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Research Article
Lotus (*Nelumbo nucifera*) Tempeh Indonesia as Antioxidant and Breast Anticancer Food- A Preliminary Study

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Abstract

Background and Objective: A traditional food sources Indonesia is Tempeh, commonly used soybean. Lotus itself is rich in substantial components; lotus tempeh was made by an alternative functional food such as soybean tempeh. The objective of this preliminary study was to examine the effect of fermentation products, such as lotus tempeh on its antioxidant and breast-anticancer potential, compared to soybean tempeh (control). **Materials and Methods:** This research were used three treatments, likely A_0 (100% soybean, control); A_1 (50% soybean: 50% lotus seeds) and A_2 (100% lotus seeds). Then, all of them have been determined the identification of phytochemicals, antioxidant activity using DPPH (2,2-Diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) methods. **Results:** The results showed that all of the tempeh samples have contained alkaloid, tannin and flavonoid except saponin. Results stated that the fermentation could increase the capability product in scavenging the free radicals. The DPPH scavenging effects of all tempeh samples were 77.02% (A_0), 72.78% (A_1) and 72.58% (A_2). For genistein (isoflavone), lotus tempeh was higher than other's treatments. The capability of DPPH radical inhibition in lotus tempeh (A_2) was as same as in soybean tempeh (A_0). **Conclusion:** Conclusively, lotus (*Nelumbo nucifera*) tempeh has a potency of breast anticancer, due to antioxidant activity and isoflavone, likely a soybean tempeh.

Key words: *Nelumbo nucifera*, anticancer, antioxidant, isoflavone, phytochemical, seed, dtempeh

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One of the traditional Indonesia food products is Tempeh, commonly consumed not only in developing countries as the main sources of dietary protein[1]. Tempeh product is fermented using a *Rhizopus* sp. Sarti *et al.*[2] have already made tempeh from lotus (*Nelumbo nucifera*). Wherein, the making of lotus tempeh has as same process as soybean tempeh. Lotus seeds are abundant during the rainy season, used commonly as food and nutraceutical ingredients. Lotus plants have been widely cultivated especially in South Asia. Lotus itself is rich in mineral and starch content, antioxidant compounds namely flavonoids, tannins and saponins. Lotus itself has been used extensively, which include rhizome roots to seeds for a food product focused on a starch-based processed food.

Several types of research on lotus seeds have shown that they contained many phytochemical compounds. The fermentation process has produced many changes in the content of nutrient, texture, color and protein digestibility. Badu *et al.*[3] and Messina[4] stated that tempeh is a product of high nutritional substances especially soybean by solid-state fermentation of cooked seeds, it likes a compact and sliceable cake product.

Actually, many advantages of common beans were as a fermentation product but a few researches have focused on the fermentation process of lotus seeds. In view of a previously studied by Sarti *et al.*[2], lotus tempeh has met the quality requirement of soybean tempeh. In addition, lotus seeds have a high carbohydrate and protein which low digestibility. The fermentation has increased the protein, protein bioavailability and enhanced antiradical (2,2-diphenyl-1-picrylhydrazyl [DPPH$^{\bullet}$], ABTS$^{\bullet+}$) capacity with increased a variety phytochemical[3,5,6] compared to the controls. The soybean tempeh produced by the fermentation is acclaimed as a breast anticancer. Consequently, this study was designed to determine lotus tempeh as the source of antioxidant and breast anticancer, likely phytochemical and isoflavone compounds. The data of this research was the beginning study to examine the effect of fermentation products such as lotus tempeh on its antioxidant and breast-anticancer potential compared to soybean tempeh (control).

MATERIALS AND METHODS

Study area: This project was started in March until November 2019 at Fisheries Product Technology, Faculty of Agriculture, Sriwijaya University, Indonesia. The lotus seeds were bought in local farmers in Indralaya, Ogan Ilir districts, South Sumatera, Indonesia. The seeds selected were ripe, grayish black color and hard texture (smooth outer skin of pericarp). Then, all seeds were packaged in vacuum plastic, stored in -4°C until used.

Tempeh preparation: All tempeh, included lotus, were conducted by Sarti *et al.*[2]. The first step was following the usual practices in local soybean tempeh producers with a slight modification. All of the beans were bought from the traditional market, South Sumatera, Indonesia. The tempeh sample proportion of using beans (w/w), likely: 100% soybean (A_0, control); 50% soybeans: 50% lotus seeds (A_1) and 100% lotus seeds (A_2). Wherein, all beans were boiled for 15 min, then inoculated using 0.75% ratio (w/w), fermented at 28±2°C for 24-36 hrs.

Extraction of beans samples: All tempeh samples were extracted using 96% ethanol, wherein tempeh samples (250 g) were extracted by 96% ethanol liquid (1.5 L) in water bath shaker (Memmert, WNB 14 type) for 36 h at room temperature, then added 1.5 L of 96% ethanol. After that the first extract was evaporated using a rotary evaporator (EXRE-2002 type) at 40°C, 230 rpm, 175 mbar. The concentrated extract from tempeh samples was stored in a dark brown glass bottle covered at ±4°C.

Phytochemical analysis: All samples were analyzed phytochemical components with qualitatively for the identification of bioactive chemical constituents. The identification of them was carried out standard procedures as described by Moriasi *et al.*[7].

Antioxidant activity: Antioxidant activity using DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging method was adapted for microplates as described by Cardador-Martinez *et al.*[8] with slight modification. The absorbance of samples was read at 517 nm using Microplate Reader Rayto Elisa (Rayto, RT-2100C). A percentage of DPPH radical inhibition was the reduction of results between OD control and OD samples divided by OD control. Wherein, OD is the optical density.

The antioxidant activity using ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging was conducted by Cano *et al.*[9] method with several modifications. ABTS solution (5 mL, 7 mM) was reacted with $K_2S_2O_8$ solution (88 µL, 140 mM). The solution was allowed to stand in a dark place for 12-16 hrs on room temperature or until resulted the

ABTSÂ+ solution dark blue. Those solutions could be used after the added ethanol solution (99.5% purity) until the absorbance value was 0.7±0.02 in 734 nm wavelength. For the sample solution, 10 mg samples were homogenized with 1 mL DMSO (Dimethyl sulfoxide), then blended ABTSÂ+ solution (1 mL) continued to incubate for 5 min in 30°C. The solution was measured by the optical density in 734 nm. Wherein, Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) used as a reference.

Isoflavone determination: This compound was determined by Wang *et al.*[10] methods with slight modification. A total of sample (2 g) was put into a 50 mL Erlenmeyer, then 6 mL of 1 M HCl was added and 24 mL acetonitrile. Samples in Erlenmeyer was stirred for 30 min and then allowed to stand for a few moments until there are 2 layers of sediment and supernatant. The obtained supernatant is then diluted with a mobile phase of 10x or 5x the dilution factor. Then, it was filtered with 0.45-micron membrane filter. Samples that pass the filter are samples that are ready to be analyzed using HPLC (Shimadzu LC-6A) with a UV-VIS detector SPD-10AV, 254 nm and c18 column in the mobile phase of methanol: ammonium acetate 1 mM (6: 4). The speed of the injection of samples into HPLC is 1 mL min^{-1} with a 20 mL loop injector. The standards used in this analysis are genistein and daidzein with concentrations of 0.625-10 ppm as a standard curve reference. Statistical analysis: The results of the data obtained are displayed as the Mean±Standard Deviation (SD) of three data repetitions. Then, all of the data was analyzed using SPSS (Statistical Package for the Social Sciences) software version 14.0 for window evaluation.

RESULTS

All of the tempeh samples had contained alkaloid, tannin, flavonoid, except saponin (Table 1). Although knowing, soybeans have become a significant source of plant protein. In this study, lotus tempeh (A_2) has a phytochemical compound as other treatments (A_0 and A_1). These compounds have more potential as an antioxidant and anticancer compound than saponin.

Next on the antioxidant activity, results showed that the fermentation could increase the capability product in scavenging the free radicals. The DPPH scavenging effects of all tempeh samples were 77.02% (A_0), 72.78% (A_1) and 72.58% (A_2) seen in Table 2. Plus, the ABTS scavenging activity were 85.04% (A_0), 67.94% (A_1) and 51.67% (A_2). All of the treatments were significantly different for two antioxidant analyses. Wherein, the differences between DPPH test and the ABTS test

Table 1: The identified phytochemical of tannin, alkaloid, flavonoid and saponin from tempeh samples

	Samples															
	A_0					A_1					A_2					
	Color					Color					Color					
Constituents	Initial	Final	Sediment	Foam	Results	Initial	Final	Sediment	Foam	Results	Initial	Final	Sediment	Foam	Results	
Alkaloid	Yellow faded	Reddish brown	Yellowish white	-	+	Yellow faded	Reddish brown	Yellowish white	-	+	Yellow faded	Reddish brown	Yellowish white	-	+	
Flavonoid	Yellow faded	Reddish brown	Yellowish white	-	+	Yellow faded	Reddish brown	Yellowish white	-	+	Yellow faded	Reddish brown	Yellowish white	-	+	
Saponin				+	+				-	-				-	-	
Tannin				+	+				+	+				+	+	

A_0: 100% soybean, A_1: 50% soybean and 50% lotus seeds, A_2: 100% lotus seeds

Table 2: The antioxidant properties of tempeh samples

Samples	DPPH scavenging activity (%)	ABTS scavenging activity (%)
A_0	77.02±0.65^a	85.04±0.13^a
A_1	72.78±1.25^b	67.94±0.78^b
A_2	72.58±1.85^b	51.67±0.67^c

Different letters in the same column show significantly different values ($p<0.05$), A_0: 100% soybean, A_1: 50% soybean and 50% lotus seeds, A_2: 100% lotus seeds

Table 3: The isoflavones component of tempeh samples

	Isoflavones (μg g^{-1})	
Samples	Daidzein	Genistein
A_0	75.84±1.02^a	114.44±1.05^a
A_1	13.72±1.03^b	25.17±1.02^b
A_2	0.57±1.05^c	2.59±1.03^c

Different letters in the same column show significantly different values ($p<0.05$), A_0: 100% soybean, A_1: 50% soybean and 50% lotus seeds, A_2: 100% lotus seeds

were in their reaction mechanism. In DPPH antioxidant has seen based on ability antioxidant composition to donate hydrogen. While on the ABTS antioxidant was based on the ability of antioxidant compositions to free radical compilation with donating radical protons. Although, there have different antioxidant activity values, the difference in this increase of the antioxidant ability was not reaching two times between treatments.

The antioxidant activity and anticancer in the isoflavone component was observed. In this study, the number of lotus aglycones daidzein and genistein were 5.7 and 25.9 mg/100 g (Table 3) respectively. The treatments of soybean tempeh (A_0) has higher of daidzein and genistein than A_1 and A_2, likely 758 mg/100 g (daidzein) and 1144 mg/100 g (genistein). All tempeh have extracted using 96% ethanol, A_0 treatment produced more than twice the isoflavone content, compared to lotus tempeh (A_1 and A_2).

DISCUSSION

The nutritive value of lotus seeds was 1.93% crude fat; 10.60% protein; 72.17% carbohydrate and 4.50% ash by Pal and Dey[11]. Sarti *et al.*[2] have reported that their values had increased due to the fermentation process, agreed to Mani and Ming[12]. This means that these microbes were more used the carbohydrates as the sources of carbon. However, the proximate analysis of lotus tempeh has met the standard national reference pattern of tempeh, which was made from soybean. Fermentation could help to improve the nutrition component, digestibility of protein and carbohydrates, etc.

All of tempeh samples had alkaloid, tannin, flavonoid, except saponin, disagreed with Pal and Dey[11]; Kredy *et al.*[13], Uwem *et al.*[14], Toyoda[15] stated that *Nelumbo nucifera's* seed are plentiful of asparagine, fat, protein, starch and tannin. The substantial value of these were composed of 4.50% (total ash); 1.93% (crude carbohydrate); 10.60% (crude fiber); 72.17% (fat) and 2.70% (protein) declared by Indrayan *et al.*[16], while tannins content was 3.91 mg g^{-1} by Bhat and Sridhar[1].

The preliminary study of phytochemical screening indicated that tempeh samples have the antioxidant potential, in a consequence of their bioactive. Pal and Dey[11] test exhibited negative for saponin not tannin. Because the complexity of polysaccharides including insoluble tannins was difficult apart from that basic complexity based on 13C-NMR's results were investigated by Das *et al.*[17]. The protein digestibility has reduced due to tannins, they blocked the activity of enzymes. Saponin in soybean and lotus common are incompletely absorbed. Sapogenins was not clearly released during fermentation. According to Mani and Ming[12] the process of soaking, boiling and steaming during the process of making tempeh can reduce the saponin contents. Although, saponins are found in many green plants[18,19]. In addition, the soya saponin content was 12.96 and 11.15 mg g^{-1} in raw yellow soybeans and raw black soybeans determined by Zhou *et al.*[19]. It was observed that the fermentation product has contained an array of aglycones, especially daidzein and genistein by Ferguson[20], Gil-Izquierdo *et al.*[21], Ahmad *et al.*[22]. Both of them were easily be permeated in our body cells, in consequence of its not as much block structure and smaller molecular weight. In preventing degenerative disease, isoflavone from the fermentation process is be assured to minimize the risk of that, proved by Gil-Izquierdo *et al.*[21], Ahmad *et al.*[22] too.

As shown in Table 2 that results were as high as to Ahmad *et al.*[22] and Hu *et al.*[23]. Plus, the ABTS scavenging activity for the antioxidant test is more sensitive than DPPH test. Even though, the number of lotus aglycones daidzein and genistein were only 5.7 mg/100 g and 25.9 mg/100 g (Table 3), lower than soybean tempeh. Difference results with Gil-Izquierdo *et al.*[21], the aglycones of all tempeh in the current research were higher than them. Wherein, the genistein content of Gil-Izquierdo *et al.*[21] was lower than this research, likely 24.03 mg/100 g, while in lotus tempeh was more than that.

The higher genistein and daidzein were in tempeh product as known 99% of isoflavones soy contained 64% genistin, 23% daidzin and 13% glycine (in aglycon form). The difference with lotus tempeh, the sources of isoflavones were in glycone form such as daidzin, genistin dan glisitin (lotus seed was rich in carbohydrates)[12,21,24,25]. The amount of daidzein and genistein were increased after fermentation of soybean during formation of tempeh. At least partial converting in the glycosides occurred transformation of that

could be slip off the potent antioxidant substances. Afterwards, the number of that reduced while the number of aglycones risen. A recent research concerned that the total phenolics have increased during fermentation which correlated with the increasing of DPPH free radical scavenging activity designated by discoloration of DPPH solution. The isoflavone of aglycones was favored due to their abundance in soybean. The conjugated form of glycosides are aglycones, the hydrolysis process broken down the sugar moiety. The phenols and amines have generally shown antioxidative activity. One of the major phenols is isoflavonoids components. When fermentation took place, p-glucosidase has liberated a part of isoflavonoids, lipophilic aglycone and increased the stability of auto-oxidation tempeh[26,27].

Research of Zhou *et al.*[19] determined that the antioxidant capacity of raw yellow soybean have 3.20 μ mol TE g^{-1} for DPPH value and 24.57 μmol TE g^{-1} for ABTS. Whereas, the DPPH and ABTS radical scavenging activity were 80.01 and 93.02% for a dried lotus seed extracted by 80% ethanol by Kim and Shin[26]. Zhao *et al.*[27] has reported EC_{50} in dried lotus seeds was 185.8% too. Unfortunately, these results were higher than the current study lotus results, likely 72.58% for DPPH value and 51.67% for ABTS value. DPPH inhibition was 90.9% for dried seed extracted by methanol[13]. At least, the capability of DPPH radical inhibition in lotus tempeh (A_2) was as same as in soybean tempeh (A_0). In this research wet seeds were used, different from other studies described by Zhou *et al.*[19], Kaur *et al.*[24], Kim and Shin [26], Zhao *et al.* [27] and Chen-Tien *et al.*[28].

Total phenolic content in lotus extracts correlates with the DPPH and ABTS activities[29,30]. Although, there was a statement that their chemical structure of phenolic compounds couldn't determine the radical scavenging activity[31,32]. In addition, Jung *et al.*[25] stated that the results of the previous and current study have be different as the potential differences of growing region, dryness and extraction condition, that could interfere the degree of oxidation and extraction efficiency of bioactive compounds. In the dried lotus seeds, the total phenolics content was 22.03 mg g^{-1}, 27.42 mg g^{-1} for total flavonoid; 38.81 mg g^{-1} for alkaloids by Zhao *et al.*[27]. Different from Reyes-Bastidas *et al.*[5], the phenolics content of common bean flour which fermented was 6.09 mg g^{-1} with 43% antiradical activity (DPPH method). Furthermore, a both of aglycones and glycones have a capability for detaining a free radical. This capability could be one of the ways to prevent breast cancer[29,30,31]. The flavonoid groups are effective as hydroxyl scavenger radical and peroxyl radical[30,31].

The results of this study, soybean tempeh was a still better source of protein. However, lotus tempeh can be used an alternative vegetable protein because its activity of radical inhibition was higher than common bean[5,31] and was as same as soybean tempeh of other's research. Hence, the lotus tempeh can be an alternative vegetable protein based on the phytochemical screening, antioxidant activity and isoflavone compounds. This study is a preliminary step and still needed further research.

CONCLUSION

The lotus (*Nelumbo nucifera*) tempeh has the potential to be a functional food, such as soybean tempeh (control). Lotus tempeh has all phytochemical substances except saponin and the antioxidant activity was 72.58% of DPPH and 51.67% of ABTS. Then, the number of lotus aglycones daidzein and genistein were 5.7 and 25.9 mg/100 g. Different from soybean tempeh as control, but their values were still in the average of soybean tempeh values. The aglycones component was a bioactive compound for breast anticancer.

SIGNIFICANCE STATEMENT

With the increasing needs for healthy foods, this study discovered the lotus tempeh (*Nelumbo nucifera*) could be alternative functional food, likely soybean tempeh that can be beneficial for food security. The lotus tempeh could be antioxidant and breast anticancer foods.

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