Vol. 12, No. 2 (2023) : 302 - 311
DOI : http://dx.doi.org/10.23960/jtep-l.v12i2.302-311

JURNAL TEKNIK PERTANIAN LAMPUNG

ISSN 2302-559X (print) / 2549-0818 (online)
Journal homepage : https://jurnal.fp.unila.ac.id/index.php/JTP

Effect of Packaging Material Types on Antioxidant Activity, Fat Content, and Ash Content in the Imported and Local Yellow Soybean Tempeh

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Article History :

Received : 23 October 2022
Received in revised form : 13 February 2023
Accepted : 4 March 2023

Keywords :

Antioxidant activity,
Ash content,
Fat content,
Soybean,
Tempeh.

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ABSTRACT

Tempeh known as indigenous food that has high nutrients and antioxidant compounds. Tempeh was produced from the fermentation of soybean with Rhizopus sp. Tempeh usually was wrapped with plastic or banana leaf. The purpose of this study to know the effect of soybean originated (import and local soybean) and the packaging material types (plastic, teakwood leaf, hibiscus leaf, and banana leaf) on antioxidant activity, fat content, and ash content of tempeh. The methods of this research were antioxidant activity analyses with DPPH (1,1-diphenyl-2- picrylhydrazil) Radical Scavenging Activity method, fat analyses with soxhlet method, ash analyses with dry method. The results of this research showed that the tempeh from local soybeans with waru leaf packaging had the highest antioxidant activity (53,43%), tempeh from import soybeans with plastic packaging had the highest fat content (48,89%), and tempeh from import soybeans with waru leaf packaging had the highest ash content (2,19%). We found that the soybean origin (import and local soybean) and the packaging types (plastic, waru leaf, jati leaf, and banana leaf) had significant effect (p<0,05) on antioxidant activity, fat content, and ash content of tempeh.

1. INTRODUCTION

Indonesia is a developing nation whose economic growth is causing an increase in environmental pollution, income fluctuations, and changes in people's lifestyles, such as low physical activity and an unbalanced diet (Ayuningtyas, 2018). This causes an increase the degenerative diseases, such as hypertension, diabetes mellitus, atherosclerosis, stroke, and cardiovascular disease (Handayani, 2013).

Over the last 60 years, much research has demonstrated that substituting soybean for animal products can lower atherosclerosis, hyperlipoproteinemia, and coronary heart disease (Gilbert & Liu, 2013). Furthermore, soybeans contain antioxidants that can be employed to protect against oxidative damage. Soybeans have a high level of bioactive compound in the form of isoflavones. Soybean isoflavones are glycosides composed of genistin, daidzin, and glycitin (Yonata *et al*., 2022). The active form of glycosides (aglycone) is found in fermented soybeans (Sayuti & Yenrina, 2015). Soybean isoflavones lower the risk of coronary heart disease and cancer (Puspasari *et al*., 2021).

Types of soybeans that are often used in food processing is imported soybeans and local soybeans (Utama & Baroto, 2018). Imported soybeans are imported from foreign nations and are usually GMO (Genetically Engineered Organisms) type. GMO soybeans are genetically modified where a modified phosphinothricin acetyltransferase (PAT) gene from a soil bacteria, *Streptomyces viridochromogenes*, was added to GMO soybeans. Consequently, GMO soybeans have a higher oleic acid content and greater seed size. In the United States, GMO soybeans are widely grown. Local soybeans are those grown by Indonesian farmers (Ratnaningsih *et al*., 2016). The best varieties of local soybeans are Argomulyo, Grobogan, and Anjasmara (Milani, 2013). Local soybeans, namely the Grobogan variety, have almost the same or even greater size and weight as imported soybeans (Ratnaningsih et al., 2016). Since of the enormous size and weight of the soybeans, the average yield of tempeh will be larger.

Tempeh is a food product fermented by the *Rhizopus sp.* mold. Tempeh can potentially be a source of antioxidants capable of combating free radicals. It can assist avoid degenerative diseases like coronary heart disease, diabetes, cancer, and atherosclerosis (Gilbert *et al*., 2013). Due to the enzyme activity of the tempeh mold, the nutritional content of tempeh, namely the content of fat, protein, carbohydrates, and minerals, has a better biological value when compared to soybeans (Sanjukta & Rai, 2018). Tempeh has more dissolved solids, nitrogen, free amino acids, free fatty acids, digestibility, protein efficiency, and protein score than soybean (Setyani *et al*., 2017).

Tempeh which is frequently encountered in the market, is typically wrapped with plastic wrap (Razie & Widawati, 2018). Packaging tempeh with plastic and leaves has fulfilled the requirements of tempeh packaging, which include being able to maintain the moisture of soybean during fermentation, ensuring good aeration thus, there is enough oxygen for mold growth; however, not providing too much air, and maintaining the cleanliness and good appearance of the tempeh produced (Steinkraus & Joshi, 2015).

Tempeh, commonly consumed in Indonesia, is typically prepared from imported GMO soybeans and packed in plastic (Ratnaningsih *et al*., 2016). In Indonesia, few producers employ indigenous soybeans to produce tempeh (Razie & Widawati, 2018). Tempeh producers mostly use imported soybeans to make tempeh. Furthermore, little is known about the nutritional value and appearance of better types of indigenous soybean tempeh. Most people still believe that the current soybeans are solely local Wilis soybeans, which are smaller and less yellow than imported soybeans. Most tempeh makers package tempeh in plastic since the average consumer prefers tempeh wrapped in plastic (Razie & Widawati, 2018). This is owing to a lack of exact information comparing the nutritional composition of tempeh packaged in plastic with tempeh packaged in leaves, particularly the contents of antioxidants, lipids, and minerals. The objective of this research is to evaluate the effect of employing local and imported soybeans on antioxidant activity levels, lipid content, and ash content in tempeh packaged with four different types of packaging materials, namely plastic, banana leaves, teakwood leaves, and hibiscus leaves.

2. RESEARCH METHOD

2.1. Materials

Local Grobogan soybeans, imported GMO soybeans, and tempeh starter (instant yeast) obtained from LIPI under the trademark Raprima Inokulum Tempeh were used in the production of tempeh. Waru Laut (*Hibiscus tiliaceus*) leaves, a type hibiscus leaf, were utilized in the packing of tempeh. Plastic from Gading Mas Supermarket, banana leaf of the type Pisang Batu (*Musa balbisiana*), and the teakwood leaves of the type Jati Gantjah from Bantul, Yogyakarta, were gathered in the Faculty of Animal Husbandry, Gadjah Mada University (UGM). Diphenylpicrylhydrazyl (DPPH) solution, technical methanol, petroleum ether, aquadest, and ascorbic acid were utilized for the chemical analysis.

2.2. Location and Time

The study was carried out at the Department of Food Technology and Agricultural Products, Food and Nutrition Laboratory, Food Chemistry and Biochemistry Laboratory, and Process and Processing Engineering Laboratory. All were belong to the Gadjah Mada University. The research was conducted in March-June 2017.

2.3. Research procedure

2.3.1 Tempeh Fermentation

The imported soybeans were obtained from the Kweni Tempeh Industry, whereas the local soybeans were obtained from the local tempeh home industry. The imported soybean and the local soybean were washed and soaked for 2 h. Subsequently, soybeans were boiled for 30 min and soaked again for 24 h. The next step was peeling the husk followed by steaming for 30 min. After cooling to room temperature, the soybean were then inoculated with 0.2% (w/w) instant yeast.

Furthermore, four packaging types are used such as plastic, banana leaves, teak leaves, and hibiscus leaves. Each of packaging was filled 110 grammes of inoculated soybeans by instant yeast. Before being utilized as packaging, the plastic is perforated at a distance of around 2 cm and then sealed using a sealer. In a new approach to leaf packaging, inoculated soybeans by instant yeast were first wrapped in banana leaves, hibiscus leaves, and teak leaves before being knotted with raffia rope and punctured at a distance of around 2 cm. Following the completion of the packaging process, all tempeh was incubated at temperature 25 ^{o}C for 48 h.

2.3.2 Sample Preparation

Thin slices of tempeh with a thickness of 0,5 mm, were dried in a cabinet dryer at 50 ^{o}C for 8 h. Subsequently, using a blender, crush the dry tempeh until smooth, and sift 60 mesh of the created tempeh powder until the desired amount of tempeh powder is acquired. After that, tempeh powder was analysed antioxidant activity, lipid content, and ash content of tempeh.

2.3.3 Analysis of Antioxidant Activity

The DPPH method was used to test antioxidant activity. The powdered tempeh sample was placed in an Erlenmeyer and weighed 0,5 grammes before being dissolved in 80 ml of methanol and sonicated for 30 minutes. Subsequently, 1 mL of sample extract was placed in a test tube that has been covered by alumunium foil, followed by 3 mL of technical methanol and 1 mL of DPPH solution, and left to stand for 30 minutes. The absorbance was read using a Spectronic 200 spectrophotometer with a 514 nm wavelength (Puspasari *et al*., 2021).

2.3.4. Analysis of Fat Content

The fat content was determined using the Soxhlet technique. Tempeh was ground with a mortar, and the resulting powder was weighed up to 2 g. Tempeh powder was placed in the Soxhlet flask. Previously, the Soxhlet flask was dried in a Memmert V30 electric oven and weighed to a constant to calculate the soxhlet flask's constant weight. The solvent was added to the petroleum ether in the Soxhlet set after the sample was entered, and the flask was assembled on the Soxhlet set. Subsequently, the extraction was carried out for three hours. After the extraction process was completed, the oven continued for 24 hours. Furthermore, the soxhlet flask was weighed every 30 minutes until it reached a steady weight (AOAC, 2016).

2.3.5 Analysis of Ash Content

The ash content test was performed on fresh tempeh after a 48-hour incubation period. Fresh tempeh was pounded in a mortar first, and the direct approach was used to determine the ash content (dry method). Fresh tempeh that had been mashed was weighed up to 2 g using an analytical balance Shimadzu AW 120. The sample was then burned with an electric burner until it was smokeless and placed in a muffle furnace, where it was ashed until it was brittle or grey-white (AOAC, 2016).

2.4. Research Design

This study employed a complete randomized factorial design with two experimental replications and two analytical replications. The independent variables utilised variations in types of soybeans, namely imported soybeans (GMO soybeans) and local soybeans and variations in packaging types, namely banana leaves, hibiscus leaves, teak leaves, and plastic. The dependent variables used were antioxidant activity, fat content and, ash content. The data obtained were analysed using SPSS General Linear Model (Univariate ANOVA). The difference real (significant) the DMRT test was carried out at a test level of 5%.

3. RESULTS AND DISCUSSION

3.1. Antioxidant Activity

Antioxidant activity analysis aimed to determine the antioxidant activity of tempeh from local soybeans and imported soybeans packaged in four types of packaging. The analysis results of the antioxidant activity in tempeh made from local soybean varieties of Grobogan and imported soybeans packaged in four packaging types: plastic, banana leaves, teak leaves, and hibiscus leaves, can be seen in Table 1.

Table 1. The antioxidant activity (%wb) of tempeh from local and imported soybeans with four packaging material variations

Soybean	Packaging Materials				Antioxidant Activity (%)
	Teak	Banana	Plastic	Hibiscus	
Import	34.76^{s}	30.37^{r}	10.06^{p}	40.69^{u}	28.97^{x}
Local	36.61^{t}	34.56^{s}	24.78^{q}	53.43^{v}	37.34^{y}
Antioxidant Activity (%)	35.68^{c}	32.46^{b}	17.42^{a}	47.06^{d}	

Note: - The average value was obtained from 2 treatment repetitions and 2 analysis repetitions
- The same superscript in the same column or row shows no significant difference ($p<0.05$)

Table 1 demonstrates that in all packages and soybean types significantly effected antioxidant activity. The average antioxidant activity of tempeh prepared from imported soybeans differed significantly from tempeh made from local soybeans. The average antioxidant activity observed is consistent with earlier research, in which the average antioxidant activity of tempeh is approximately 48,61% (%wb) (Istiani *et al.*, 2015). In all packages, local soybean tempeh has a higher antioxidant activity (37.34%) than imported soybean tempeh (28,97%). Changes in antioxidant activity in soybean varieties and growing conditions influence this difference, which also causes differences in the number of isoflavones in soybeans and tempeh (Puspasari *et al.*, 2021).

Table 1 also reveals average antioxidant activity of tempeh in four packaging materials for all soybeans: plastic, banana, teak, and hibiscus leaves. All tempeh wrapped with leaves (banana, teakwood, and hibiscus) demonstrated considerably varied antioxidant activity for all types of soybeans. Compared to other soybean types, tempeh wrapped with hibiscus leaves had the highest average antioxidant activity (47.06%). When compared to other forms of soybeans, tempeh packaged in plastic had the lowest average antioxidant activity (17.42%); when the interplay of two variables, namely the type of soybean and the form of packaging, tempeh made from local soybeans packaged in hibiscus leaves had the highest antioxidant activity (53.43%).

Since hibiscus leaves provided a suitable environment for the growth of tempeh molds such as *Rhizopus oryzae, Rhizopus stolonifera,* and *Rhizopus oligosporus*, tempeh packaged with hibiscus leaves exhibited the highest antioxidant activity compared to another packaging (Kembaren *et al*., 2013). Hibiscus leaves feature trichomes (heavy hairs) on the underside that easily bind to the fungus. Thus, hibiscus leaves generate yeast in tempeh, known as *usar* (Sarwono, 2018).

Apart from the effect of activity from the added instant yeast, tempeh packaged with hibiscus leaves also effects fermentation activity from molds such as *Rhizopus oligosporus* and *Rhizopus oryzae* clinging to the hibiscus leaves, allowing the fermentation to proceed more efficiently. The tempeh molds will produce superoxide dismutase, an antioxidant enzyme (Astawan *et al*., 2013).

Furthermore, the more optimal the fermentation, the more protein is degraded into amino acids by proteolytic enzymes produced by mold activity (Castro & Sato, 2015). An increase in the number of amino acids is accompanied by an increase in tempeh antioxidant peptides, resulting in the maximum antioxidant activity of the hibiscus (Sanjukta & Rai, 2016). Hibiscus leaves contain flavonoid chemicals, anthocyaninin, saponins, and phenols, which are suggested to boost the antioxidant content of tempeh (Utama *et al*., 2018; Astawan, 2013). Tempeh packed with teak and hibiscus leaves has the highest antioxidant activity due to the presence of bioactive compound and pigments, specifically anthocyanins (Kembaren *et al*., 2013). Anthocyanin from hibiscus and teak leaves disperse in tempeh, resulting in brownish-red blotches in hibiscus leaves and teak leaves tempeh (Putri *et al*., 2018). There are also fine hairs (trichomes) on the surface of teak leaves, though not as many as on hibiscus leaves (Sulistiyono *et al*., 2016).

Since banana leaves contain antioxidant chemicals in the form of polyphenols, then tempeh packed in banana leaves has more higher antioxidants than tempeh packed in plastic. Polyphenol molecules are also responsible for tempeh's aroma, and this chemical diffuses throughout tempeh to provide a fragrant aroma (Mastuti & Handayani, 2013). Tempeh, packaged in plastic, has lowest of antioxidant activity since it is only fermented using molds from added instant yeast.

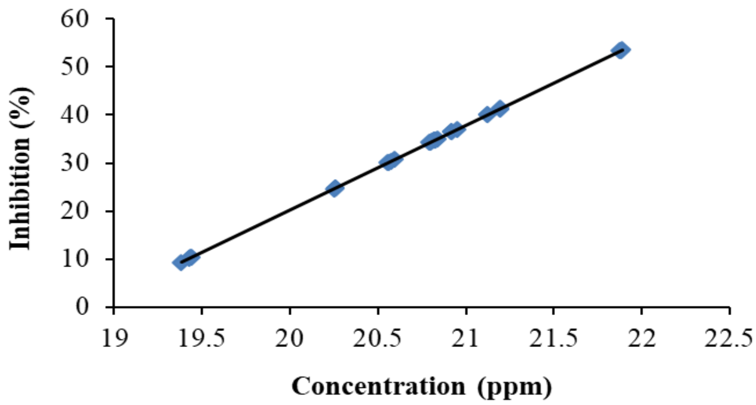

Figure 1. Relation between antioxidant concentration and sample inhibition percentage

Figure 3.1 depicts the sample's antioxidant component concentration and antioxidant activity (% Inhibition). The more antioxidant molecules there are in tempeh, the higher the percentage of antioxidant activity (% Inhibition) (Meenashare *et al*., 2014). The relation between antioxidant concentration (*x*) and inhibition percentage (*y*) is represented by:

$$y = 17.64x - 332.6 \qquad (R^2 = 0.99) \qquad (1)$$

3.2. Fat Content

Lipids are fats and oils, and the fat content of soybeans indicates the quality of the soybeans. Table 2 demonstrates the fat content of tempeh prepared from Grobogan local soybean varieties and imported soybeans bundled in four different packaging methods: plastic, banana leaves, teak leaves, and hibiscus leaves. All packaging materials and soybean types significantly effected fat content.

Table 2. The fat content (%db) of local and imported soybean tempeh using four different packaging materials

Soybean	Packaging Materials				Fat Content (%)
	Teak	Banana	Plastic	Hibiscus	
Import	42.33^{u}	43.06^{v}	48.89^{w}	41.28^{t}	43.89^{x}
Local	38.55^{q}	39.67^{r}	40.14^{s}	30.38^{p}	37.15^{y}
Fat Content (%)	40.23^{b}	41.61^{c}	44.52^{d}	35.83^{a}	

Note: - The average value was obtained from 2 treatment repetitions and 2 analysis repetitions
- The same superscript in the same column or row shows no significant difference ($p<0.05$)

Table 2 illustrates that the average fat content of tempeh made from imported soybeans in all packages differs significantly from the average fat content of tempeh made from local soybeans in all packages, with tempeh from imported soybeans having a higher average fat content (43.89%) than tempeh from local soybeans (37.15%). In general, imported soybeans contain more fat than local soybeans (Carlim *et al*., 2019). Furthermore, variations in fat content are driven by environmental and genetic variables (Gilbert *et al*., 2013). Compared to SNI tempeh, the fat content of tempeh is at least 28.57% (bk) (SNI, 2015).

Table 2 also illustrates the average fat content of tempeh wrapped in four different forms of packaging for all types of soybeans: plastic, banana leaves, hibiscus leaves, and teak leaves. Tempeh, packaged in plastic, teak leaves, hibiscus leaves, and banana leaves, had considerably varying fat content for all types of soybeans. Compared to leaf packing, tempeh packaged in plastic has the highest average fat content (44.52%) of all of soybeans. When the interaction of two variables, namely the soybean and the type of packaging, is taken into account, the tempeh with the highest fat content is tempeh made from imported soybeans with plastic packaging (48.89%). This is because the leaf packing is light-resistant; aeration happens since air can circulate, allowing oxygen to be exchanged quickly. Humidity is maintained, allowing tempeh molds to grow appropriately. Temperatures of 30 ^{o}C, pH of 6.8, and humidity levels of 70%-80% are ideal for fermenting soybeans into tempeh (Radiati, 2016).

Furthermore, teak and hibiscus leaf packaging is a favourable medium for the growth of tempeh molds since these two leaves include fine hairs (trichomes) that can be connected to tempeh molds attached to the leaf. Thus, more tempeh molds will participate in fermentation, producing ideally synthesised lipase enzymes, which hydrolyse triacylglycerol into free fatty acids. Fatty acids will provide energy for mold growth (Astawan *et al.*, 2013). Thus, tempeh wrapped with leaves has lower fat content than tempeh packaged in plastic.

3.3. Ash Content

The ash content of a dietary material indicates its mineral content; the higher the ash content of a substance, the more minerals it contains. Table 3 illustrates the ash content of tempeh prepared from imported soybeans and indigenous soybeans of the Grobogan variety packaged in four different packaging methods: plastic, banana leaves, teak leaves, and hibiscus leaves. All packaging materials and soybean types significantly affected ash content.

Table 3. The ash content (%db) of local and imported soybean tempeh with four different packaging materials

Soybean	**Packaging Materials**				**Ash Content (%)**
	Teak	Banana	Plastic	Hibiscus	
Import	2.15^{r}	1.98^{q}	1.98^{q}	2.19^{r}	2.07^{x}
Local	1.76^{p}	1.74^{p}	1.67^{p}	1.88^{q}	1.77^{y}
Ash Content (%)	1.96^{b}	1.86^{a}	1.83^{a}	2.03^{b}	

Note: - The average value was obtained from 2 treatment repetitions and 2 analysis repetitions
- The same superscript in the same column or row shows no significant difference (p<0.05)

Table 3 reveals that the average ash level of tempeh made from imported soybeans in all packages (2.07%) is significantly higher than the average ash content of tempeh made from local soybeans in all packages (1.77%). This demonstrates that the mineral content of the tempeh from imported soybeans is higher than that of local soybeans.

Varieties and environmental conditions alter tempeh's mineral content (Gilbert *et al.*, 2013). The work of the phytase enzyme generated by *Rhizopus oligosporus*, which can hydrolyse phytic acid in fermented soybeans, results in an increase in mineral content, such as phosphorus, during the fermentation of soybeans into tempeh (Sarwono, 2018). Compared to SNI tempeh, the obtained ash content is consistent with SNI, with a maximum of 4.28% db (SNI, 2015). If the ash content in tempeh surpasses the maximum specified SNI (4.28% db), the tempeh flavour will taste harsh due to the excessive mineral content. This could happen if the tempeh producer employs a metal cooker that is not good enough to dissolve the metal component in the soybeans.

Table 3 also indicates the average ash level of tempeh, which is packaged in four different ways for all types of soybeans: plastic, banana leaves, teakwood leaves, and hibiscus leaves. Tempeh, wrapped in plastic (1.83%) and banana leaves (1.86%), had a similar average ash percentage for all types of soybeans. Similarly, tempeh had an average ash concentration that was not substantially different when bundled with teak leaves (1.96%) and hibiscus leaves (2.03%) for all types of soybeans. Meanwhile, for all types of soybeans, tempeh wrapped with plastic leaves had a lower average ash level (1.83%) than hibiscus leaf packing (2.03%).

When the interplay of two variables, namely the soybean and the type of packaging is considered, the tempeh with the highest ash level is tempeh prepared from imported soybeans with hibiscus leaf packaging at 2.19%. Since hibiscus leaves have numerous fine hairs (trichomes) on their surface that can be connected to tempeh molds, tempeh wrapped in hibiscus leaves will be fermented by tempeh molds from instant yeast and tempeh molds attached to the hibiscus leaves (Sarwono, 2018).

Tempeh wrapped with banana leaves had a lower average ash concentration (1.83%) than tempeh packaged with teak leaves (1.96%). This is due to the hairs (trichomes) on the surface of the teak leaves. Since tempeh molds can stick to downy hairs, the fluff is an excellent medium for their growth. Thus, the activity of the instant ragi and tempeh molds attached to the teak leaves will causing more phytase enzymes to be created to hydrolyse phytic acid in soybeans for fermentation (Sarwono, 2018). Meanwhile, tempeh wrapped in banana leaves only receives the tempeh activity from instant ragi. Factors of variety, environment and waru leaf surface which has fine hairs (trichomes) cause the growth of more diverse tempeh molds. These tempe mold can produce the optimum phytase enzyme to hydrolyze phytic acid into minerals such as phosphorus during the tempe fermentation process (Sarwono, 2018).

4. CONCLUSION AND SUGGESTION

The following conclusions can be derived from the findings of the analysis that the type of soybeans used, namely local and imported soybeans, and the type of packing materials, namely plastic, banana leaves, hibiscus leaves, and teak leaves effect significantly the increasing of the antioxidant activity, lipid content, and ash content of tempeh.

The results of this research were about antioxidant activity, fat content and ash content. Thus, Isoflavones content and shelf life of imported soybeans and local soybeans tempeh wrapped in four different packaging types: hibiscus leaves, teak leaves, banana leaves, and plastic must be analysed in the next research for development of local tempeh product with four different packaging types: hibiscus leaves, teak leaves, banana leaves, and plastic.

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