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Effect of Tempe Processing on Genistein Content

Siti Uswatun Hasanah[1], Sukrasno[2], Rika Hartati[3], Diki Prayugo W[4*]
[1]dikiprayugo@stfi.ac.id
[1,4]Department of Pharmaceutical Biology, Indonesian School of Pharmacy,
[1,2,3]Department of Pharmaceutical Biology, School of Pharmacy, Institut Teknologi Bandung

*Corresponding Author: Diki Prayugo W
Email: dikiprayugo@stfi.ac.id

ABSTRACT

Tempe is one of the traditional foods consumed by the Indonesian people, and is produced from soybean fermentation using Rhizopus oligosporus. Furthermore, soybeans contain a high concentration of isoflavones such as genistein. Therefore, this study aimed to determine the genistein content in tempe. A soybean fermented for 46 hours showed three times higher genistein, compared to its seeds. The results of the research showed that boiling, steaming and frying of tempe increased its genistein content. The highest result was from frying, which had 7 times more genistein content.

Keywords: *Boiling, Genistein, Fermentation, Frying, Soybean, Steaming*

INTRODUCTION

The soybean (Glycine max (L) Merr) contains different forms of isoflavone and is consumed in most Asian countries. Isoflavones are generally similar to phytoestrogens and estradiol-17β (Setchell, 1998). Isoflavones are flavonoids, which are generally found in soybeans. They are divided into 4 groups, namely: aglycones (genistein, daidzein, and glycitein); glycosides (genistin, daidzin and glycycin); malonyl glycosides (malonyl genistin, malonyl daidzin and malonyl glycine) and acetyl glycosides (acetyl genistin, acetyl daidzine and acetyl glycine) (Dhaubhadel, 2011).

Tempe is a fermented food made from soybeans and was first developed in Central Java (Karyadi & Lukito, 2009). According to Steinkraus (1988), tempeh is a white mass covered by mold mycelium and is obtained from cotyledon fermentation. It was also reported that R. Microsporus and R. Orizae are good molds for making tempeh (Jurus & Sundberg, 1976). Purwoko showed that R. microsporus var UICC 521 and R chinensis UICC 524 Orizae achieves isoflavone biotransformation in tempeh (Purwoko et al., 2001). However, the results of UICC 521 chinensis is higher than Orizae UICC 524. Nout and Kiers stated that R. oligosporus is the mold strain widely used for tempeh fermentation (Nout & Kiers, 2005). They further explained that its nutritional value was higher using R. oligosporus, when compared with Orizae.

Glycosides can deglyosilate into aglycone isoflavones in the form of genistein and daidzein (Huynh et al., 2014). These compounds have better thyroxinase inhibiting activity than isoflavone glycosides (Chang et al., 2005). A study showed that isoflavones play a role in preventing cancer by reducing the levels of IGF-I, which is responsible for proliferation, fermentation, and apoptosis. Also, an increased concentration of IGF-I is related to a high risk of breast and prostate cancer (Matthies et al., 2012).

In addition, genistein (4, 5, 7-trihydroxy isoflavones) is one of the main isoflavones present in soy, and has good biological activity. This compound decreases the activity of genes that regulate proliferation, cell cycle, stimulate apoptosis, inhibit NFkB activity, and have antioxidant effects (Yang et al., 2012).

Therefore, this study aimed to investigate the genistein content in tempeh made from different fermentation times, and also examine the effects of processing on its genistein content.

LITERATURE REVIEW

Raw Materials and Chemicals

Soybean (Glycine max) varieties such as *detam*, *demas*, *dering* and *gema* are obtained from BALITKABI, East Java. The genistein standards were obtained from SIGMA Aldrich Chemicals with analytical grades.

Tempe Production

100g of soybeans were soaked in clean water for 18 hours, washed, and separated from their outermost covering. The clean soybeans were then boiled (250 mL water) for 30 mins and then left to cool. This was followed by adding 200mg of yeast, evenly stirred and wrapped. The soybeans were fermented for 31, 36, 41, 46, and 51 hours until a rotten tempeh was obtained at 91 hours. The resulting product was then fried to brown color using hot oil, boiled for 15 mins, and steamed for 30 mins.

Extraction of Soy Bean, Tempe and Processed Tempe

Processed tempeh was (± 10 grams) extracted by reflux, using methanol (80 ml) solvent, and the maceration filtrate was evaporated to obtain a concentrated methanol extract.

Fractionation

A classical column chromatographic method was used to fractionate 50 mg extract. The stationary phase of the column was silica gel 60 (1:50), while the mobile phases were n-hexane (100%), n-hexane-ethyl acetate (80%: 20%), n-hexane (saturated methanol) -ethyl acetate (50%: 50%) and n-hexane-ethyl acetate (20%: 80%).

Making Genistein Calibration Curves

A standard solution was made with a concentration of 3 mg/l, 6 mg/l, 12 mg/l, 24 mg/l, 40 mg/l and 60 mg/l. Each concentration was eluted using n-hexane-ethyl acetate (4: 5). Also, the stains were analyzed with a 254 nm UV lamp and scanned with TLC Spectrophoto Densitometry at a maximum wavelength of 262 nm.

RESEARCH METHODOLOGY

Analysis of Genistein with Spectrophoto Densitometry

The standard and sample were 5 µL and eluted using the mobile phase n-hexane-ethyl acetate (4: 5). The stains were analyzed with a 254 nm UV lamp, and then scanned with TLC Spectrophoto Densitometry at maximum wavelength. The scanner used was a 3 CAMAG TLC.

RESULT AND DISCUSSION

Tempe Production

Each fermentation stage's variations in tempe were represented in Figure 1. After 31 hours, the mushrooms started to grow, and a thin layer of white filament could be visible on the soybeans' surface. Once the fungus reached the acceleration phase, the cells began to divide quickly. The fungus reached the exponential process at 36–51 hours, during which cell activity significantly increased. The fungus reached the deceleration phase after 56 hours of fermentation, during which the cell

started to grow but was less active. After 61-71 hours of fermentation, the fungus reached the stationary phase, during which live and dead cells alternated. Mucus was seen on the surface at this phase, indicating decomposition had actually occurred. The fungus began to disappear around 76-91 hours of fermentation, which was followed by thick mucus and an unpleasant smell. Ammonia was produced as a result of ongoing protein degradation. Therefore, the ideal fermentation time for consumption was 46 hours.

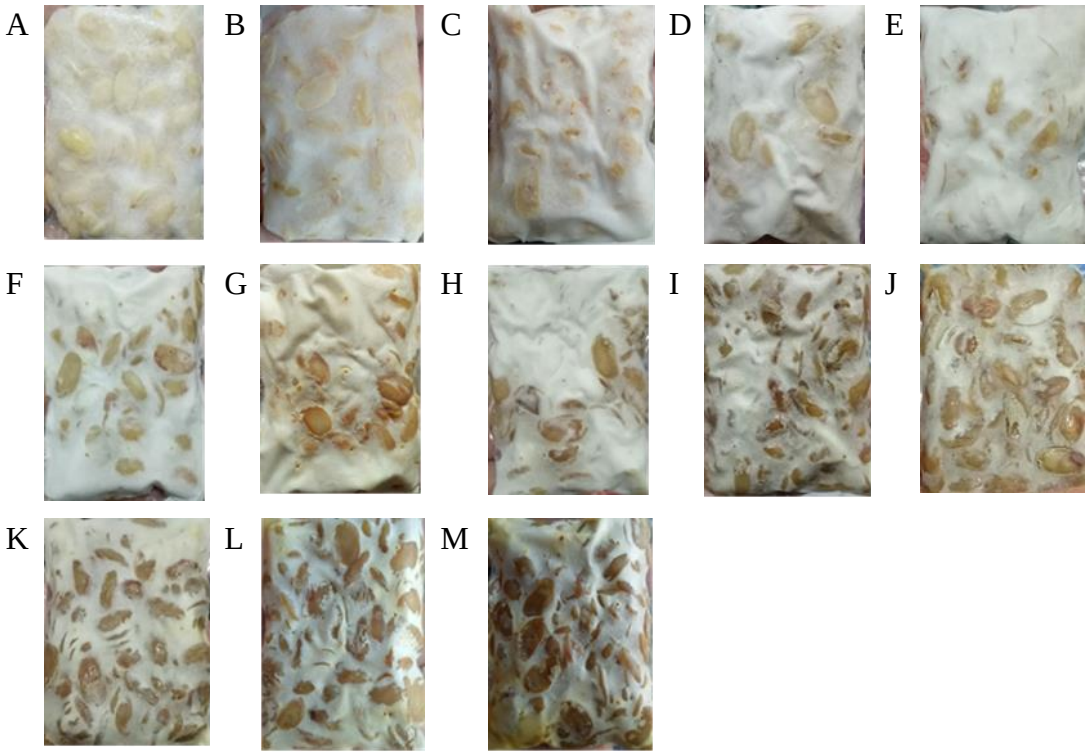


Figure 1. The Duration of Fermentation-processed Tempe

Description: (A) 31, (B) 36, (C) 41, (D) 46, (E) 51, (F) 56, (G) 61, (H) 66, (I) 71, (J) 76, (K) 81, (L) 86, (M) 91 hours

The Extraction of Soybean and Tempe

An enhanced extract was obtained at each harvest as a result of fermenting tempe for 31-91 hours. Additionally, because treated tempe was softer, the solvent could more easily permeate its pores and extract the chemicals, increasing yield. Steamed tempe had the second-highest production, followed by fried tempe. The amount of absorbed oil, heat, expansion, softening, discoloration, scent, and taste all contributed to this better result. Additionally, the fried tempe's surface area enhanced how much oil it absorbed. Because there was no compound loss during steaming, the typical variety produced more than boiling. Furthermore, the compounds were reduced after boiling because some of the compounds had dissolved in the water. In Tables 1 and 2, the yield of tempe extract are included.

Table 1.
The Yield of Tempe Methanol Extract at Various Fermentation Times

No	Fermentation time (hours)	Yield of tempeh methanol extract (%)	
		Detam Varieties	Dering Varieties
1	31	8.347	9.509
2	36	7.296	10.09
3	41	10.58	9.039
4	46	9.32	10.39
5	51	11.80	11.43
6	56	12.98	12.04
7	61	12.50	15.12
8	66	12.41	14.62
9	71	13.51	15.50
10	76	14.08	14.72
11	81	14.43	15.92
12	86	14.12	18.18
13	91	15.4	18.01

Table 2.
The Yield of Processed Methanol Extract of Tempe at 46 Hours Fermentation Time

Processing type	Yields of processed methanol extract of tempeh (%)			
	Gema Varieties	Demas Varieties	Dering Varieties	Detam Varieties
Without processing	11.37	8.86	10.39	9.32
Boiling	11.34	9.67	11.83	11.66
Frying	13.97	14.83	14.80	13.88
Steaming	13.2	13.85	11.70	15.65

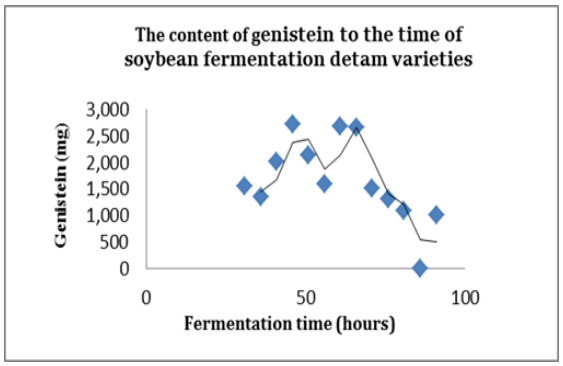
Analysis of Genistein with Spectrophoto Densitometry

The genistein calibration curve gave linear regression values of y = 94.046x + 480.71 and R2 = 0.9968. The values were used to analyze genistein in the tempe. Content analysis showed the soybean fermented for 91 hours still contained genistein (Table 3). The genistein was present as glycoside, genistein, which was converted to aglycone genistein by β-glucosidase enzyme (Huynh et al., 2014). Testing the content of two varieties of soybean seeds gave different values due to several factors such as the seed quality and time of harvest. The 61 hours of fermentation showed the highest genistein content from the two tested varieties. This was indicated in the fermentation time curve that peaked at 61 hours (Figure 2), but it is not suitable for consumption.

Table 3.
The Amount of Genistein in 100 Grams of Soybean

No	Fermentation time (hours)	Genistein content (mg)	
		Detam Varieties	Dering Varieties
1	31	1.56 ± 0.68	1.49± 1.15
2	36	1.35 ± 0.66	1.75 ± 1.27
3	41	2.02 ± 1.75	1.33 ± 1.26
4	46	2.73 ± 0.73	0.07 ± 0.23
5	51	2.14 ± 2.24	1.97 ± 1.26
6	56	1.61 ± 1.41	2.30 ± 1.15
7	61	2.69 ± 0.60	3.28 ± 1.59
8	66	2.66 ± 1.82	2.47 ± 0.54
9	71	1.51 ± 1.63	2.06 ± 0.36
10	76	1.31 ± 1.03	0.56 ± 0.29
11	81	1.10 ± 0.62	1.38 ± 0.24
12	86	0.84 ± 0.51	0.93 ± 0.36
13	91	1.01 ± 0.63	0.44 ± 0.21

A



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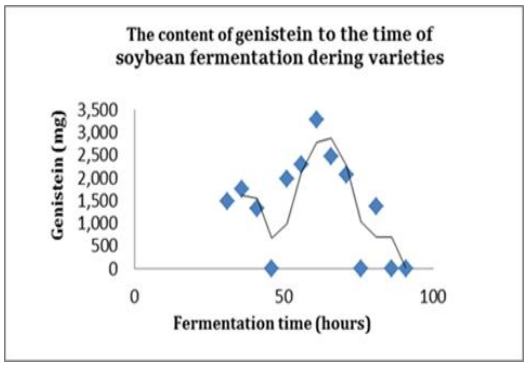

Figure 2. Tempe fermentation time curve for genistein levels in 100 gram detam variety (A) and dering variety (B) difference while results with different alphabet superscript within the row shows significant difference at $P < 0.05$.

Table 4.
The Amount of Genistein in 100 grams of Processed Tempe at 46 hours Fermentation Time

Processing type	content of Genistein (mg)			
	Gema Varieties	Demas Varieties	Dering Varieties	Detam Varieties
Without processing	2.82±0.58	0.12±0.19	0.07±0.23	2.73±0.73
Boiling	0.99±0.46	1.27±1.18	0.86±0.14	2.73±1.46
Frying	0.89±0.75	5.98±2.31	0.48±0.84	5.97±3.81
Steaming	2.86±2.47	1.86±0.43	1.09±0.38	4.59±5.36

The results of 46-hours fermentation gave an average of 1,435 mg/100g tempeh. According to previous research, genistein content in 100g of tempeh was 7.2 mg with a fermentation time of more than 24 hours (Nakajima et al., 2005). The tested genistein was lesser than the literature data, due to differences in soybean, temperature, and fermentation time. The results also showed there was diversity in the four varieties, which was caused by differences in harvesting periods (Sutoro et al., 2008). Fukutake showed that genistein content in soybean seeds was about 4.6 µg/g dry weight. When compared with the genistein in tempeh (46 hours fermentation time), there was about three times increase in the content (Fukutake et al., 1996). In addition, previous studies showed that isoflavones-aglycones increased with fermentation time (Nakajima et al., 2005).

The tempe was processed by steaming, boiling and frying, which does not eliminate the genistein, but instead increased its content. When compared with soybean seeds (based on Fukutake research, around 4.6 µg/g dry weight), there were increase in each processing, which was about seven times for fried tempe (± 33.3 ug/g), five times for steamed (± 26 µg/g), and three times for boiled (± 14.6 µg/g). This was because hydrolysis was still ongoing during the processing (Table 4), which was catalyzed by β-glucosidase enzyme. According to Riseh et al, the β-glucosidase enzyme works optimally at a temperature of 50°C (Riseh et al., 2012).

CONCLUSION

After 31 hours of fermentation, genistein in tempeh began to increase until it reached a high at 61 hours, at which period it began to decrease. Furthermore, the 46-hour fermentation of soybean seeds resulted in a higher quantity of genistein. As a result, genistein content increased whether tempeh was boiled, steamed, or fried, with frying providing the maximum value.

CONFLICT OF INTEREST

The researchers declare that they have no conflict of interest.

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