

# A comparative assessment of antioxidant compounds and their activities in tempeh made from different Taiwanese soybean varieties

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## ABSTRACT

Tempeh is one of the most popular soybean-based food products and is known to have several health benefits. Although several studies have analyzed the antioxidant properties of tempeh, the effect of different soybean varieties on the antioxidant properties of tempeh has not been studied. The aim of the present study is to evaluate the effect of different soybean varieties in Taiwan, namely Kaohsiung No. 9, Kaohsiung No. 10, Tainan No. 10 and Tainan No. 4, on the antioxidant compounds (phenolic and isoflavone contents) and antioxidant activities (ferrous ion chelating activity and ABTS radical scavenging activity) of tempeh. Tempeh made from Tainan No. 10 and Kaohsiung No. 9, which had bigger seed size, had the highest mold growth, while Tainan No 4 produced tempeh with the least mold. Tainan No. 10 showed the highest increase in total phenolic compounds after fermentation, while Tainan No. 4 showed the lowest increase. Moreover, pre-fermented soybean and tempeh made from Kaohsiung No. 9 showed the highest ferrous ion chelating properties but the lowest ABTS radical scavenging activity, while Tainan No. 4 showed the highest ABTS radical scavenging activity but

lowest ferrous ion chelating properties. Of the four soybean varieties, Tainan No. 4 is the least suitable soybean for tempeh production.

**KEYWORDS:** soybean varieties, tempeh, antioxidant capacities, phenolic content, isoflavone content.

## 1. INTRODUCTION

Soybean, *Glycine max* L. Merr., is a plant belonging to *Glycine* genus in Fabaceae family. Soybean comes in different varieties, which can be classified based on the length of breeding time, fruiting period, color and size of the seeds [1]. In general, the shape of soybean seed is oval, and the color of seed skin is milky yellow or black. Taiwan produces high-quality non-GMO (genetically modified organism) soybeans every year and it has high potential to be processed further into different products like miso, natto, soy sauce, tofu and tempeh.

Tempeh is a fermented food originating from Indonesia and is bound together by the mycelium of a mold [2]. Besides its unique flavor, soy-tempeh has been reported to have many health benefits. The antioxidant activity of tempeh was found to increase significantly after fermentation and was due to 3-hydroxyanthranilic acid (HAA)

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formation [3]. Moreover, the fermentation time increased the antioxidant activity of tempeh [4]. Tempeh also possesses anti-microbial activity [5, 6] and change in gut microbiota was observed following tempeh consumption [7]. In addition, other health benefits have been reported such as hepatoprotective activity [8, 9], neuroprotective activity [10], anti-amnesic activity [11], anti-cancer activity [12] and anti-hypertensive activity [13].

Earlier studies have explored the effect of different processing methods and parameters on the change of bioactivities of tempeh; however, the effect of various soybean varieties on the bioactivities of tempeh has not been studied. Therefore, the present study was conducted to evaluate the effect of different Taiwanese soybean varieties on the bioactivities of tempeh and find out the best soybean variety for making tempeh by comparing its phenolic content, isoflavone content, and antioxidant activities.

## 2. MATERIALS AND METHODS

### 2.1. Sample preparation

Non-GMO soybean varieties (Kaohsiung No. 9 (big seed size), Kaohsiung No. 10, Tainan No. 10, and Tainan No. 4) were purchased from the traditional market in Pingtung, Taiwan. Vernier scale was used to measure the size of the soybean seeds and water activities were measured by water activity meter (CX-2, AquaLab, WA, USA). To make tempeh, 200 g of soybeans was soaked in water for 4 h and dehulled. After removing the hull, soybeans were added into boiling water containing 1% lactic acid and cooked for 30 min. These cooked soybeans were called pre-fermented soybeans, which were cooled down and added with tempeh starter containing  $10^6$  CFU/gram *R. oligosporus*, packaged into perforated plastic bag and incubated at 30 °C for 2 days. Water activity was measured from the obtained tempehs. Afterwards, pre-fermented soybeans and tempehs produced were freeze-dried and ground into powder using a grinding machine and stored in a -20 °C freezer for further analysis. Samples were extracted using distilled water prior to analysis.

### 2.2. Total phenolic content

Total phenolic content was determined based on the method described by Strycharz and Shetty [14].

Briefly, 1 ml of sample was added with 1 ml of 95% ethanol, 5 ml of distilled deionized water and 0.5 ml of 50% (v/v) Folin-Ciocalteu reagent and reacted for 5 min. Afterwards, 1 ml of 5% (w/v)  $\text{Na}_2\text{CO}_3$  was added and reacted for 1 h. The color generated was measured by spectrophotometer at 725 nm and the concentration of phenolic content was calculated based on gallic acid (0-100  $\mu\text{g/ml}$ ) standard curve.

### 2.3. Determination of isoflavone compounds by HPLC

The contents and compositions of isoflavones in pre-fermented soybean and tempeh were determined quantitatively by HPLC following methods described by Yang *et al.* [15] (2009). A Mightysil RP-18 GP column (4.6 x 250 mm) (Kanto Chemical Co. Inc., Tokyo, Japan) set at 40 °C was attached to the HPLC system (Hitachi, Tokyo, Japan) consisting of a Chromaster 5110 pump, a Chromaster 5210 autosampler, and a Chromaster 5430 diode array detector. The mobile phase for HPLC consisted of solvent A [0.1% (v/v) acetic acid in filtered MilliQ water], and solvent B [0.1% (v/v) acetic acid in acetonitrile]. Gradient for solvent B was 15-25% for 35 min, 25-26.5% for 12 min, and 26.5-50% for 30 s, followed by isocratic elution for 14.5 min. The flow rate was 1.0 ml/min, sample amount was 20  $\mu\text{L}$  and the absorbance was measured at 254 nm. The isoflavone content of the samples was calculated by interpolation of the standard curves of the isoflavone standards.

### 2.4. Antioxidant activities

Antioxidant activities measured in this study included ABTS radical scavenging activity and ferrous ion chelating activity. ABTS radical scavenging activity was measured according to Re *et al.* [16] and ferrous ion chelating ability was determined according to the method by Huang *et al.* [17].

### 2.5. Statistical analysis

All measurements were carried out in at least three replicates. Values were expressed as mean  $\pm$  standard deviation (SD). Statistical analysis was done by Pearson's correlation analysis, paired student t-test was used for two-group sample and one way analysis of variance (ANOVA)

followed by post hoc testing (Duncan's test) for more than two-group sample at  $p < 0.05$  by using SPSS (Statistical Package for Social Sciences) 21.

### 3. RESULTS AND DISCUSSION

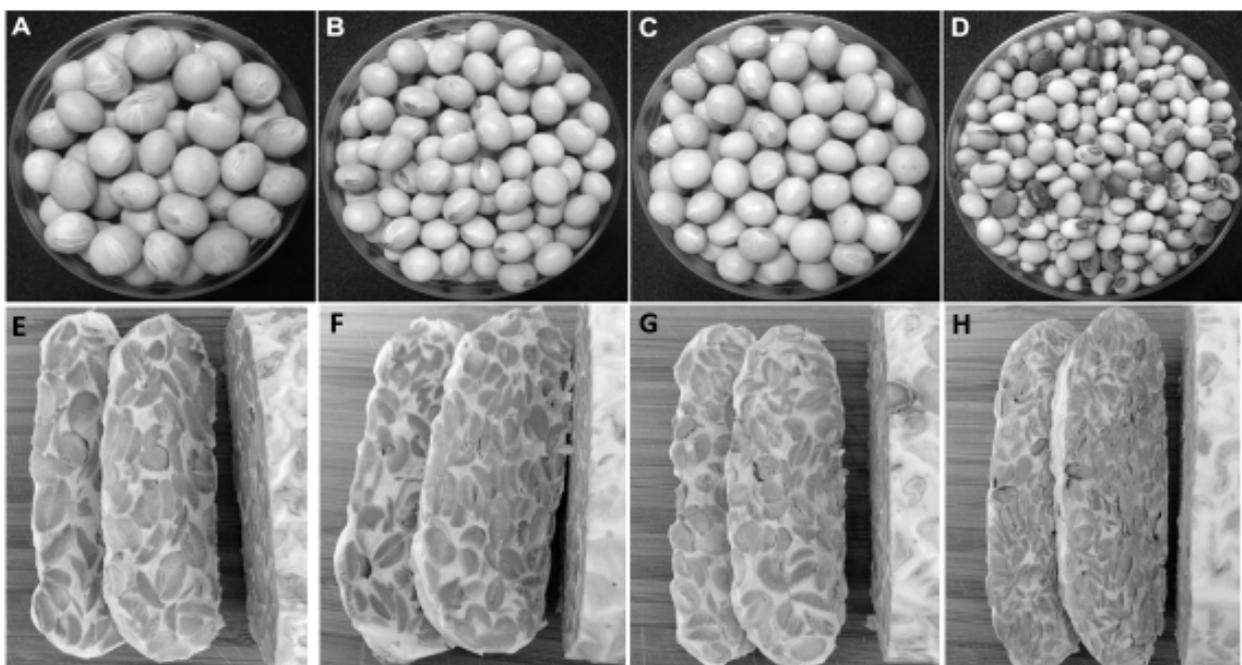
Figure 1 shows the different soybeans used (A-D) and tempeh produced (E-H). Kaohsiung No. 9 and Tainan No. 4 had greenish skin color, while other soybeans had yellowish skin color. Regarding seed size, Kaohsiung No. 9 had the biggest size, followed by Tainan No. 10, while Tainan No. 4 had the smallest size.

All tempehs collected after 48 h fermentation were fully covered with white mold and bound into a firm cake. The cross-sectional appearance of tempehs can be seen in Figure 1. The tempeh made from Kaohsiung No. 9 and Tainan No. 10 showed the highest white portion (*Rhizopus* mold), while tempeh made from Tainan No. 4 showed the highest yellow portion (soybean). Kaohsiung No. 9 and Tainan No. 10 had bigger seed size which provided bigger space and more oxygen to facilitate better mold growth.

The length, width, thickness and weight of different varieties of soybeans used are shown in Table 1.

Based on the weight, Kaohsiung No. 9 was the heaviest, while Tainan No. 4 was the lightest. Difference in water activity was also observed among different soybean seeds. The highest water activity was found in Kaohsiung No. 9 seed, while the lowest water activity was found in Tainan No. 4 seed.

The results of water activity and total phenolic contents of tempehs compared to their pre-fermented soybeans are shown in Table 2. Tempeh made from Tainan No. 4 showed the lowest water activity (Table 2). Compared to their pre-fermented soybeans, all type of soybeans showed increase in water activity after fermented into tempeh, except Tainan No. 4. Water activity is an important factor for a substrate to be used as mold growth media and the optimum Aw for the growth of *Rhizopus oligosporus* was 1.00 [18]. After cooking, Tainan No. 4 only had Aw of 0.758 which made the growth of mold in Tainan No. 4 not as good as in other soybeans. The number of phenolic compounds increased significantly after fermentation. However, the phenolic compound profile observed by HPLC was not changed significantly (data not shown). Therefore, it was



**Figure 1.** Different varieties of soybean seeds used (A-D) and tempeh produced (E-H) (Kaohsiung No. 9 (A&E), Kaohsiung No. 10 (B&F), Tainan No. 10 (C&G), and Tainan No. 4 (D&H)).

**Table 1.** The length, width, thickness, weight and water activity of different varieties of soybean seeds.

Soybean varieties	Length (mm)	Width (mm)	Thickness (mm)	Weight (g)	Water activity
Kaohsiung No. 9	10.381 ± 0.486 <sup>a</sup>	9.794 ± 0.385 <sup>a</sup>	8.190 ± 0.379 <sup>a</sup>	0.532 ± 0.056 <sup>a</sup>	0.603 ± 0.010 <sup>a</sup>
Kaohsiung No. 10	7.808 ± 0.384 <sup>b</sup>	7.151 ± 0.217 <sup>c</sup>	5.782 ± 0.140 <sup>b</sup>	0.208 ± 0.017 <sup>c</sup>	0.486 ± 0.002 <sup>c</sup>
Tainan No. 10	8.051 ± 0.414 <sup>b</sup>	7.779 ± 0.400 <sup>b</sup>	6.196 ± 0.417 <sup>b</sup>	0.249 ± 0.044 <sup>b</sup>	0.510 ± 0.006 <sup>b</sup>
Tainan No. 4	5.800 ± 0.447 <sup>c</sup>	4.892 ± 0.297 <sup>d</sup>	3.839 ± 0.199 <sup>c</sup>	0.072 ± 0.012 <sup>d</sup>	0.475 ± 0.001 <sup>d</sup>

<sup>a-d</sup>Results expressed are mean ± SD from 10 replications for length, width, thickness, and weight and from 3 replications for water activity. Data with different small letters were significantly different at  $p < 0.05$  (observed by analysis of variance (ANOVA) with Duncan's post hoc).

**Table 2.** Comparison of water activity and total phenolic content (mg/kg dried sample) in pre-fermented soybeans and tempeh.

Soybean varieties	Pre-fermented soybean		Tempeh		Changes	
	Water activity	Total phenolic content	Water activity	Total phenolic content	Water activity	Total phenolic content
Kaohsiung No. 9	0.924 ± 0.001 <sup>a</sup>	1.205 ± 0.058 <sup>b</sup>	0.991 ± 0.001 <sup>a</sup>	9.294 ± 0.570 <sup>b</sup>	+0.067*	+8.089*
Kaohsiung No. 10	0.852 ± 0.005 <sup>b</sup>	1.051 ± 0.045 <sup>c</sup>	0.988 ± 0.001 <sup>a</sup>	9.667 ± 0.330 <sup>b</sup>	+0.136*	+8.616*
Tainan No. 10	0.848 ± 0.014 <sup>b</sup>	1.197 ± 0.083 <sup>b</sup>	0.990 ± 0.001 <sup>a</sup>	10.778 ± 0.344 <sup>a</sup>	+0.142*	+9.581*
Tainan No. 4	0.758 ± 0.003 <sup>c</sup>	1.401 ± 0.065 <sup>a</sup>	0.758 ± 0.003 <sup>b</sup>	9.119 ± 0.163 <sup>b</sup>	0.00	+7.718*

<sup>a-c</sup>Results expressed are mean ± SD from 3 replications. Data with different small letters were significantly different at  $p < 0.05$  (observed by analysis of variance (ANOVA) with Duncan's post hoc). \*Results of tempeh were significantly different compared to its pre-fermented soybean (observed by paired t-test (two-tailed) at  $p < 0.05$ ).

suspected that the increase in the number of phenolic compounds was due to the formation of 3-hydroxyanthranilic acid, which was detected as a phenolic compound using Folin-Ciocalteu reagent, because it also contains phenol group. Of all soybean varieties, pre-fermented Tainan No. 4 showed the highest total phenolic content, but after fermentation, it showed the lowest total phenolic content. Moreover, the highest increase in total phenolic content after fermentation was found in Tainan No. 10.

Table 3 shows the composition of isoflavones and total isoflavones (the sum of daidzin, genistin, daidzein, and genistein) in pre-fermented soybeans and tempehs produced. Daidzin and genistin belong to isoflavone glycosides, and daidzein and genistein belong to isoflavone aglycones. After fermentation, daidzin and genistin contents reduced

in all types of soybeans; in contrast, daidzein and genistein contents increased in all soybeans. This is caused by the transformation of glycoside isoflavones into aglycone isoflavones by glucose chain removal. Regarding total isoflavones, Kaohsiung No. 10 showed the highest total isoflavones in pre-fermented soybean and Tainan No.4 showed the lowest total isoflavones. This finding is supported by Wei *et al.* [1] who reported that Kaohsiung No. 10 had the highest isoflavone content (346 mg/100 g), while Tainan No. 4 had the lowest isoflavone content (142 mg/100 g). However, total isoflavones reduced a little after fermentation and Kaohsiung No. 10 showed the highest reduction of total isoflavones after fermentation. Reduction of isoflavone aglycones may be due to further fermentation. Daidzein can transform into dihydrodaidzein,

**Table 3.** Comparison of isoflavone contents (daidzin, genistin, daidzein and genistein) of pre-fermented soybean and tempeh (mg/kg dried sample).

Soybean varieties	Isoflavone contents				Total isoflavones
	Daidzin	Genistin	Daidzein	Genistein	
<b>Pre-fermented soybean</b>					
Kaohsiung No. 9	0.249 ± 0.028 <sup>b</sup>	0.262 ± 0.018 <sup>b</sup>	0.026 ± 0.000 <sup>b</sup>	0.024 ± 0.000 <sup>b</sup>	0.561 ± 0.026 <sup>b</sup>
Kaohsiung No. 10	0.295 ± 0.019 <sup>a</sup>	0.316 ± 0.010 <sup>a</sup>	0.073 ± 0.001 <sup>a</sup>	0.063 ± 0.001 <sup>a</sup>	0.747 ± 0.027 <sup>a</sup>
Tainan No. 10	0.233 ± 0.010 <sup>b</sup>	0.214 ± 0.010 <sup>c</sup>	0.011 ± 0.000 <sup>c</sup>	0.013 ± 0.000 <sup>c</sup>	0.471 ± 0.020 <sup>c</sup>
Tainan No. 4	0.188 ± 0.012 <sup>c</sup>	0.160 ± 0.008 <sup>d</sup>	0.001 ± 0.000 <sup>d</sup>	0.003 ± 0.000 <sup>d</sup>	0.351 ± 0.018 <sup>d</sup>
<b>Tempeh</b>					
Kaohsiung No. 9	0.019 ± 0.001 <sup>b</sup>	0.122 ± 0.006 <sup>a</sup>	0.253 ± 0.003 <sup>a</sup>	0.196 ± 0.004 <sup>a</sup>	0.590 ± 0.010 <sup>a</sup>
Kaohsiung No. 10	0.017 ± 0.000 <sup>b</sup>	0.082 ± 0.001 <sup>c</sup>	0.176 ± 0.002 <sup>b</sup>	0.151 ± 0.003 <sup>b</sup>	0.426 ± 0.005 <sup>b</sup>
Tainan No. 10	0.018 ± 0.001 <sup>b</sup>	0.115 ± 0.007 <sup>b</sup>	0.134 ± 0.005 <sup>c</sup>	0.089 ± 0.002 <sup>c</sup>	0.355 ± 0.014 <sup>c</sup>
Tainan No. 4	0.024 ± 0.001 <sup>a</sup>	0.056 ± 0.005 <sup>d</sup>	0.121 ± 0.002 <sup>d</sup>	0.089 ± 0.001 <sup>c</sup>	0.291 ± 0.009 <sup>d</sup>
<b>Changes</b>					
Kaohsiung No. 9	-0.230*	-0.140*	+0.227*	+0.172*	+0.029
Kaohsiung No. 10	-0.278*	-0.234*	+0.103*	+0.088*	-0.321*
Tainan No. 10	-0.215*	-0.099*	+0.123*	+0.076*	-0.116*
Tainan No. 4	-0.164*	-0.104*	+0.121*	+0.086*	-0.060*

<sup>a-d</sup>Results expressed were mean ± SD from 3 replications. Data with different small letters were significantly different at  $p < 0.05$  (observed by analysis of variance (ANOVA) with Duncan's post hoc). \*Results of tempeh were significantly different compared to its pre-fermented soybean (observed by paired  $t$ -test (two-tailed) at  $p < 0.05$ ).

O-desmethylangolensin, and equol, while genistein can transform into dihydrogenistein, 6'-hydroxy-O-desmethylangolensin and 2-(4-hydroxyphenyl) propionic acid and 5-hydroxy-equol [19] (Matthies *et al.*, 2008). However, this study did not examine the degraded products of daidzein and genistein.

The results of antioxidant activities expressed as EC<sub>50</sub> (effective concentration to have 50% capacity) are shown in Table 4. The lower the EC<sub>50</sub> value, the higher will be the antioxidant activity. Antioxidant activity of soybean measured as ferrous ion chelating ability was too low to be expressed as EC<sub>50</sub>. However after fermentation, their antioxidant activity increased dramatically. The results of tempeh's antioxidant activities show that Kaohsiung No. 9 had the highest ferrous ion chelating ability but the lowest ABTS radical scavenging activity. In contrast, Tainan No. 4

showed the highest ABTS radical scavenging activity, but the lowest ferrous ion chelating ability. This difference was mainly caused by the antioxidant activities of the soybean itself. Pre-fermented soybean of Kaohsiung No. 9 had the highest ferrous ion chelating activity and the lowest ABTS radical scavenging activity, while Tainan No. 4 had the highest ABTS radical scavenging activities. Research done by Astawan *et al.* [20] showed that tempeh made from different soybeans had no significant different in antioxidant capacities, which were 186-191 mg AEAC (acid equivalent antioxidant capacity)/kg tempeh.

Based on this experiment, the total isoflavonoid compounds didn't change much during fermentation which prompted us to suspect that 3-hydroxyanthranilic acid is the main compound

**Table 4.** EC<sub>50</sub> of antioxidant activities of tempeh produced from different varieties of soybeans.

Soybean varieties	EC <sub>50</sub> of ferrous ion chelating activity (mg dried sample/mL)	EC <sub>50</sub> of ABTS radical scavenging activity (mg dried sample/mL)
<b>Pre-fermented soybean</b>		
Kaohsiung No. 9	113.516 ± 12.696	979.267 ± 29.563 <sup>a</sup>
Kaohsiung No. 10	>200	908.215 ± 37.404 <sup>b</sup>
Tainan No. 10	>200	729.095 ± 14.882 <sup>c</sup>
Tainan No. 4	>200	587.290 ± 11.963 <sup>d</sup>
<b>Tempeh</b>		
Kaohsiung No. 9	2.207 ± 0.271 <sup>c</sup>	76.202 ± 0.663 <sup>a</sup>
Kaohsiung No. 10	11.175 ± 0.462 <sup>b</sup>	66.526 ± 2.514 <sup>b</sup>
Tainan No. 10	10.162 ± 0.682 <sup>b</sup>	58.159 ± 2.464 <sup>c</sup>
Tainan No. 4	23.885 ± 1.436 <sup>a</sup>	48.671 ± 1.461 <sup>d</sup>

<sup>a-d</sup>Results expressed were mean ± SD from 3 replications. Data with different small letters were significantly different at  $p < 0.05$  (observed by analysis of variance (ANOVA) with Duncan's post hoc). Data from ferrous ion chelating activity of soybean was not included in the statistical analysis.

contributing to the increase in soybean's antioxidant activities during fermentation. This tryptophan derivative compound had been reported to exert both radical scavenging and iron chelating activity [21], which lead to the sharp increase in the antioxidant activities. Besides 3-hydroxyanthranilic compounds, there could still be other compounds that may change during fermentation, including tannin, saponin, phytate, vitamins and peptide, which may contribute to the change in soybean's antioxidant activities; however, the changes caused by these compounds were not observed.

#### 4. CONCLUSION

Of all soybean varieties, tempeh made from Tainan No. 10 had the highest phenolic compounds and highest increase in phenolic compounds, tempeh made from Kaohsiung No. 9 showed the highest chelating properties, while tempeh made from Tainan No. 4 had highest ABTS radical scavenging. Compared with the ABTS radical scavenging activity of pre-fermented soybean, the antioxidant capacity of tempeh was higher provided the antioxidant capacity of the pre-fermented soybean used was also higher. In other words,

antioxidant capacity of tempeh is related to the antioxidant capacity of its pre-fermented tempeh. Therefore, to produce tempeh with higher antioxidant activity, soybean with higher antioxidant activities must be chosen.

#### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

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