Jurnal Farmasi Galenika (*Galenika Journal of Pharmacy*) (e-Journal) 2025; 11 (2): 68– 77
ISSN: 2442-8744 (electronic); 2442-7284 (printed)
Journal Homepage: https://bestjournal.untad.ac.id/index.php/Galenika
DOI: 10.22487/j24428744.2025.v11.i2.17247

Daidzein and Genistein Content in Tempeh Products from Several Local Soybean Varieties

(*Kandungan Daidzein dan Genistein Dalam Produk Tempe Dari Beberapa Varietas Kedelai Lokal (Glycine Max (L.) Merrill)*)

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Article Info:
Received: 10 July 2024
in revised form: 19 July 2024
Accepted: 15 September 2025
Available Online: 31 October 2025

Keywords:
Daidzein
Soybean seed
Tempeh

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ABSTRACT

Background: Tempeh is a widely consumed food due to its health benefits, affordability, and suitability for long-term consumption. The production process of tempeh from soybeans involves several stages, including soaking, dehulling, and inoculation with *Rhizopus oligosporus* yeast. Fermentation plays a key role in enhancing the nutritional quality of tempeh, particularly by increasing the levels of aglycone isoflavones. Tempeh contains three major types of aglycone isoflavones: daidzein, glycitein, and genistein. Among these, daidzein and genistein are classified as phytoestrogens due to their structural similarity to endogenous estrogens and their associated health-promoting properties. **Objectives:** This study examined six soybean seed varieties and their corresponding tempeh products, processed using traditional methods and fermented for three different durations (42, 47, and 52 hours). **Methods:** Soybean seeds and tempeh samples were extracted using cold extraction method and analyzed for daidzein and genistein content by the standard addition method. **Results:** The results indicated variations in daidzein and genistein content across the six soybean varieties and among tempeh products fermented for different durations. The tempeh produced from the Dering 1 soybean variety had the highest daidzein content (2.59%), while the Devon 1 variety had the highest genistein content (2.13%) after 47 hours of fermentation. **Conclusions:** These findings support the potential of tempeh made from local soybean varieties as a functional food, owing to their elevated levels of bioactive isoflavones.

How to cite (APA 6th Style):
Hasanah, S. U., Fitriansyah, S. N., & Herawati, I. E. (2025). Daidzein and genistein content in tempeh products from several local soybean varieties. *Jurnal Farmasi Galenika: Galenika Journal of Pharmacy (e-Journal), 11*(2), 68-77. doi:10.22487/j24428744.2025.v11.i2.17247

INTRODUCTION

Tempeh is widely consumed for its health benefits, affordability, and long-term consumption. It can serve as a substitute for meat, as widely discussed (Godfray et al., 2018). Tempeh is a native Indonesian food. The stages of making tempeh include soaking, ripening, and fermenting the soybeans by adding the mold *Rhizopus oligosporus*. In general, soybeans are soaked for 10–12 hours at room temperature, and the skin is peeled off. The seeds are then boiled, drained, and cooled to 35-40°C, added yeast, and fermented at 37°C for 24-48 hours (Nout & Kiers, 2005). Fermentation improves the nutrition in tempeh by increasing the isoflavone aglycone content (genistein, daidzein, and glycitein). In non-fermented products, such as soy milk and tofu, the aglycone content is smaller compared to fermented products (Astuti et al., 2000). During the fermentation process, the glycosidic bonds in daidzin are hydrolyzed, releasing the sugar moiety at the 7-position, catalyzed by the β-glucosidase enzyme. The extent of conversion of glycosides to aglycones during fermentation depends on the fermentation duration. After 6 hours of fermentation, only a small fraction of glycosides converts to aglycones.

Nakajima et al. reported that tempeh contains three types of aglycone isoflavones (daidzein, glycitein, and genistein), and their levels increase after fermentation for 24 hours (Nakajima et al., 2005). Daidzein (isoflavone aglycone) is a compound found in soybeans in high amounts (Kuligowski et al., 2017). Daidzein is a phytoestrogen similar to estrogens in the body and provides benefits for women's health (Van Duursen, 2017). In other studies, daidzein may protect against osteoporosis (Jin et al., 2017), cardiovascular and cerebrovascular diseases (Jamwal, 2019; Li et al., 2014). Based on its chemical structure, daidzein is similar to the human hormone estrogen and exhibits action on estrogen and estrogen receptors. Daidzein modulates estrogen receptor signaling, which demonstrates its important role in various conditions such as cancer, diabetes, and cardiovascular diseases (Lecomte et al., 2017).

Several studies show that daidzein has anti-inflammatory activity in TNF-α–treated lung epithelial cells (Li et al., 2014) and stimulates TNF-α signaling in caco-2 cells (Peng et al., 2017). Daidzein can also suppress inflammation by inhibiting signal transduction and activating transcription 1 (STAT-1) and NF-kB (S. E. Jin et al., 2012). Daidzein shows inhibition of LPS-NO (nitric oxide) induction of macrophages by activating macrophages (Choi et al., 2012).

Considering the pharmacological activity of daidzein and genistein, as well as the increasing availability of superior soybean raw materials (the government has released 73 super soybean varieties) (Krisdiana, 2014), this study aims to determine and compare the daidzein and genistein content in tempeh, using raw materials from six local soybean varieties and various fermentation times (42, 47, and 52 hours). Traditional processing methods, commonly used by Indonesian producers, were employed, and analysis was performed using the standard addition method, in which known amounts of a reference standard were added to the sample for testing. The study was conducted to identify the soybean varieties and tempeh products with the highest daidzein and genistein content, as well as the optimal fermentation duration.

MATERIAL AND METHODS

Materials

This study used six soybean varieties (Anjasmoro, Dering 1, Deja 1, Derap 1, Devon 1, and Gepak kuning) from BALITKABI, East Java, Aquadest, methanol PA (Dwi lab), tempeh yeast (Raprima®), and standard daidzein (Sigma Aldrich). The pieces of equipment used in this research were a micropipette (Eppendrof®), a UV-Vis spectrophotometer (Thermo Scientific®), an analytical balance (Ohaus Pj1003), and glassware.

Methods

The stages in producing tempeh are soaking, peeling, boiling, and fermenting. The fermentation time used in this study was 42, 47 and 52 hours. The soybean seeds and procedsed tempeh were extracted using methanol solvent. The daidzein content was analyzed using a pure daidzein standard and the addition method, in which the daidzein standard solution was added to equalize the matrix between the sample and the standard.

Tempeh production and extraction

Soybeans (50 grams) were soaked in water for 24 hours, then peeled and washed. They were boiled for 15 minutes, drained, and cooled to room temperature (25^0C). Yeast (100 mg) was added to the cooled soybeans, and then the mix was fermented for 42 hours, 47 hours, and 52 hours. Soybean seed powder and tempeh were extracted using the maceration method, using 400 ml of methanol solvent. The liquid extract was evaporated to obtain a thick extract of soybeans and tempeh.

Analysis Procedure

1. Daidzein content

A standard solution of 4 mg daidzein in 100 ml methanol PA was used. 50 mg of Soybean seed and tempeh extracts were dissolved in 10 ml methanol. The extract solutions were poured into vials. Each vial contained 2 ml of the extract solution. Then, the standard daidzein was added to the vials in different amounts (0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ml), and methanol was added up to 5 ml. The solutions were measured in a UV-Vis spectrophotometer at a wavelength of 254 nm. The daidzein content in 50 grams of extract was determined based on the line equation obtained in percentage.

2. Genistein content

A standard solution of 4 mg genistein in 100 ml methanol PA was used. 50 mg of Soybean seed and tempeh extracts were dissolved in 10 ml methanol. The extract solutions were poured into vials. Each vial contained 2 ml of the extract solution. Then, the standard genistein was added to the vials in different amounts (0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ml), and methanol was added up to 5 ml. The solutions were measured in a UV-Vis spectrophotometer at a wavelength of 261 nm. The genistein content in 50 grams of extract was determined based on the line equation obtained in percentage.

RESULTS AND DISCUSSION

The tempeh in this study was made from different soybean varieties namely Anjasmoro, Dering 1, Deja 1, Derap 1, Devon 1, and Gepak Kuning, with fermentation times of 42 hours, 47 hours, and 52 hours. The process of making tempeh involved three stages - soaking, boiling, and fermenting. Figure 1 shows the tempeh made with various fermentation times. The soaking process was the initial stage in making tempeh. It was a hydration process so that the soybean seeds skin can be easily peeled. Additionally, during the soaking process, a natural acidification process occurred (reaching pH 4.85), which helped inhibit the growth of microorganisms that could cause spoilage (Ahnan-Winarno et al., 2021). The next stage involved boiling the soybeans to soften them and eliminate spoilage organisms that could pose a food safety hazard or disrupt the fermentation process (Dinesh Babu et al., 2009; Nout & Kiers, 2005).

1 2 3

Figure 1. Tempeh produced with a fermentation time of 42 hours (1), 47 hours (2), and 52 hours (3).

During the fermentation process, the amount of mycelial growth increased with longer fermentation, leading to a compact tempeh. Based on Figure 1, the tempeh with a 47-hour fermentation time showed a significant increase in mycelium growth, leading to a whiter color and complete coverage of the soybean seeds. This was due to the rapid growth of the fungus *Rhizopus oligosporus* during the 24-hour fermentation time at a temperature of 30-42°C. On the other hand, the tempeh with a 52-hour fermentation time had a softer texture, with mucus appearing on the surface and a pungent aroma. This was due to the release of around 40% ammonia in the tempeh, caused by alkylation, which began to form in tempeh with a fermentation time of 46 to 72 hours. The bacteria *K. pneumoniae* and *Trichosporon beigelii* produce proteolytic enzymes during fermentation that break down proteins into peptides and amino acids under anaerobic conditions, generating H_2S, ammonia, methyl sulfide, amines, and other odorous compounds. As ammonification progresses, the pH of the tempeh increases from 4.6 to 7.1, producing a slightly bitter taste.

The process of extracting tempeh and soybean seeds involved maceration. Methanol was chosen as the solvent due to its universal nature and its ability to dissolve more phenolic and flavonoid compounds compared to other solvents such as ethyl acetate, n-hexane, water, and butanol (Jan et al., 2013).
The daidzein and genistein contents were measured in the soybean seeds and tempeh methanol extracts at three fermentation times. The results are shown in Table 1.

Table 1. Results For Daidzein and Genistein Content in Soybean Seeds and Tempeh

Soybean seed varieties	Product	Daidzein* (%)	Genistein* (%)
Anjasmoro Variety	Dry seeds	0.30±0.02	0.52±0.01
	Tempeh fermented 42 hours	0.20±0.18	0.91±0.47
	Tempeh fermented 47 hours	1.78±1.02	0.44±0.29
	Tempeh fermented 52 hours	0.62±1.84	0.28±0.14
Dering 1 Variety	Dry seeds	0.67±0.02	0.57±0.02
	Tempeh fermented 42 hours	0.34±0.38	0.41±0.17
	Tempeh fermented 47 hours	2.59±0.21	0.54±0.06
	Tempeh fermented 52 hours	1.03±0.01	0.4±0.03
Deja 1 Variety	Dry seeds	1.58±0.02	0.57±0.05
	Tempeh fermented 42 hours	0.95±0.02	0.44±0.02
	Tempeh fermented 47 hours	1.05±0.01	0.44±0.01
	Tempeh fermented 52 hours	1.77±0.01	0.49±0.01
Derap 1 Variety	Dry seeds	1.87±0.01	0.58±0.01
	Tempeh fermented 42 hours	2.21±0.02	0.21±0.01
	Tempeh fermented 47 hours	2.29±0.02	0.95±0.01
	Tempeh fermented 52 hours	2.18±0.01	0.16±0.01
Devon 1 Variety	Dry seeds	2.85±0.01	0.58±0.01
	Tempeh fermented 42 hours	0.82±0.02	0.35±0.01
	Tempeh fermented 47 hours	1.38±0.02	2.13±0.02
	Tempeh fermented 52 hours	0.91±0.05	2.71±0.03
Gepak Kuning Variety	Dry seeds	3.97±0.02	0.35±0.04
	Tempeh fermented 42 hours	1.17±0.03	0.12±0.21
	Tempeh fermented 47 hours	0.82±0.02	0.15±0.02
	Tempeh fermented 52 hours	2.54±0.02	0.68±0.37

*Data is given in mean±SD and triple replication

Table 1 reveals that tempeh generally contains a higher amount of daidzein and genistein compared to soybeans. This is because the fungus *Rhizopus oligosporus* produces the enzyme β-glucosidase, which hydrolyzes glycosidic bonds in daidzin and genistin, converting them into daidzein and genistein (Dajanta, K et al., 2013; Guzmán-Uriarte et al., 2013). Daidzein and genistein are flavonoid and phenolic compounds that possess natural antioxidant properties (Cahyati et al., 2013). In soybean seeds, there are

12 different types of isoflavones (flavonoids) present in the form of aglycones and glycosides (Sun et al., 2011). Isoflavones in soybean seeds are bioactive compounds that exhibit estrogen-like activity, also known as phytoestrogens (Cahyati et al., 2013).

During the fermentation process, the glycosidic bond in daidzein and genistein are broken down by a hydrolysis mechanism, when the glycosidic bond is broken and the molecule breaks into two parts, sugar (glycone) and aglycone, in position number 7. The amount of glycoside in soybeans that turn into aglycones during fermentation depends on the duration of fermentation. After 6 hours of fermentation, only some glycoside compounds convert into aglycones. However, this amount increases as fermentation progresses. After 12 hours, the amount of glycoside decreases by approximately 25%. This number continues to decline and reaches 50% after 18-24 hours of fermentation (Ferreira et al., 2011). Several studies report that aglycone levels increase with fermentation duration and double after 24 hours (Haron et al., 2009; Nakajima et al., 2005). According to the test results, the daidzein content in the tempeh made from Anjasmoro, Dering 1, and Derap 1 soybean varieties increased by 1.2 to 6 times the amount of daidzein present in soybean seeds after 47 hours of fermentation. This finding is supported by the results of previous research that suggests that the daidzein content in soybeans increases during the fermentation process and doubles in amount after 24 hours of fermentation (Bavia et al., 2012; Ferreira et al., 2011; Haron et al., 2009; Nakajima et al., 2005). However, the daidzein content in tempeh decreases after 52 hours of fermentation. This decrease could be attributed to the growth of bacteria that break down daidzein into 7,8,3',4'-tetrahydroxy-isoflavone and 6,7,3',4'-tetrahydroxy-isoflavone (Ahnan-Winarno et al., 2021). A similar trend was observed in the genistein content, which increased sixfold in the Devon 1 variety after 47 hours of fermentation.

Different varieties of soybean seeds and the tempeh made from those seeds were tested for their daidzein content. Amongst all the varieties, gepak kuning had the highest daidzein content. In the tempeh, the Derap 1 variety had the highest daidzein content after 42 hours of fermentation, which continued to increase until 47 hours of fermentation. The highest genistein content in the tempeh was produced from the Devon 1 soybean variety, whereas the lowest was observed in the tempeh produced from the Gepak Kuning variety, both after 47 hours of fermentation. The increase in daidzein and genistein contents was not consistent across all varieties at a specific fermentation time. This was because the growth speed of *Rhizopus oligosporus*, the microbe used in the fermentation process, changes with different temperatures and humidity levels, affecting the speed of the hydrolysis process of glycoside (daidzin) into aglycone (daidzein) (Ahnan-Winarno et al., 2021). In this study, temperature and humidity levels had a significant influence because they are related to oxygen levels, which influenced the growth of *Rhizopus oligosporus* (Ahnan-Winarno et al., 2021).

According to a previous study, the daidzein and genistein contents in dry Indonesian soybean seeds were higher than those found in dry soybeans from the United States and Australia (McKenzie's). The concentrations of daidzein and genistein reported were 30.8 mg/100 g and 72.3 mg/100 g for soybeans from the United States, 127.7 mg/100 g and 83.4 mg/100 g for Indonesian soybeans, and 96.4 mg/100 g and 61.4 mg/100 g for Australian soybeans (Hutabarat et al., 2001). These findings are consistent with the data in Table 1, which show average daidzein and genistein contents of 1870 mg/100 g and 528 mg/100 g, respectively, in dry soybean seeds. This suggests that local Indonesian soybeans have superior isoflavone levels compared to imported ones (Hutabarat et al., 2001).

Furthermore, this study found that the average concentrations of daidzein and genistein in tempeh were 0.95 g/100 g and 0.41 g/100 g after 42 hours, 1.65 g/100 g and 0.775 g/100 g after 47 hours, and 1.51 g/100 g and 0.786 g/100 g after 52 hours of fermentation. Previous studies show that the daidzein and genistein levels in tempeh made from local Indonesian soybeans were lower (21.5 mg/100 g and 24.5 mg/100 g, respectively) compared to tempeh produced from imported soybeans (Nutrisoy, Australia), which contained 4.7 mg/100 g genistein (Hutabarat et al., 2001). Variations in daidzein content between local soybean seeds and the tempeh reported in earlier studies and those used in the current study may be influenced by several factors, including seed size, pod maturity, plant age (Hasanah et al., 2020), as well as fermentation duration, and temperature (Kuligowski et al., 2017).

CONCLUSION

Tempeh is a traditional fermented food product recognized for its high levels of isoflavones, particularly daidzein and genistein, which are associated with various potential health benefits. The fermentation process significantly enhances the levels of these isoflavones, with optimal increases observed between 42 to 47 hours of fermentation. In this study, a fermentation duration of 47 hours yielded the highest concentrations of both daidzein and genistein.

Tempeh produced from local soybean varieties generally exhibited superior isoflavone content compared with those made from imported soybeans. Specifically, the Dering 1 variety yielded the highest daidzein content (2.59%), while the Devon 1 variety yielded the highest genistein content (2.13%). These findings suggest that tempeh derived from local soybean varieties has strong potential as a functional food, owing to its elevated levels of bioactive isoflavones.

ACKNOWLEDGEMENT

This study would not have been possible without the grant funding from the Hazanah Foundation. The authors would like to thank Siti Aminah, Hasna Fauziah, and Yuliana Anggraeni for their assistance. Their belief in the importance of this study and dedication to advancing knowledge in this area were vital.

CONFLICT OF INTEREST

The authors state that there is no potential conflict of interest.

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