

Changes in Macronutrient, Total Phenolic and Anti-Nutrient Contents during Preparation of Tempeh

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Abstract

Background: The effects of processing towards changes in contents of macronutrient, total phenolic and anti-nutrient during preparation tempeh was studied. Tempeh preparation was carried out using the usual method used in the tempeh cottage industry in Malaysia.

Methods: The samples studied were in the form of raw, soaked, cooked soya bean and tempeh. Macronutrients, total dietary fiber (TDF), total phenolic, phytate and oxalate were determined using AOAC method, Folin-Ciocalteu assay, Anion Exchange Column (AEC) and Oxalate kit, respectively. Each analysis was carried out with minimum of four replicates for every sample.

Results: Raw soya bean contained significantly lower ($p < 0.05$) content of moisture (10.11%) but highest in ash (5.27%), protein (32.80%), fat (10.60%), carbohydrate (41.22%), phytate (615.00 mg/100 g) and oxalate (43.43 mg/100 g) contents when compared to tempeh. Phenolic content (8.90 mg GAE/100 g) increased significantly after fermentation process and it was not significantly different compared to the one in raw soya bean. Fermentation process also caused increment moisture content but reduction in ash, protein, fat, carbohydrate, phytate and oxalate contents in tempeh.

Conclusions: Nutrient and phenolic contents reduced after soaking and boiling process. However, total phenolic increased after fermentation process. Anti nutrients like phytate and oxalate contents were reduced significantly with fermentation. Tempeh produced in this study can be considered as food containing low amount of oxalate content (< 10 mg/ serving).

Keywords: Tempeh; Macronutrients; Total Phenolic; Phytate; Oxalate

Introduction

Soya bean is a unique legume due to its high content of protein, complex carbohydrate, fiber, vitamin B and isoflavone [1]. Tempeh can also be served as meat substitutes due to its high protein content [2]. Tempeh processing involved steps such as soaking, boiling/heating, drying and fermentation [3]. Soya bean and starter culture such as *Rhizopus oligosporus* are the main ingredients used in making tempeh. Soya bean needs to be soaked for more than 36 hours followed by dehulling process. The hull needs to be removed in order to allow fungal development [4]. This will be followed by boiling process usually takes place about 30 minutes at 100°C [5] to make it easier for fungal penetration and digestion process. This process also need to be done in order to remove beany and bitter taste of the beans [6,7]. Soya beans need to be dried before inoculation process in order to prevent the contamination of bacteria. Inoculation with microorganism is optimum at 10,000 colony forming units/ gram [6]. Perforated plastic is preferred for tempeh packaging that enables enough oxygen supply for the growth of fungal. The plastic should have pores and the space between the pores should not be close in order for the fungal to grow slowly. This is also to ensure that substrate temperature will not exceed optimum level [7].

Main soya protein consisted of β -conglycinin and glycinin. The other protein (glycoprotein) including lipoxygenase, lectin, tripsin inhibitor and α -amilase [8]. In fermented soya products, only part of protein hydrolyzed due to inability of protease to merge the glycoprotein, phosphoprotein, other modified species or domain that containing more disulfide bridge [9]. Raw soya bean contained highest content of fat compared to the other legumes due to the low unsaturated fat and contain both essential fatty acid [10]. Soya bean contains about

26 to 30% carbohydrate content. Soya bean processing leads to the loss of many soluble carbohydrate materials when compared with the fermentation process [11].

Legumes product especially soya bean contain high amount of anti-nutrient such as phytate and oxalate that may lead to poor mineral bioavailability [12,13]. However, anti-nutrient contents will reduce as the product undergoes few processing method [12,13] such as washing, soaking, dehulling, heating and fermentation [14]. Formation of phytate occurs naturally during plant and cereal seed maturation [15]. Biochemical changes in tempeh processing largely occur during the fermentation stage [16]. Thus, this study was conducted to determine the changes of macronutrient, total phenolic and anti-nutrient contents in tempeh during tempeh processing that was carried out based on the laboratory scale.

Materials and Methods

Tempeh processing

Tempeh was prepared in laboratory scale. Soya bean (*Glycine Max*

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sp.) used was imported from China and it was purchased from Sofaz Enterprise Puchong, Selangor. Tempeh's starter (*Raprima*) or *Rhizopus oligosporus* originated from Bandung, Indonesia was purchased from the same company. The method for making tempeh was also adopted from the same company. The process started with soaking the soya beans for more than 36 hours, followed by boiling process for 30 minutes and dried in an open air for about an hour. Tempeh starter were added into the dried soya bean with ratio soya bean: tempeh starter (100 g: 0.2 g). The soya bean was packed in plastic bags with pores and incubated for about two days at room temperature with good ventilation.

Sample preparation

All samples (raw, soaked, cooked soya bean and tempeh) were grinded using blender (Panasonic MX 7985) and kept in freezer (-20°C) until next analysis. For analyses of total dietary fiber and fat, the samples were freeze dried using floortop freeze drier (Labconco, USA) and kept in freezer (-20°C) prior to analysis. Sample for tempeh was analysed using six replicates while the other sample (raw soya bean, soaked soya bean and cooked soya bean) was analysed with 4 replicates.

Macronutrient content

Moisture, ash, protein and fat content were determined using AOAC method [17] while total dietary fiber was based on AOAC 985.29 [18].

Total phenolic content

Determination of phenolic content was based on Singleton and Rossi [19] and Song et al. [20] while preparation of sample was according to Fu et al. [21]. This analysis was based Folin-Ciocalteu assay and using gallic acid as standard. About 1 g of sample was added with 9 ml ethanol: water (50:50, v/v) and was shaken with shaking water bath for an hour followed by filtration by using filter paper (Whatman 41). About 500 µl sample was added into 2500 µl diluted Folin-Ciocalteu (1:10). The mixture was left for 4 minutes and then 2 ml of natrium carbonate (75 g/L) were added. The mixture was incubated at room temperature for 2 hours. The absorbance was read at 760 nm by using UV-spectrophotometer (SECOMAM CE, France). Phenolic content was expressed as gallic acid equivalent (mg GAE/ 100 g) wet weight. Standard concentration for calibration curve were prepared at 25 to 200 µg/ml. A calibration curve using gallic acid standard was shown in Figure 1.

Phytate content

Determination of phytate was based on Ma et al. [22] by using Anion Exchange Column (AEC) method. Sample was weight at 1.0 g and mix with 40-50 ml mixture of sodium sulphate (100 g/L)-hydrochloric acid

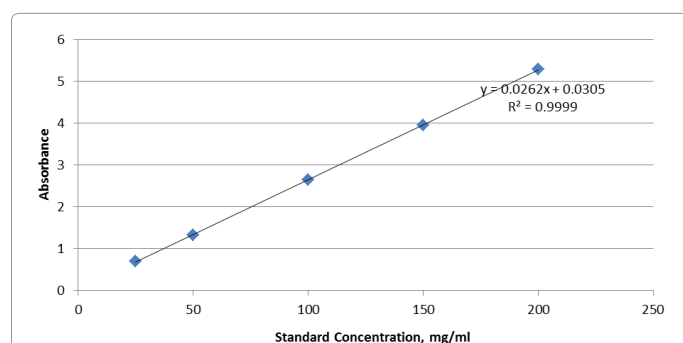


Figure 1: The calibration curve for total phenolic content based on Gallic acid equivalent.

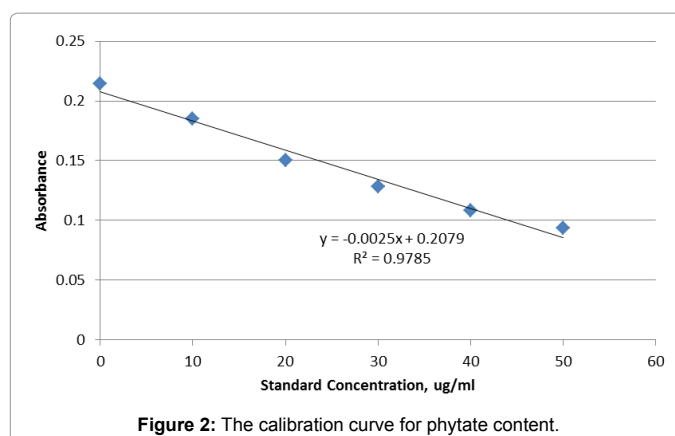


Figure 2: The calibration curve for phytate content.

(1.2%). Supernatant was then filtered using filter paper 5A (Advantec, Japan).

About 10 ml of filtrate was diluted to 30 ml with distilled water after adding 1 ml of 0.75 M NaOH. The mixture was passing through the column resin (resin, AG1-X4, ~100-200 mesh; column polypropylene, 0.8 × 4 cm, Bio-Rad Laboratory, Inc., CA). Before passing through the filtrate, the column was clean first with 0.5 M NaCl and deionized water followed by 15 ml distilled water and 20 ml 0.05 M NaCl to remove inorganic phosphate. The trapped phytate content was then removed by using 0.7 M NaCl. As much as 4 ml of Wade reagent (0.03% ferric chloride (FeCl₃) and 0.3% sulphosalicylic acid (SSA) were added into 5 ml phytate eluate and was centrifuged at 3000 rpm for 10 minutes. The absorbance was determined at 500 nm by using spectrophotometer (SECOMAM CE, France). A calibration curve using sodium phytate was obtained and shown in Figure 2.

Oxalate content

Determination of oxalate was using Ilarslan et al. [23]. Oxalate kit was obtained from Trinity Biotech (Wicklow, Ireland). The kit consisted of reagent A, reagent B, EDTA and 0.5 mmol/L oxalate standard. Approximately 1 g of sample was needed and was added with 5 ml deionized water followed by 5 ml of EDTA. The mixture was adjusted at pH 5 to 7. Sample was transferred into centrifuge tube (containing charcoal) and centrifuged at 2000 rpm for 5 minutes. Filtrate was collected using filter paper 5A (Advantec, Japan). Tubes were labelled for blank, standard, control and sample. As much as 1 ml of reagent A was added into all tubes, followed by 50 µl filtrate into sample tube. About 50 µl of deionized water and oxalate standard were added into blank and standard tubes, respectively. This was followed by adding 1 ml of reagent B into all tubes. All tubes were incubated at 18 to 37°C for 5 minutes. The absorbance of the samples was read at 590 nm.

Statistical Analysis

Results were reported as mean and standard deviation. Analysis of variance (ANOVA) with post-hoc Tukey was conducted (SPSS for Windows, Version 21) to determine the difference among means. Statistical significance was considered at P<0.05.

Results and Discussion

Macronutrient contents

The studied raw soya bean contained 10.11% of moisture, which was similar to yellow soya bean from [24,25] which were reported to contain 9.82% and 8.50% moisture, respectively. Moisture content was

significantly highest ($p < 0.05$) in soaked soya bean (64.08%) compared to other samples. Table 1 showed soaking, drying and fermentation process have caused moisture content in raw soya bean to increase up to 83% in tempeh. Tempeh produced in this study contained 61.39% moisture which was in range with the one (60-66%) reported in Malaysia Food Composition Database (FCD) [26] and USDA [27].

Ash content was significantly higher ($p < 0.05$) in raw soya bean (5.27%) compared to soaked (1.46%), cooked (0.99%) and tempeh (1.08%) samples. Redondo-Cuenca et al. [24] have also reported similar ash content (4.81%) in their raw soya bean sample. Soya bean samples that underwent the process of tempeh making have reduced ash content. The reduction of ash content was 79.51%, after the fermentation process but ash reduction has started since the process soaking and boiling as showed in Table 1. Mo et al. [28] have reported the same pattern reduction for ash content in their raw (4.47%), soaked (1.08%), cooked soya bean (0.79%) and tempeh (0.77%). Malaysia FCD [26] and USDA [27] also reported tempeh to contain 0.9% and 1.62% ash, respectively.

Table 1 showed raw soya bean contained high amount of protein (30.01%). Other studies [24,29,30] have reported higher protein content in raw soya bean, which were in the range of 38-42%. Protein content in soya bean has decreased as much as 43.65% when it has turned into tempeh. There was a 51% of reduction after underwent soaking process and slightly increased after boiling and fermentation. Van der Riet et al. [11] also reported slight increase of protein content after soya bean underwent fermentation process. The results from present study was not much different compared to Mo et al. [28] (reported tempeh contained 17.34% protein) and both Malaysia [26] and USDA [27] food composition databases also stated that tempeh contained 15.90 and 18.54% respectively.

Raw soya bean contained 10.60% fat and the value reduced after underwent soaking and boiling process. The fat content in tempeh after fermentation process was 8.39%. Total reduction of fat content from raw soya bean to tempeh was about 21%. Other studies [24,30,31], reported higher fat content in raw soya bean which was in the range of 18-19%. Mo et al. [28] also reported that soaked soya bean (11.29%) has higher fat content compared to tempeh (10.84%). Fat content in tempeh reported in Malaysia FCD [26] and USDA [27] were 7.5% and 10.80%, respectively. Fat content was reduced during tempeh processing since lipase enzyme hydrolyses the triglyceride into free fatty acid [32]. It was

also due to the microorganisms that use the fat as an energy source [33].

Table 1 showed that carbohydrate content in raw soya bean (3.63%) was significantly ($p < 0.05$) highest compared to soaked (2.63%), cooked soya bean (2.64%) and tempeh (2.67%). Carbohydrate was largely reduced (91.95%) after undergo soaking process. Reduction of carbohydrate content was due to the utilisation of carbohydrate by microorganism [11]. Carbohydrate content reported by Malaysia FCD [26] was 6.80% while, USDA [27] reported tempeh containing 9.39% of carbohydrate content.

Raw soya bean contained 10.69% TDF and it was reduced to 9.58% after fermentation process. Redondo-Cuenca et al. [24] and Souci et al. [25] have reported higher amount of TDF (16.5- 22%) in their raw soya bean. In this study, TDF has increased as much as 32.46% during soaking process and decreased about 11.30% after boiling process. The increased of TDF in soaked bean was also observed by Vidal-Valverde et al. [34], Azizah and Zainon [35], Kutos et al. [36] and Ramadan [37]. Total dietary fiber consisted of soluble and insoluble dietary fiber [38]. Since insoluble fiber was not soluble in water, it become more concentrated in soaked [39] bean and increased the TDF content in soaked bean. The reduction of TDF (23.73%) continued even after fermentation process. According to Hutkins [40], fiber is part of degraded compounds that is released during tempeh processing. A loss of fiber usually takes place during soaking process and a small loss after fermentation. TDF was not mentioned in both Malaysia FCD [26] and USDA [27] but in Malaysia FCD [26], tempeh contained 2.9% of crude fiber. According to Zeman [41], TDF can be estimated with an estimation of two to six times the value of crude fiber. Therefore, the TDF content in tempeh of this study was in the range with the one in Malaysia FCD, estimated to be in the range of 5.8-17.4%.

Total phenolic content

A calibration curve for total phenolic content ($R^2 = 0.9999$) and the result were shown in Figure 1. Phenolic content (8.90 mg GAE/100 g) in raw soya bean was the highest, as shown in Table 2. There was a reduction of phenolic content after soaking (3.65 mg GAE/100 g) and boiling (2.38 mg GAE/100 g) process where the reduction were 58.99% and 34.79%, respectively. This was supported by Boateng et al. [42] who reported phenolic content was reduced after soaking process. Phenolic content (6.83 mg GAE/100 g) in tempeh has increased as much as 65% after boiling process.

Sample	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Total Dietary Fiber (%)	Carbohydrate(%)
Raw soya bean ¹	10.11 ± 0.48 ^c	5.27 ± 0.20 ^a	30.01 ± 0.94 ^a	10.60 ± 0.74 ^a	10.69 ± 0.46 ^b	3.63 ± 0.50 ^a
Soaked soya bean ¹	64.08 ± 1.07 ^a	1.46 ± 0.07 ^b	14.80 ± 0.56 ^c	4.48 ± 0.33 ^c	14.16 ± 1.08 ^a	2.63 ± 1.69 ^b
Cooked soya bean ¹	61.95 ± 0.78 ^b	0.99 ± 0.08 ^c	16.48 ± 0.42 ^{bc}	4.66 ± 0.20 ^c	12.56 ± 1.10 ^a	2.64 ± 1.14 ^b
Tempeh ²	61.39 ± 0.77 ^b	1.08 ± 0.13 ^c	16.91 ± 1.29 ^b	8.39 ± 0.17 ^b	9.58 ± 0.77 ^b	2.67 ± 1.67 ^b

¹4 replicates

²6 replicates

Different alphabet in the same column showed significant differences ($p < 0.05$) based on ANOVA (post-hoc Tukey)

Table 1: Macronutrient contents in soya bean samples during tempeh processing.

Sample	Total Phenolic Content (mg GAE/100g)
Raw soya bean ¹	8.90 ± 2.26 ^a
Soaked soya bean ¹	3.65 ± 0.31 ^b
Cooked soya bean ¹	2.38 ± 0.15 ^b
Tempeh ²	6.83 ± 1.81 ^a

4 replicates

²6 replicates

Different alphabet in the same column showed significant differences ($p < 0.05$) based on ANOVA (post-hoc Tukey)

Table 2: Total phenolic contents in soya bean samples during tempeh processing.

Sample	Phytate (mg/100g)	Oxalate (mg/100g)
Raw soya bean ¹	615.00 ± 12.25 ^a	43.43 ± 4.62 ^a
Soaked soya bean ¹	455.00 ± 30.00 ^b	23.90 ± 5.14 ^b
Cooked soya bean ¹	450.00 ± 50.50 ^b	9.03 ± 1.77 ^c
Tempeh ²	255.00 ± 45.50 ^c	6.72 ± 1.51 ^c

¹4 replicates

²6 replicates

Different alphabet in the same column showed significant differences ($p < 0.05$) based on ANOVA (post-hoc Tukey)

Table 3: Anti-nutrient contents in soya bean samples during tempeh processing

Taylor [16] reported that phenolic content reduced significantly during tempeh processing (soaking, boiling and fermentation). The reduction was due to the rehydration of the beans as well as the effect of heating and leaching of phenol into boiling water. Soaking process weakens the cell wall tissues of the soya bean which lead to solubilisation of bound polyphenol and cause the polyphenol to be lost in water [42]. According to Pastor-Cavada et al. [43], dehulling the beans also may lower the phenolic content because the hull is more abundant in phenolic compounds than the cotyledon. Oomah et al. [44] mentioned that the reduction was about 45% after dehulling.

According to Xu and Chang [45] and Boateng et al. [42], phenolic content reduced as much as 17% after the heating process. However, the present study reported a higher reduction (35%) of phenolic content after boiling which may due to the duration of heating applied. Boateng et al. [42] also reported that the rate of increment in phenolic content may depends on method of tempeh preparation and types of legume used. Granito et al. [46] and Oboh et al. [47] also reported the same finding as in this study where the fermentation of soya bean into tempeh increased the phenolic content.

Phytate

Figure 2 showed the calibration curve for phytate standard ($R^2 = 0.9785$). Phytate content (615 mg/100 g) was significantly ($p < 0.05$) highest in raw soya bean compared to other sample, as shown in Table 3. Phytate content reduced after soaking (455 mg/100 g) and boiling (450 mg/100 g), where the reduction was 26%. According to Karkle and Beleia [48], significant reduction (23-30%) in phytate content occurred after soaking and boiling does not caused additional reduction. Phytate was reduced further after fermentation process (255 mg/100 g). In this study, the reduction of phytate content from raw soya bean to tempeh was 58.54%. Egounlety and Aworh [3] reported that phytate content was reduced to about 31% after fermentation. Different phytate content from different studies may due to the different cultivars, weather condition and years [49].

According to van der Riet et al. [11], phytate content was reduced during fermentation. Reduction of phytate in soya bean after fermentation was 30.7%, as reported by Egounlety and Aworh [3]. Earlier study by Sudarmadji and Markakis [50] reported that about 33% of phytate was reduced after soya bean fermentation. Phytate content leached out during soaking, boiling and fermentation [51]. Reduction of phytate gives significant increases on minerals availability such as calcium, zinc and ferum [51,52].

Oxalate

Results of oxalate content were as shown in Table 3. Oxalate content was significantly higher ($p < 0.05$) in raw soya bean compared to other samples. Amount of oxalate (43.43 mg/100 g) in raw soya bean was reduced as much as 44.97% after soaking process (23.90 mg/100 g). Cooked soya bean contained 9.03 mg/100 g of oxalate content and it

reduced about 62.22% after boiling process. Oxalate content continues to decrease after fermentation process (6.72 mg/100 g). The reduction from raw soya bean to tempeh was about 84.53%.

Different treatment will give different effects on oxalate oxalate content and it also depends on soya bean cultivar used [53]. Oxalate content in soya bean (Red Mill) and tempeh (White Wave and Turtle Islan) were reported [53] to contain oxalate which was 54 mg/100 g, 47 mg/100 g and 65 mg/100 g, respectively. American Dietetic Association [54] stated that high oxalate foods refer to foods that contain more than 10 mg per serving. Hence, tempeh produced in this study was considered low oxalate containing food due to the oxalate content which were 6.72 mg/100 g or 4.77 mg oxalate/serving (1 serving of tempeh=71 g).

Conclusions

Macronutrients, total phenolic and anti-nutrient contents in tempeh were lower compared to the raw soya bean. It was due to the digestion of soya bean by *Rhizopus*, as the digested material may be used as nutrients for its growth. Phenolic contents decreased after soaking and boiling processes but slightly increased as fermentation takes place. Fermentation process significantly reduced the phytate and oxalate contents in raw soya bean as it turned into tempeh.

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