeh. Moreover, Wolkers-Rooijackers, Endika & Smid (2018) added that there is a significant increase in vitamin B<sub>12</sub> in lupin tempeh with a starter combination of *Rhizopus oryzae* and *Propionibacterium freudenreichii*. Therefore, in this study, we contribute to the existing the literature by testing several comparisons between soybeans and lupin, by using mix starters and evaluating their impact on the characteristics and quality of the tempeh produced. In addition, we also conducted an organoleptic test to analyze the sensory acceptance level of the produced tempeh.

## MATERIALS AND METHODS

Soybeans and lupin were utilized in this study as raw materials for producing a substituted tempeh. The tempeh and LIPI starter were selected as starters in this study. There are various strains used in the tempeh starter, including *Rhizopus oligosporus*, *Rhizopus oryzae*, *Rhizopus stolonifer*, and *Rhizopus arrhizus*, while the LIPI starter combines rice with tempeh starter so that the growth of *Rhizopus oryzae* is higher than other strains for fermenting carbohydrates in the substituted tempeh. The chemicals used for analysis of protein content were kjehdahl salt, selenium black, concentrated H<sub>2</sub>SO<sub>4</sub>, aquadest, 30 % NaOH, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, Zn granules, 0.1N HCL, phenolphalein, 0.1N NaOH, formol titration for protein content analysis, oxalate, phenolphalein, 40 % formalin, crude fiber content analysis of H<sub>2</sub>SO<sub>4</sub> 0.3 N through gravimetric method, CHCl<sub>3</sub>, alcohol, and aquadest. This study is divided into two main stages of preliminary study and pilot testing. The preliminary study was conducted to determine the immersion method to reduce the saponin content in lupin, which will be used in the pilot testing. The process utilized Gas Chromatographic Mass Spectrometry (GCMS) method with a 24-hour immersion time while the pilot testing utilized treatment, experimental, and response design.

### Treatment design

The treatment design in this study employed three factors, type of starters (factor A), ratio of soybeans to lupin (factor B), and interactions between both factors. Factor A consisted of

tempeh starter ( $a_1$ ) and LIPI starter ( $a_2$ ). While factor B was divided into seven ratio levels between soybeans and lupin. The listed ratios (soybeans: lupin) were  $b_1 = 1:0$ ,  $b_2 = 1:1$ ,  $b_3 = 1:2$ ,  $b_4 = 1:3$ ,  $b_5 = 0:1$ ,  $b_6 = 2:1$ , and  $b_7 = 3:1$ .

**Experimental design**

We employed a Randomized Complete Block Design (RCBD) as an experimental design model in this study with three replications in each combination of treatments. Overall, 42 treatments were obtained, with a combination of experiments (Table 1). The experimental design was continued with randomization treatment to obtain a pattern layout in the randomized block design as indicated in Tabel 2.

Tabel 1. RCBD Conbinations

Starter types	Ratios (soybean: lupin)	Repeated measure designs		
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
Tempeh starter ( $a_1$ )	$b_1$ (1:0)	$a_1b_1$	$a_1b_1$	$a_1b_1$
	$b_2$ (1:1)	$a_1b_2$	$a_1b_2$	$a_1b_2$
	$b_3$ (1:2)	$a_1b_3$	$a_1b_3$	$a_1b_3$
	$b_4$ (1:3)	$a_1b_4$	$a_1b_4$	$a_1b_4$
	$b_5$ (0:1)	$a_1b_5$	$a_1b_5$	$a_1b_5$
	$b_6$ (1:2)	$a_1b_6$	$a_1b_6$	$a_1b_6$
	$b_7$ (1:3)	$a_1b_7$	$a_1b_7$	$a_1b_7$
LIPI starter ( $a_2$ )	$b_1$ (1:0)	$a_2b_1$	$a_2b_1$	$a_2b_1$
	$b_2$ (1:1)	$a_2b_2$	$a_2b_2$	$a_2b_2$
	$b_3$ (1:2)	$a_2b_3$	$a_2b_3$	$a_2b_3$
	$b_4$ (1:3)	$a_2b_4$	$a_2b_4$	$a_2b_4$
	$b_5$ (0:1)	$a_2b_5$	$a_2b_5$	$a_2b_5$
	$b_6$ (1:2)	$a_2b_6$	$a_2b_6$	$a_2b_6$
	$b_7$ (1:3)	$a_2b_7$	$a_2b_7$	$a_2b_7$

Tabel 2. RCBD Layout

1 <sup>st</sup> repeated group						
$a_1b_2$	$a_2b_4$	$a_5b_6$	$a_6b_3$	$a_3b_1$	$a_4b_5$	$a_6b_7$
2 <sup>nd</sup> repeated group						
$a_6b_3$	$a_2b_4$	$a_1b_2$	$a_6b_7$	$a_3b_1$	$a_5b_6$	$a_4b_5$
3 <sup>rd</sup> repeated group						
$a_6b_7$	$a_6b_3$	$a_5b_6$	$a_3b_1$	$a_2b_4$	$a_1b_2$	$a_4b_5$

The data obtained was analyzed using Analysis of Variance (ANOVA) through the following linear models (Toutenburg, 2009):

$$Y_{ij} = \mu + \tau_i + \beta_j + \epsilon_{ij} \quad (1)$$

Where:  $Y_{ij}$  = The observed values of the group- $i$  on the group- $j$ ;  $\mu$  = The actual average value ;  $\tau_i$  = The additional effect due to the effect of the second treatment;  $\beta_j$  = The additional effect due to the addition of group- $j$ ;  $\epsilon_{ij}$  = error

The hypothesis tested is  $H_0$  = There is no diversity in the treatment population and  $H_1$  = there is diversity in the treatment population. The results were evaluated through F-test. When the results indicated the higher F-calculation than F-table, it will be followed by the Duncan test to determine the extent of the differences in each treatment.

**Response design**

The design of the response to be performed on lupin includes chemical and organoleptic responses. Chemical responses include analysis of protein content with the kjedahl method, analysis of amino acid levels with the formol method, analysis of water content by the gravimetric method, and analysis of fiber content by the gravimetric method while the organoleptic response includes an analysis of the response to 15 panelists. Quality attributes chosen are color, aroma, taste, and texture by using the hedonic quality test method. The scale used is five points Likert scale (1= dislike very much, 2= dislike, 3= neutral, 4= like, 5= like very much).

**Tempeh production procedure**

The procedure for making the substituted tempeh is set up with sorting and soaking soybeans, then peeling the lupin from its skin. Soaking is accomplished to separate good quality with poor quality beans. Good quality beans will sink into the water, while those with poor quality will float on the water surface. In addition, this activity serves to cleanse beans from dirt or skin carried from the stripping process or during harvesting. The second stage is continued by a draining step which aims to remove water attached on the beans. Subsequently, immersion is performed to produce softer soybeans and lupin by using clean water and drained afterwards. The fourth stage is continued by boiling the beans for 30 minutes with boiling temperature, which aims to expand the surface and ripen the beans. After that, the beans are washed and re-boiled to reduce the water content so that the life span is longer and optimize the effects at the time of giving yeast. The sixth stage was steaming the beans at a temperature of 1000 °C for 25 minutes. After that, the temperature was reduced until it reached room temperature 260 °C. The eighth stage was inoculated by using LIPI starter with a predetermined comparison, which is 0.3 % of the

amount of raw material used. In addition, 0.2 % tapioca flour was added and the stirring process was carried out.

## RESULTS AND DISCUSSIONS

Analysis using GC-MS (Gas Chromatography-Mass Spectroscopy) was carried out as a preliminary study to measure the type and content of compounds in the extract both qualitatively and quantitatively. Based on the chromatogram data, it was found that the components of the saponin contained in the extract were OCTADEC-9 ENOIC ACID with a retention time of 24,457 minutes, an area of 29.04 % and a height of 23.96 %, which had the highest and widest peak. Glycerin had a retention time of 9.568, an area of 2.97 % and a height of 1.56 %. Hexadecanoic acid, Methyl ester (CAS) Methyl palmitate had a retention time of 22.416 minutes, an area of 4.29 % and a height of 5.55 %. Ascorbic acid 2,6-dihexadecanoate had a retention time of 22,744 minutes, an area of 8.05

% and a height 9.14 %. Octadecadienoic acid (Z-Z) -, Methyl ester (CAS) and Methyl linoleate had a retention time of 24,076 minutes, an area of 10.31 % and a height of 12.64 %. Octadecadienoic and Methyl ester had a retention time of 24,119 minutes, an area of 18.97 % and a height of 21.10 %. Octadecadienoic, Methyl ester, and Methyl stearate had a retention time of 24.332 minutes, an area of 2.57 % and a height of 2.64 %. Octadecadienoic acid (Z, Z) - (CAS) Linoleic acid had a retention time of 24.419 minutes, an area of 11.37 % and a height of 12.27 %. Octadecanoic acid had a retention time of 24.637 minutes, an area of 4.87 % and a height of 4.57 %. Lastly, Octadecanoic, 1,2,3-propanetriyl ester, (E, E, E) had a retention time of 29.013 minutes, an area of 7.55 % and a height of 6.57 % (Table 3).

Table 3. Results of GC-MS analysis

Peak	R Time	Area	Area (%)	Height	Height (%)	A/H	Components
1	9.568	55199	2.97	14010	1.56	3.94	Glycerin
2	22.416	79643	4.29	49728	5.55	1.60	Hexadecanoic acid, methyl ester (CAS) methyl
3	22.744	149439	8.05	81892	9.14	1.82	1-(+)-Ascorbic acid 2,6-dihexadecanoate
4	24.076	191321	10.31	113167	12.64	1.69	9.12-Octadecadienoic acid (Z,Z)-, methyl ester
5	24.119	352096	18.97	188975	21.10	1.86	9-Octadecenoic acid, methyl ester, (E)-
6	24.332	47652	2.57	23619	2.64	2.02	Octadecanoic acid, methyl ester (CAS) methyl
7	24.419	210994	11.37	109898	12.27	1.92	9.12-Octadecadienoic acid (Z,Z)-(CAS) Linol
8	24.457	538858	29.04	214568	23.96	2.51	OCTADEC-9-ENOIC ACID
9	24.637	90341	4.87	40911	4.57	2.51	Octadecanoic acid
10	29.013	140095	7.55	58885	6.57	2.38	9-Octadecenoic acid, 1,2,3-propanetriyl ester

Further analysis was obtained through 24-hour immersion treatment. The responses were tested through chemical responses of water content, protein content, amino acid levels, and fiber content while organoleptic response tested using the hedonic test method including attributes of taste, flavor and texture.

### Chemical response to water content

It was found that the type of starter, bean's ratio, and the interactions of the type of starter and ratio significantly affect water content.

Furthermore, Duncan's Multiple Range Test (DMRT) test were used to test differences in all treatment pairs. The results indicated that the two types of starter significantly affect the water content of the substituted tempeh with different sizes. This result supports the study of Jayasena, Chih, & Nasar-Abbas (2011). The LIPI starter fermented more carbohydrates in the substituted tempeh, so that the water content in the treatment of  $a_2b_1$  (1: 0) is higher than that of treatment  $a_2b_5$  (0: 1) (Table 4).

Table 4. The DMRT test results of the effect of A, B, and AB interactions on water conten

Factor A (Starter types)	Factor B (Ratios (soybeans: lupin))						
	b <sub>1</sub> (1:0)	b <sub>2</sub> (1:1)	b <sub>3</sub> (2:1)	b <sub>4</sub> (3:1)	b <sub>5</sub> (0:1)	b <sub>6</sub> (1:2)	b <sub>7</sub> (1:3)
a <sub>1</sub> (Tempeh starter)	58.190	74.410	71.350	71.373	59.307	62.330	62.250
a <sub>2</sub> (LIPI starter)	63.240	75.280	71.353	71.453	59.560	62.680	62.827

In addition, according to Kristianto, Fitriah, & Astuti (2015), in the process of making tempeh, there is a process of boiling, steaming and fermentation. The steaming and boiling process can increase the water content of tempeh because most of the cooking water goes and stays into the food matrix. During the fermentation process, an increase in water content is caused by the release of water trapped by the components of soybean seeds due to the activity of mold. During fermentation, some of the nutrients in soybeans will be metabolized by mold and will release water. The presence of heat by the metabolic process produces a lot of water vapor and it is trapped through the tempeh packaging material. Therefore, these processes can increase the water content of food.

### Chemical response to protein content

The ANOVA results indicated that the type of starter, bean's ratio, and the interaction of the type of starter and ratio significantly affect protein contents. Further, a DMRT test was used to test differences in all treatment pairs (Table 5). The results indicated that the bean's ratio significantly affects protein contents. Soybeans have a protein level of 34.9 %, while lupin are 41 % (Cahyadi, 2009). When processed, the protein content in a soybean tempeh decreased by 20.8 %, while the protein content in the substituted tempeh decreased by 23.8 %. According to Wianarko (2002), quantitatively, the nutritional value of tempeh is slightly lower than soybeans. However, qualitatively, the nutritional value of the soybean tempeh is higher because it has a better digestive value. This is because the levels of water-soluble proteins will increase due to the activity of proteolytic enzymes. For the substituted tempeh, this is because the beans used are split or skinless beans, which result in a decreased protein content.

Table 5. The DMRT test results of the effect of B on protein content

Factor B (Ratios (soybeans: lupin))	Protein content %	Alpha 5 %
b <sub>1</sub> (1 : 0)	19,66	Ab
b <sub>2</sub> (1 : 1)	19,73	C
b <sub>3</sub> (2 : 1)	19,73	C
b <sub>4</sub> (3 : 1)	19,77	Ab
b <sub>5</sub> (0 : 1)	19,88	Bc
b <sub>6</sub> (1 : 2)	20,05	A
b <sub>7</sub> (1 : 3)	20,08	Ab

The results in Table 5 indicated that the high protein content is obtained in b<sub>7</sub>, i.e. with the composition of soybeans (3) and lupin (1). While the lowest protein content was obtained in b<sub>1</sub> with the composition of soybeans (1) and lupin (0). There are several factors that cause protein loss, including immersion and cooking process, as well as fermentation (Steinkraus, Hwa, Van Buren, Provvidenti, & Hand, 1960; Winarno, Fardiaz, & Fardiaz, 1980).

### Chemical response to amino acid levels

The ANOVA results indicated that the type of starter, bean's ratio, and the interaction of the type of starter and ratio do not significantly affect the amino acid levels in tempeh. Therefore, further testing with DMRT cannot be performed.

### Chemical response to fiber content

The ANOVA results indicated that the type of starter, bean's ratio, and the interaction of the type of starter and ratio significantly affect the fiber content of the substituted tempeh. Further, a DMRT test was used to test differences in all treatment pairs. The results are indicated in Table 6.

Table 6 The DMRT test results of the effect of A, B, and AB interactions on fiber content

Factor A (Starter types)	Factor B (Ratios (soybeans: lupine beans))						
	b <sub>1</sub> (1:0)	b <sub>2</sub> (1:1)	b <sub>3</sub> (2:1)	b <sub>4</sub> (3:1)	b <sub>5</sub> (0:1)	b <sub>6</sub> (1:2)	b <sub>7</sub> (1:3)
a <sub>1</sub> (Tempeh starter)	5.53	5.63	5.82	5.75	4.68	5.76	5.78
a <sub>2</sub> (LIPI starter)	5.54	5.64	5.39	5.71	4.50	5.49	5.78

The interaction of soybeans and lupin with the type of starter on fiber content indicated that a<sub>1</sub>b<sub>3</sub> (2:1) and a<sub>1</sub>b<sub>6</sub> (1:2) are significantly different from a<sub>1</sub>b<sub>1</sub> (1:0), a<sub>1</sub>b<sub>2</sub> (1:1), a<sub>1</sub>b<sub>5</sub> (0:1), a<sub>1</sub>b<sub>4</sub> (3:1), and a<sub>1</sub>b<sub>7</sub> (1:3), while a<sub>2</sub>b<sub>1</sub> (1:0), a<sub>2</sub>b<sub>2</sub> (1:1), a<sub>2</sub>b<sub>3</sub> (2:1), a<sub>2</sub>b<sub>4</sub> (3:1), a<sub>2</sub>b<sub>5</sub> (0:1), a<sub>2</sub>b<sub>6</sub> (1:2), and a<sub>2</sub>b<sub>7</sub> (1:3) are not significantly different. According to Kasmidjo (1990) during tempeh fermentation, the growth of *Rhizopus sp.* continues to increase by producing mycelia on the soybean surface which are getting thicker and longer to form a denser tempeh period.

### Organoleptic response

The organoleptic responses analyzed in this study include responses to aroma, texture, and taste. It was found that the type of starter, bean's ratio, and the interaction of the type of starter and ratio do not significantly affect aroma, texture, and taste in the substituted tempeh. First, the tempeh aroma is caused by the presence of volatile compounds. The volatile compounds that can be isolated from tempeh are aldehyde, ketone and hydrocarbon types. In addition, the distinctive aroma in tempeh is produced from fermented products that produce alcohol. In

addition, during the steaming and boiling process, the resulting aroma is reduced due to the evaporation of volatile compounds so that the aroma does not have a significant effect. There is no difference between each treatment interaction because it comes from the different panelists. Second, a texture can be defined as the way various components and structural elements are carried out structuring and combining into micro and macro structure both in terms of flow and deformation (Deman, 1997). The test results indicated that there is no difference in organoleptic response to differences in bean's ratio, types of starters, and interactions that occurred. The similar point prevailed to the taste. This result supports prior study of Priatni, Devi, Kardono, & Jayasena (2013), that tempeh substitution using lupin is sensory acceptable.

### The best treatment results

The overall results of the tests are summarized in Table 7 which includes the chemical response of water content, protein content, amino acid levels, fiber content, and organoleptic responses which include taste, aroma and texture.

Table 7. Results of chemical and organoleptic response

Treatments	Chemical responses (%)				Organoleptic responses		
	Water content	Fiber content	Protein content	Amino acid level	Taste	Aroma	Texture
a <sub>1</sub> b <sub>1</sub>	58.187 <sup>a</sup>	5.530 <sup>b</sup>	19.707 <sup>a</sup>	0.770 <sup>a</sup>	3,18 <sup>a</sup>	3,73 <sup>a</sup>	2,80 <sup>a</sup>
a <sub>1</sub> b <sub>2</sub>	74.410 <sup>k</sup>	5.633 <sup>b</sup>	20.054 <sup>a</sup>	0.780 <sup>a</sup>	3,22 <sup>a</sup>	3,31 <sup>a</sup>	3,22 <sup>a</sup>
a <sub>1</sub> b <sub>3</sub>	71.350 <sup>i</sup>	5.820 <sup>b</sup>	20.104 <sup>a</sup>	0.740 <sup>a</sup>	3,38 <sup>a</sup>	3,44 <sup>a</sup>	3,60 <sup>a</sup>
a <sub>1</sub> b <sub>4</sub>	71.453 <sup>j</sup>	5.707 <sup>b</sup>	19.795 <sup>a</sup>	0.613 <sup>a</sup>	3,20 <sup>a</sup>	3,06 <sup>a</sup>	3,18 <sup>a</sup>
a <sub>1</sub> b <sub>5</sub>	59.557 <sup>c</sup>	4.680 <sup>a</sup>	19.931 <sup>a</sup>	0.560 <sup>a</sup>	3,87 <sup>a</sup>	2,97 <sup>a</sup>	3,38 <sup>a</sup>
a <sub>1</sub> b <sub>6</sub>	62.333 <sup>e</sup>	5.760 <sup>b</sup>	19.489 <sup>a</sup>	0.553 <sup>a</sup>	3,76 <sup>a</sup>	3,13 <sup>a</sup>	3,40 <sup>a</sup>
a <sub>1</sub> b <sub>7</sub>	62.827 <sup>g</sup>	5.780 <sup>b</sup>	19.720 <sup>a</sup>	0.747 <sup>a</sup>	3,60 <sup>a</sup>	3,33 <sup>a</sup>	3,36 <sup>a</sup>
a <sub>2</sub> b <sub>1</sub>	63.240 <sup>h</sup>	5.537 <sup>b</sup>	19.745 <sup>a</sup>	0.713 <sup>a</sup>	3,60 <sup>a</sup>	3,26 <sup>a</sup>	3,78 <sup>a</sup>
a <sub>2</sub> b <sub>2</sub>	75.280 <sup>l</sup>	5.637 <sup>b</sup>	20.040 <sup>a</sup>	0.640 <sup>a</sup>	3,37 <sup>a</sup>	3,33 <sup>a</sup>	3,11 <sup>a</sup>
a <sub>2</sub> b <sub>3</sub>	71.353 <sup>i</sup>	5.390 <sup>b</sup>	20.066 <sup>a</sup>	0.663 <sup>a</sup>	3,67 <sup>a</sup>	3,20 <sup>a</sup>	3,31 <sup>a</sup>
a <sub>2</sub> b <sub>4</sub>	71.37 <sup>i</sup>	5.757 <sup>b</sup>	19.661 <sup>a</sup>	0.403 <sup>a</sup>	3,71 <sup>a</sup>	3,51 <sup>a</sup>	3,31 <sup>a</sup>

a <sub>2</sub> b <sub>5</sub>	59.307 <sup>b</sup>	4.507 <sup>a</sup>	19.826 <sup>a</sup>	0.720 <sup>a</sup>	3,68 <sup>a</sup>	3,40 <sup>a</sup>	3,37 <sup>a</sup>
<b>a<sub>2</sub>b<sub>6</sub></b>	<b>62.680<sup>f</sup></b>	<b>5.493<sup>b</sup></b>	<b>19.836<sup>a</sup></b>	<b>0.773<sup>a</sup></b>	<b>3,77<sup>a</sup></b>	<b>3,26<sup>a</sup></b>	<b>3,46<sup>a</sup></b>
a <sub>2</sub> b <sub>7</sub>	62.247 <sup>d</sup>	5.780 <sup>b</sup>	19.819 <sup>a</sup>	0.837 <sup>a</sup>	3,91 <sup>a</sup>	3,37 <sup>a</sup>	3,62 <sup>a</sup>

Where : a = type of starter; a<sub>1</sub> = Tempeh starter, a<sub>2</sub> = LIPI starter, b = Ratios (soybeans: lupin); b<sub>1</sub>= 1:0, b<sub>2</sub>= 1:1, b<sub>3</sub>=1:2, b<sub>4</sub>= 1:3, b<sub>5</sub> = 0:1, b<sub>6</sub> =2:1, and b<sub>7</sub> = 3:1.

Based on statistical analysis of the chemical and organoleptic response in the main study, the chosen treatment for the substituted tempeh is a<sub>2</sub>b<sub>6</sub> treatment with a ratio of soybeans: lupin (1: 2) by using the LIPI starter. The substituted tempeh has a low water and fiber content, therefore it is appropriate for consumption. Moreover, the digestibility of the nutritional value can be absorbed effectively, while the protein content is higher so that it is suitable for daily consumption.

### CONCLUSION

The results of the ANOVA test indicated that the type of starter, bean's ratio, and the interactions significantly affect water and fiber content of the substituted tempeh. While the result of the protein content test indicated that the type of starter and the interactions have no effect on the protein content, while the effect of the bean's ratio is significant. Conversely, the results of the amino acid content test, indicated that the three treatments do not have significant effects. The same results are also provided by organoleptic responses. Based on the chemical and organoleptic analysis, it is concluded that the treatment of a<sub>2</sub>b<sub>6</sub> is the best treatment with a ratio of soybeans (1), lupine beans (2) and using a LIPI starter.

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