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Physical characteristics of the seeds of soybean (*Glycine max*) varieties and the effect of fermentation time on the chemical characteristics of *tempeh*

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Abstract. *Suhartanti PD, Handajani S, Nandariyah. 2019. Physical characteristics of the seeds of soybean (*Glycine max*) varieties and the effect of fermentation time on the chemical characteristics of tempeh. Cell Biol Dev 3: 13-18.* Soybean is the main raw material for making *tempeh*, and so far, the raw material for *tempeh* is imported soybeans. Therefore, Indonesia needs to develop soybean varieties to overcome this matter. The soybean varieties are expected to be processed into *tempeh* with good physical and chemical properties. This study aims to determine the effect of different varieties on the physical characteristics of soybean seeds and fermentation time on the chemical characteristics of *tempeh*. The research design was a completely randomized design (CRD) with a factorial pattern consisting of two factors, namely soybean varieties (Grobogan, Argomulyo, Seulawah, Anjasmoro, Burangrang, and Galunggung) and variations in fermentation time (30, 42, and 54 hours). The results showed that the difference in varieties did not affect the color of the *tempeh*. Different varieties affect seed weight, water absorption, and swelling power of soybeans. Soybean varieties that have the best physical characteristics (highest water absorption and swelling power) are Grobogan. Variations in fermentation time affect the chemical properties of *tempeh*. Longer fermentation time will increase the tempeh's water, ash, and total protein content but decrease the fat and carbohydrate content. The soybean variety with the best chemical characteristics (highest protein content) is Galunggung. The soybean seed coat is yellow and greenish-yellow, and the color of the *tempeh* is white. The biggest weight is the Grobogan var of 24.14 g, and the largest water absorption capacity of the Grobogan var is 188%. Therefore, the biggest swelling power of Grobogan var is 150%. Based on the time of fermentation, the highest water content of *tempeh* was found in Grobogan var (54 hours), with 67.33%. The highest ash content of *tempeh* was in Anjasmoro var (30 hours), with 1.97%. The highest fat content of *tempeh* was in Galunggung var (30 hours), with 8.89%. The highest protein content of *tempeh* was in the Galunggung var. (54 hours) with 25.17%%. The highest carbohydrate content was in the Seulawah var (42 hours), with 11.43%.

Keywords: Chemical characteristics, fermentation time, *Glycine max*, seeds, soybean, *tempeh*, varieties

INTRODUCTION

According to botanists, the soybean is a plant from Manchuria and parts of China, where there are wide varieties of wild soybeans. Then it spreads to tropical and subtropical areas. After breeding, superior varieties of soybeans are produced. Soybean harvest age varies depending on the variety but generally ranges between 75 and 105 days. Regarding food and nutrition, soybean is the cheapest protein in the world and the highest protein compared to other beans (Septiani et al. 2004; Koswara 2005).

Soybean is the main raw material for making *tempeh*. So far, *tempeh* producers have met the needs of *tempeh* raw materials on imported soybeans, even though their price is increasing daily. According to Sisworo (2008), of the domestic demand for soybeans of two million tons per year, as much as 1.4 million tons are met from imports. If the world soybean price jumps above 100% from the normal Rp 2,500.00 per kg (August-September 2007), the soybean price will become Rp 7,500.00 per kg (Early January 2008).

The Indonesian developed soybean varieties to overcome their dependence on imported soybeans. The soybean varieties are expected to be processed into *tempeh* with better physical, chemical, and antioxidant activity than imported soybeans. The local soybean varieties used in this study were Grobogan, Argomulyo, Seulawah, Anjasmoro, Burangrang, and Galunggung. Indonesia has wide local soybean varieties due to individual variability. Furthermore, this individual variability is caused by internal, genetic, external, and environmental factors, such as soil conditions and types, nutrients, climate, temperature, humidity, etc. *Tempeh* is one of the authentic Indonesian foods made from soybeans by fermentation. In the *tempeh* production process, the ingredients are boiled soybean seeds and microorganisms in the form of *tempeh* molds: *Rhizopus oligosporus, Rhizopus oryzae, and Rhizopus stolonifer* (a combination of two/three species). Also, the supportive environment consists of a temperature of 30°C, initial pH of 6.8, and 70-80% relative humidity (Sarwon 1996).

According to SNI 01-3144-1992, soybean tempeh is a food product that is fermented from soybeans by certain molds, is in a compact solid form, and has a distinctive odor of white or grayish color (BSN 2010).

Tempeh is a highly nutritious food, so it has a strategic meaning and is very important for nutritional fulfillment. In addition, *tempeh* has other advantages: antioxidant content, simple producing technology, low price, good taste, and easy to cook.

The antioxidant content of *tempeh* can counteract free radicals, including vitamin E, carotenoids, superoxide dismutase, isoflavones, and so on. Therefore, the consumption of antioxidants in *tempeh* could mobilize antioxidant activity in the body. For example, there are three antioxidant compounds in soybeans in the form of isoflavone compounds: daidzein, genistein, and glycitein. In addition, it can prevent diseases caused by free radicals in the body, such as atherosclerosis, coronary heart disease, diabetes mellitus, cancer, etc., by serving as an antidote to free radicals (radical scavenger) (Haslina and Pratiwi 1996). Moreover, several local soybean varieties are used as raw materials for making *tempeh* as an alternative to imported soybeans with the same physical and chemical characteristics with, or even better than, imported soybeans at an affordable price.

The aims of this study were: (i) to determine the effect of different varieties on the physical characteristics of the seeds of several soybeans (Grobogan, Argomulyo, Seulawah, Anjasmoro, Burangrang, and Galunggung), including weight, seed coat color, cooking quality such as swelling power, and water absorption; (ii) to determine the effect of fermentation time (30 hours, 42 hours, and 54 hours) on the chemical characteristics of *tempeh* (contents of protein, fat, water, ash and carbohydrates) from several soybeans (Grobogan, Argomulyo, Seulawah, Anjasmoro, Burangrang, and Galunggung).

MATERIALS AND METHODS

Materials

The main ingredients in the making of *tempeh* are local soybeans, namely Grobogan, Seulawah, Burangrang, and Galunggung, and the introduced varieties Anjasmoro and Argomulyo obtained from the *Balai Penelitian Kacang-Kacangan dan Umbi-Umbian* (Malang, East Java, Indonesia), and Raprima *tempeh* yeast produced by PT. Aneka Fermentasi Industri (Bandung, West Java, Indonesia).

Research design

The experimental design of this study was a completely randomized design (CRD) with a factorials pattern consisting of 2 factors repeated 2 times. Factor 1: Soybean varieties (Grobogan, Argomulyo, Seulawah, Anjasmoro, Burangrang, and Galunggung) (K1, K2, K3, K4, K5, K6), so there were 36 replications. Factor 2: Duration of fermentation (30 hours, 42 hours, and 54 hours) (P1, P2, P3), so there were 36 replications.

Physical characteristics test of soybeans

Weight analysis was carried out by weighing 100 seeds using an analytical balance in duplicate, and the seed coat color was observed visually. The observed cooking quality included (i) swelling power (Plhak et al. 1989) and (ii) water absorption (Plhak et al. 1989). Boiling quality analysis was performed by calculating each sample's weight (a gram) and volume (b mL). Furthermore, the seeds were put in a glass filled with water 10 times the volume of seeds and soaked for 12 hours. Next, the seeds were boiled with constant heat for 20 minutes. Finally, the seeds were drained, and the weight (d gram) and volume (e mL) were calculated.

Swelling power = e-b/a x100%

Water absorption = d-a/a x100%

Tempeh making

The stages of making *tempeh* were according to Syarief's (1999) method. First, seed sorting is done traditionally by choosing good and plump soybeans. In the container, soybean seeds are mixed with dirt, such as sand or wrinkled and porous seeds (Ali 2008). The washing used clean running water. The immersion I, used clean water as much as 500 mL for 12 hours, and the boiling I, used 500 mL of clean water for 20 minutes. The immersion II used 500 mL of clean water for 12 hours. Next, stripping the epidermis was done by squeezing the soybean seeds; soybean skin was added to make tempeh. Boiling II used 500 mL of clean water for 20 minutes. Finally, draining was done by placing soybeans in a winnowing tray. Soybeans were inoculated using Raprima *tempeh* yeast, and the packaging used banana leaves. Fermentation was carried out with three kinds of treatment: 30 hours, 42 hours, and 54 hours.

Analysis method

According to Apriyantono et al. (1989), the analysis includes: (i) moisture content using the gravimetric method, (ii) ash content using a kiln, (iii) protein content using the micro-Kjeldahl method, (iv) fat content using the Soxhlet method, (v) carbohydrate content using the by difference method.

Data analysis

The research data were analyzed by SPSS, using analysis of variance (ANOVA) to determine whether there was a difference in treatment at the level of $\alpha = 0.05$. Then, DMRT (Duncan Multiple Range Test) was followed at the $\alpha = 0.05$ level.

RESULTS AND DISCUSSION

Physical characteristics

Table 1 shows that the colors of soybean seeds are yellow and greenish-yellow; moreover, Soybean seed coat color does not affect the *tempeh* color. Therefore, Rhizopus sp. needs energy and nutrients to change the yellow soybean seeds to become covered with white fungal mycelia to grow and develop.

The *tempeh* was white because fungal mycelia covered it. Good *tempeh* is characterized by a surface covered by mold mycelium (fine threads) evenly, compact and white. The soybean granules are filled with mycelium with strong and even bonds so that when the *tempeh* is sliced, the *tempeh* is not crushed.

The seed weight of 100 soybeans ranges from 7.66 g-24.14 g and varies for several soybean varieties. In general, the weight of 100 seeds was 7-10 g and was not significantly different for Argomulyo, Anjasmoro, and Burangrang varieties. The biggest weight was the Grobogan variety at 24.14 g, and the smallest was the Seulawah variety at 7.66 g. The size of the seed influences the amount of produced *tempeh*. A large seed size will produce more *tempeh*.

The highest water absorption was in Grobogan soybean with 188%, and the lowest was in Seulawah soybean with 106% (Table 1). All varieties showed significant differences. During soaking, soybeans will absorb water. The immersion temperature affects the rate of water absorption by soybean seeds. The higher the immersion temperature, the greater the rate of water absorption. On the other hand, the water absorption rate will decrease in proportion to the increase in the moisture content of soybean seeds. It seems that the absorption rate is also not significantly affected by the state of the soybean seeds.

Water absorption by legume seeds is very important concerning its utilization business. The amount of water absorption has something to do with softening of legumes. According to Kamil in Handajani and Atmaka (1993), several factors that affect the rate of water absorption are seed coat/seed membrane permeability, solution concentration, temperature, hydrostatic pressure, the surface area of seeds in contact with water, intermolecular forces, species, variety, maturity level, chemical composition, and the age of the seeds. Meanwhile, according to Bewley and Black in Handajani and Atmaka (1993), several factors that affect water absorption are seed coat anatomy, the external environment (soil, light, moisture), genetic factors, and others, including seed size.

In Table 1, the highest swelling power value was 150% of Grobogan soybeans or 1.5 times the initial volume of soybeans. Table 1 also shows that the lowest swelling power value was 82% for the Seulawah variety soybean. All varieties showed significant differences. The swelling power of legumes increases the seed volume because the absorbed air is replaced by water during water absorption. In this case, the swelling power is determined more by the swelling of the seed coat and not by the seed's flesh, so the seed's softening will occur.

The correlation between water absorption and swelling power shows that the greater the water absorption capacity, the greater the swelling power. Therefore, according to Nabessa et al. in Handajani and Atmaka (1993), seeds swell during water absorption, increasing seed volume.

Water content

Water content is one of the most important characteristics of foodstuffs because water can affect the appearance, texture, and taste of foodstuffs. The water content in foodstuffs determines the freshness and durability of these foodstuffs. High water content makes it easy for bacteria, molds, and yeasts to breed, so there will be changes in foodstuffs (Wiryadi 2007). Based on the results of the study, the water content (%) in several soybean varieties with several fermentation time treatments is shown in Table 2. The water content of *tempeh* ranges from 55.80-67.33% and varies with the treatment of fermentation time and differences in soybean varieties. Table 2 shows that the longer the fermentation time, the higher the water content of *tempeh* for several soybean varieties.

All fermentation times showed significant differences, except for the Galunggung variety at 42 and 54 hours. In the Seulawah variety, 42 hours of fermentation decreased. The decrease was caused by the water content, which was still quite large at the time of immersion, making it difficult to drain these small and hard seeds.

The highest water content was in Grobogan soybean *tempeh*, with a fermentation time of 54 hours, which was 67.33%. The lowest water content was in Burangrang soybean *tempeh*, with a fermentation time of 30 hours, 59.03% (Table 2). The water content of *tempeh* increased with increasing fermentation time. According to Sudarmadji (1977), soybeans experienced an increase in water content after 40 hours of fermentation during the *tempeh* process.

Referring to Steinkraus (1995), some water is produced from the breakdown of carbohydrates by microbes during tempeh fermentation. According to Rokhmah (2008), water is a product of aerobic fermentation, and in *tempeh* fermentation, microbes digest the substrate and produce water, carbon dioxide, and large amounts of energy (ATP). Furthermore, in fermentation, the Rhizopus mold will destroy the matrix between the bacterial cells so that the soybeans will become soft on the third day. In the next stage, the cells in soybeans are destroyed by water, which results from the breakdown of carbohydrates and causes *tempeh* to become mushy and watery (Syarief 1999).

Table 1. Physical characteristics of seeds of several soybean varieties

Varieties	Characteristics			
	Seed coat color	Weight of 100 seeds (g)	Water absorption (%)	Swelling power (%)
Grobogan	Yellow	24.14^{d}	188^{f}	150^{f}
Argomulyo	Yellow	13.44^{b}	144^{c}	110^{c}
Seulawah	Greenish-yellow	7.66^{a}	106^{b}	82^{b}
Anjasmoro	Yellow	14.02^{b}	175^{e}	132^{e}
Burangrang	Yellow	13.44^{b}	157.5^{d}	120^{d}
Galunggung	Yellow	16.01^{c}	119^{a}	100^{a}

Note: Numbers in the same column followed by the same letter show no significant difference ($\alpha < 0.05$)

Table 2. Water content (%) of *tempeh* with various fermentation time

Varieties	Water content (%)		
	Fermentation time (hour)		
	30	42	54
Grobogan	64.73^{fg}	65.65^{i}	67.33^{j}
Argomulyo	65.77^{h}	65.96^{i}	66.24^{i}
Seulawah	64.77^{fg}	62.72^{d}	64.19^{ef}
Anjasmoro	64.58^{fg}	64.92^{h}	65.94^{i}
Burangrang	59.03^{a}	62.07^{c}	63.72^{e}
Galunggung	61.16^{b}	62.12^{c}	62.15^{c}

Note: Numbers followed by the same letter show no significant difference ($\alpha < 0.05$)

Fermentation time is one of the most important factors causing the increase in water content, so with increasing fermentation time, the water content will also increase (Mulato and Widyotomo 2003; Wiryadi 2007).

Water, as one of the products of metabolism, is very influential on other components, including mold growth as microorganisms that play a role in *tempeh* fermentation (Rokhmah 2008).

Protein content

The protein content determination test was carried out using the Micro-Kjeldahl method, calculated as total N. The total protein content of *tempeh* from several soybean varieties with variations in the time of fermentation treatment can be seen in Table 3.

The protein content of *tempeh* ranges from 16.65-25.19%, varies between treatments of fermentation time and differences in soybean varieties. In all varieties and fermentation time, it tended to increase but showed no significant difference, except for Argomulyo and Galunggung varieties, 42 and 54 hours of fermentation showed significant differences.

Galunggung soybean *tempeh*, with 54 hours of fermentation time, had the highest protein content of 67.33%, but *tempeh* was in an over-fermented condition, so it was not preferred. At the same time, the lowest protein content in soybean *tempeh* Grobogan during 30-hour fermentation was 16.65% (Table 3).

Tempeh protein content increases as fermentation time increases (30, 42, and 54 hours). These results are per the opinion of Astuti et al. (2000). Due to processing soybeans into *tempeh*, the total nitrogen, cellulose, and ash content increase significantly.

Vitamin B complex formation occurs in soybean tempeh fermentation, except for thiamin, which decreases (Astuti 2000). Vitamin B_{12} is produced by the bacterium *Klebsiella pneumoniae*, a desirable microorganism that may be required in the natural *tempeh* fermentation process (Steinkraus in Steinkraus 1983). It is suspected that during fermentation, *tempeh* also undergoes the formation of vitamin B_{12}, so the increase in the amount of protein is thought to come from the nitrogen in the vitamin B complex.

Many fungi are active during *tempeh* fermentation, but researchers generally assume that Rhizopus sp is the most dominant fungus. The fungus that grows on soybeans produces enzymes that can break down complex organic compounds into simpler compounds so that these compounds can be quickly used by the body (Pangastuti and Triwibowo 1996). In addition, *R. oligosporus* produces protease enzymes. The breakdown of protein complex compounds into simpler compounds is important in *tempeh* fermentation. Furthermore, it is one of the main factors determining the quality of *tempeh*, namely as a source of vegetable protein with a high digestibility value (Pangastuti and Triwibowo 1996).

Ash content

These mineral elements are also inorganic substances or ash content (Winarno 2002). According to Winarno (2002), ash is an inorganic substance from the combustion of organic material.

Tempeh ash content ranged from 0.92-1.97% and varied during fermentation. The longer the fermentation time, the ash content of *tempeh* increases. Even though the ash content of the *tempeh* samples of the Grobogan variety decreased at 42 hours of fermentation, the decrease was not significant (Table 4).

There was no significant difference in all fermentation times except for the Argomulyo variety at 42 and 54 hours. Likewise, the Anjasmoro variety at 30 and 42 hours of fermentation showed a significant difference.

The increase in ash content during *tempeh* fermentation is to Astuti et al. (2000), which stated that the total nitrogen content increased from processing soybeans into *tempeh* slightly, and the cellulose content and ash content increased significantly. This increase in ash content is probably due to the fermentation of molds producing enzymes for their metabolism. Enzymes are protein compounds containing the mineral element nitrogen (N), and the N is counted as ash.

In addition, the increase in ash content is thought to come from vitamins formed by bacteria that grow during *tempeh* fermentation, such as *K. pneumoniae* (Ferlina 2009), especially vitamin B. Astuti et al. (2000), stated that during *tempeh* fermentation, the amount of vitamin B complex increased except for thiamin. As mentioned earlier, vitamin B12 is produced by the bacterium *K. pneumoniae* in the *tempeh* fermentation process (Steinkraus in Steinkraus 1983).

Table 3. Protein content (%) of *tempeh* with various fermentation time

Varieties	Protein Content (%)		
	Fermentation time (hour)		
	30	42	54
Grobogan	16.65^{a}	17.93^{abcd}	18.61^{cde}
Argomulyo	16.8^{ab}	18.28^{bcd}	21.06^{ghi}
Seulawah	17.70^{abc}	17.75^{abc}	18.69^{cde}
Anjasmoro	19.35^{def}	19.87^{efg}	20.21^{fgh}
Burangrang	20.21^{fgh}	21.51^{hi}	22.47^{ij}
Galunggung	21.96^{i}	23.47^{j}	25.19^{k}

Note: Numbers followed by the same letter indicate no significant difference (α < 0.05)

Table 4. Ash content (%) of *tempeh* with various fermentation time

Varieties	Ash Content (%)		
	Fermentation time (hour)		
	30	42	54
Grobogan	1.29^{bc}	1.28^{bc}	1.34^{bcd}
Argomulyo	1.45^{cd}	1.69^{de}	1.97^{f}
Seulawah	0.99^{ab}	1.06^{ab}	1.24^{abc}
Anjasmoro	0.92^{a}	1.35^{bcd}	1.52^{cde}
Burangrang	1.34^{bcd}	1.47^{cd}	1.59^{cde}
Galunggung	1.29^{bc}	1.54^{cde}	1.84^{ef}

Note: Numbers followed by the same letter indicate no significant difference (α < 0.05)

During soybean fermentation, the increase in vitamin B_{12} levels can reach 33 times, the increase in riboflavin reaches 8-47 times, the increase in pyridoxine ranges from 4-14 times, the increase in niacin ranges from 2-5 times, the increase in biotin ranges from 2-3 times, the increase in folic acid ranges from 4-5 times and the increase in pantothenic acid reaches 2 times (Ferlina 2009). All these compounds contain the element nitrogen (N). Vitamin B_{12} also contains an atom of cobalt (Co) bonded similar to that of iron-bound in hemoglobin or magnesium in chlorophyll (Winarno 2002). Thus, the increase in ash is thought to come from nitrogen – nitrogen and cobalt (Co in vitamin B_{12}) contained in the vitamin B complex.

The lowest ash content was in Anjasmoro soybean *tempeh* at 30 hours of treatment with 0.92%%, and the highest ash content was in Argomulyo soybean *tempeh* at 54 hours of treatment with 1.97%.

Fat content

Table 5 shows the total fat content of *tempeh* samples of several local soybean varieties such as Grobogan, Seulawah, Burangrang, and Galunggung introduced varieties Anjasmoro and Argomulyo with variations in the length of fermentation treatment in this study ranging from 6.33-8.89%. Table 5 also shows that the fermentation treatment length affected the tempeh samples' fat content. The Grobogan, Argomulyo, and Galunggung varieties showed significant differences in all fermentation times. Meanwhile, the Anjasmoro variety showed a significant difference between 30 and 42 hours of fermentation. Meanwhile, the Burangrang variety showed a significant difference between 42 and 54 hours of fermentation. In other varieties and fermentation time, there was no significant difference.

In the Grobogan variety, fat content decreased at all fermentation times of 30, 42, and 54 hours, and all three showed significantly different. In the Argomulyo variety, fat content was decreased at all fermentation times of 30, 42, and 54 hours. At 30 hours and 42 hours of fermentation, there was no significant difference in fat content, while at 54 hours of fermentation, there was a significant difference. The decrease in fat content also occurred in the Seulawah variety at all fermentation times of 30, 42, and 54 hours but did not show a significant difference.

In the Anjasmoro variety, there was also a decrease in fat content at 30, 42, and 54 hours of fermentation. However, there was no significant difference between 42 hours and 54 hours of fermentation, while there was a significant difference between 30 hours of fermentation. The decrease in fat content also occurred in the Burangrang variety at 42 and 54 hours of fermentation (8.43% and 7.87%), and there was a significant difference. Finally, in the Galunggung variety, there was a decrease in fat content at 30, 42, and 54 hours of fermentation, but there was no significant difference.

The fat content of *tempeh* in several local soybean varieties of Grobogan, Seulawah, Burangrang, and Galunggung and introduced varieties of Anjasmoro and Argomulyo with variations in the length of fermentation treatment (30, 42, and 54 hours) in this study tended to experience an insignificant decrease (Table 5). It is because fat is not easily used directly by microbes compared to protein and carbohydrates (Ketaren 1986; Wiryadi 2007). A significant decrease in fat content occurred in Anjasmoro *tempeh* at 42 hours of fermentation. In Kasmidjo (1990), it is stated that the fat content of soybeans will decrease due to fermentation into *tempeh*. More than 1/3 neutral fat from soybean was hydrolyzed by lipase enzyme during 3 days of fermentation by R.oligosporus at 37°C. After 48 hours of fermentation, all fat will be hydrolyzed.

In this study, it was found that the highest fat content was found in samples of *tempeh* of the Galunggung variety with a fermentation time of 30 hours (8.89%), while the lowest fat content was found in samples of *tempeh* of the Anjasmoro variety with a fermentation time of 54 hours (6.33%).

Carbohydrate content

Table 6 shows carbohydrate levels in *tempeh* from several soybean varieties with variations in the length of fermentation treatment.

In the results of statistical analysis, it can be seen that the treatment of fermentation time and differences in soybean varieties have a significantly different effect on the carbohydrate content of *tempeh* samples. Furthermore, it can be seen from the different notations behind the carbohydrate content numbers. For example, the highest carbohydrate content was in the *tempeh* of Seulawah variety in 42 hours of treatment with 11.43%, and the lowest carbohydrate content was in the *tempeh* of Grobogan variety in 54 hours of treatment with 3.34%.

Table 5. Fat content (%) of *tempeh* with various fermentation time

Varieties	Fat content (%)		
	Fermentation time (hour)		
	30	42	54
Grobogan	8.22^{hi}	7.82^{f}	7.48^{e}
Argomulyo	8.31^{i}	8.19^{ghi}	7.39^{de}
Seulawah	7.24^{cde}	7.04^{bc}	6.87^{b}
Anjasmoro	8.40^{i}	6.52^{a}	6.33^{a}
Burangrang	8.43^{i}	8.43^{i}	7.87^{fg}
Galunggung	8.89^{j}	7.95^{fgh}	7.07^{bcd}

Note: Numbers followed by the same letter indicate no significant difference ($\alpha < 0.05$)

Table 6. Carbohydrate Content (%) of *Tempeh* with Various Fermentation Time

Varieties	Carbohydrate content (%)		
	Fermentation time (hour)		
	30	42	54
Grobogan	9.11^{e}	7.32^{o}	5.24^{e}
Argomulyo	7.63^{m}	5.89^{f}	3.34^{a}
Seulawah	9.29^{p}	11.43^{r}	9.02^{n}
Anjasmoro	6.77^{j}	7.39^{l}	6.00^{g}
Burangrang	10.99^{q}	6.52^{h}	4.36^{c}
Galunggung	6.70^{i}	4.92^{d}	3.76^{b}

Note: Numbers followed by the same letter indicate no significant difference ($\alpha < 0.05$)

According to Kim, Smit, and Nakayma in Kasmidjo (1990), during the soaking process, monosaccharides increased, but in soaking for 24 hours at 25°C with a seed: water ratio of 1: 3 and 1:10, there was no decrease in oligosaccharides. According to Mulyowidarso (1988), sucrose decreased by 84%, while stachyose, raffinose, and melibiose decreased by 64% from the content in the seeds during soaking.

The reduction of stachyose, raffinose, and melibiose compounds and the increase of monosaccharides provide microbiological and nutritional advantages in the manufacture of *tempeh*. However, *R. oligosporus* cannot metabolize these compounds; on the contrary, it can utilize monosaccharides well. In addition, glucose is a sugar compound that encourages the germination of *R. oligosporus* spores.

Stachyose, raffinose, and sucrose, the main carbohydrate sources in beans, are carbon sources for *tempeh* yeast to grow. Therefore, the treatment of soaking and boiling can cause a reduction in the main sugar content. The decrease in carbohydrate levels during the fermentation process is due to the use of monosaccharides by *tempeh* yeast to grow so that the fermentation process can run. Stachyoses will be reduced further during fermentation by *tempeh* mushrooms, remaining only 30% of the stachyose content of raw soybeans after 48 hours and only 7% remaining after 72 hours of fermentation. Meanwhile, the relative raffinose content will be the same during fermentation.

In conclusion, (i) The difference in varieties does not affect the color of the *tempeh* produced. However, different varieties affect seed weight, water absorption, and swelling power of soybeans. Heavy seed weight will produce more *tempeh*, and high-water absorption will increase the swelling power. The soybean variety that has the best physical characteristics is Grobogan. (ii) Variations in the fermentation time treatment affect the chemical properties of *tempeh*. The longer fermentation time will cause the tempeh sample's water content, ash content, and total protein content to increase while the fat and carbohydrate content to decrease. The soybean variety that has the best chemical characteristics is Galunggung.

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