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Does cooking affect the phytate content in local soy based dishes?

Shimi, G. and *Hasnah, H.

Nutritional Science Programme, School of HealthCare, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

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Abstract

This study aimed to determine the effect of cooking on phytate content and the inhibitory effects of phytate on the bioavailability of minerals in eight Malaysian soy based dishes. Phytate was analyzed by using anion-exchange chromatography while minerals were analyzed by using Atomic Absorption Spectrophotometer. Molar ratios were obtained by dividing the mole of phytate to minerals. Phytate content was reduced in cooked dishes compared to the raw ones but it was not significantly different ($P > 0.05$). Raw, cooked and whole dish soy products contained 257.14-900.00, 182.14-803.57 and 289.29-910.71 mg/100 g phytate, respectively. Boiling and steaming have reduced most phytate content in the food samples. Molar ratios for phytate/minerals in these samples (phytate/Ca >0.17; phytate/Fe >1) indicated that phytate content inhibited the absorption of calcium and iron. However, the ratio for Ca × phytate/Zn in all samples was less than 200 which showed that phytate did not affect the bioavailability of zinc.

Introduction

The emergence of interest in healthier nutrition in the recent years has given a greater trend towards the use of plant food products with multiple functional properties. Soybean (*Glycine max*) is an ancient legume which is traditionally used to make both fermented and non-fermented foods. It is the staple food for the Asian populations (Omoni and Aluko, 2005). Nowadays, soybean products have attracted a lot of interest due to their health benefits to nutrition and health (Paucar-Menacho *et al.*, 2010). On an average, Asians consume 20 to 80 g of soy foods daily which consisted of tofu, miso and *tempeh* (Omoni and Aluko, 2005). The Food Consumption Statistics for Malaysia (2006) reported that *tempeh* and bean curd intakes among the Malaysian population were 6.91 g/day and 19.40 g/day, respectively. This showed that Malaysians do consume soy products in their daily intake.

Many studies have highlighted the importance of the nutritional values in soy products. Soy bean and its by-products are excellent sources of nutritionally basic macro- and micro-minerals, even though their availability may be seriously compromised by the presence of phytic acid, polyphenols and oxalate or the particular structure of soybean proteins (Giami, 2002; Karr-Lilienthal *et al.*, 2004; Rani *et al.*, 2008). Soy beans are different from other legumes in terms of higher in fat and protein but lower carbohydrate contents (Messina and Messina, 2010). On the average, dry soy bean contains about 40% protein, 20% oil, 35% soluble and insoluble dietary fiber and 5% ash (Jooyandeh, 2011).

Phytate (InsP6) is a compound that exists in plant food like legumes, cereals, nuts, oil seeds. It has both beneficial and detrimental effects on human health (Horner *et al.*, 2005). Phytic acid has long been considered as an anti-nutrient due to its ability to complex with several metal ions such as iron, zinc, and calcium, thus reducing their bioavailability (Horner *et al.*, 2005; Mejborn and Tetens, 2011). Therefore, many food processing and preparation techniques are the main efforts made to decrease the amount of phytate in foods (Egli *et al.*, 2002). Apparently, the cause of mineral deficiency in Malaysia was due to its low bioavailability in the diet. Phytate was one of the factors that affect the bioavailability. The incidence of anemia due to deficiency of iron was nearly one million cases (969,645); osteoporosis as a result of calcium deficiency is 2,421,432 cases while data on Zn status in Malaysia is not available (Norhaizan and Nor Faizadatul, 2009).

The intake of phytate in Malaysia is not available due to the lack of database on phytate content in local food. Although the phytate content in other Asian foods might be available, some factors such as differences in cooking methods, food processing techniques and the variety of food consumed by Malaysians compared to other countries may attribute to the unsuitability of data being used to assess the phytate intake of Malaysians. Therefore, this study

*Corresponding author.
Email: *hasnah@fsk.ukm.my*
Tel: (603) 9289 7511; Fax: (603) 2694 7621

Table 1. Name of eight soy based dishes and their cooking instructions in this study

Sample name (n=8)	Cooking instructions
Sambal tempeh (Fried tempeh with chilli paste)	• Tempeh,peanuts and anchovies were fried (temperature: 70°C, time: 4- 5 minutes). • Chilli paste was sauted. Then sliced onions were added. • Fried tempeh, peanuts and anchovies were added and mixed well. • Sugar and salt were added and stirred thoroughly. • Cooking temperature for whole dish: 65°C, time: 9:02 minutes.
Tempeh goreng kicap (Tempeh fried in soy sauce)	• Tempeh and anchovies were fried (temperature: 80°C, time: 5 minutes). • Onions, garlic small chilly, green and red peppers were sauted. • Tempeh and anchovies were added and stirred well. • Soy sauce and salt were added and mixed well.
Sambal Goreng Jawa (Fried tempeh with tofu and fujook, the Java style)	• Tempeh, tofu, potatoes and liver were fried (tempeh frying temperature: 75°C, 6-7 minutes, tofu frying temperature: 80°C, 6 minutes, fujook frying temperature: 50°C, 5 minutes). • Onions, garlic, lemongrass, green and red peppers were sauted. • Fried ingredients were added and mixed well. • Vermicelli, long beans, fujook and salt were added and mixed well. • The overall cooking temperature: 50°C, 15 minutes.
Masak lodeh (Tofu, fujook and tempeh cooked in coconut milk)	• Garlic, candlenut, ginger, anchovies, fennel, chili paste and onions were blended. • Blended ingredients were sauted together with lemongrass and galanga. • Coconut milk was added and stired slowly. • Tempeh, tofu, fucuk, carrots, long beans and white shrimp were added. (Temperature for boiling soy products: 80°C, time: 10 minutes) and stired gently until the ingredients were cooked. • Vermicelli, eggplant, turnip, cabbage, red green peppers were added and stired for a while. • Cooking temperature for whole dish: 80°C, time: 25 minutes.
Sambal tofu (Tofu cooked in chilli paste)	• Tofu was fried(temperature: 94°C, time: 8 minutes). • Chilli paste was sauted. Onion was added and mixed well until the onion was cooked. • Tofu was added(temperature of tofu in sauce: 68°C, time: 4 minutes). • Salt and sugar were also added and mixed well. • Overall cooking temperature: 68°C, time:15 minutes.
Steamed soft tofu (Steamed soft tofu cooked with tauchu)	• Onion, garlic and ginger were sauted. • Soy bean paste and soy sauce were added and mixed well. • In a separate steaming pan, soft tofu was organized. Carrot, red and green peppers were sprinkled on the soft tofu. • The sauce cooked previously was poured onto the soft tofu • The soft tofu was steamed for 20 minutes (temperature: 51°C).
Egg tofu soup (Egg tofu cooked in soup)	• Egg tofu was fried (temperature: 88°C, time: 4 minutes). • Garlic and ginger were sauted. Water was added and left to be boiled. • Eggs were added and left to be cooked. • Egg tofu, cauliflower and carrots were added. • Corn flour and salt were added and mixed well. • Cooking temperature after the egg tofu included: 71°C ,time: 6 minutes.
Stir fried green mustard with egg tofu (Egg tofu fried with mustard)	• Egg tofu was fried (temperature: 90°C, time: 5:30 minutes). • Garlic, green, red peppers and anchovies were sauted • Fried egg tofu and green mustard (sawi)were added, followed by oyster sauce. • Then mixed thoroughly. • Stir-frying temperature for egg tofu: 62°C, time: 4 minutes

aimed to determine the effect of cooking on phytate content and the inhibitory effects of phytate on the bioavailability of minerals in Malaysian soy based dishes. Analysis of the nutrients was also carried out since the list of nutrients in cooked soy based dishes of the local food composition table is rather limited.

Materials and Methods

Food sampling and preparation

A total of eight types of soy based dishes in Malaysia were home cooked. The description for each dish was described in Table 1. Soy products like *tempeh*, tofu, soft tofu and Japanese tofu/ egg tofu were obtained from the local wet market area. Samples were divided into three groups, consisted of whole cooked dish, separated cooked soy products from the whole dish and raw soy products used in preparing the whole dish. All samples were homogenized and stored at -20°C prior to analysis.

Proximate composition

The method of AOAC (1997) was used to determine the proximate compositions of the whole dish samples only; such as moisture content, protein and total ash. Total carbohydrate content was calculated as by difference (100% - percent of total fat, protein, moisture and ash values).

Total Dietary Fiber

A combination of enzymatic and gravimetric methods based on the method published in the 16th Edition of the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC

985.29) was used to determine the total dietary fiber (TDF) content.

Mineral contents

Calcium, natrium, magnesium, zinc and iron contents in samples were measured by using Atomic Absorption Spectrophotometer (AAS). Standard stock solutions of calcium, natrium, magnesium, zinc and iron were prepared from AAS grade chemicals (Fisher scientific, UK) with appropriate dilutions.

Phytate content

This analysis was carried out based on the method by Ma *et al.* (2005) for whole dish samples, raw and cooked soy products. Approximately, 1 g of each sample (whole dish, cooked and raw soy products) was weighed and added into 50 ml of Na_2SO_4 (100 g/ l) - HCl (1.2%) in a 100 ml conical flask. The flask was then covered with a stopper. The extraction of phytate was performed by stirring the sample using magnetic stirrer for 2 hours at room temperature. The supernatant was collected through the filtration of filter paper. A total of 10 mL of filtered extract was diluted to 30 mL using 1 mL of 30 g/L NaOH and 19 mL of distilled water. It was then passed through an anion resin column (resin, AG1-X4, 100-200 mesh, Bio- Rad Laboratory, Inc., CA; column, 0.8 ×4 cm, Beijing Glass Instrumental Factory). The column was rinsed before use with 20 ml of 0.5 M of NaCl and deionised water. Following the application of the samples the column was washed with 15 ml of distilled water and 20 ml of 0.05 M NaCl to remove the inorganic phosphate. Then the retained phytate was eluated by 25 ml of 0.7 M of NaCl. To prepare the Wade reagent, 0.03% $FeCl_3$ solutions with 0.3% SSA was used .About 4 ml of reagent and 5 ml of collected eluate was vortexed and centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured at 500 nm using a spectrophotometer (SECOMAM CE, France). The standard solutions with a series of concentration within 10-50 ug/ ml sodium phytate were prepared. Phytate content in the sample was determined by using below formula:

Phytate content (mg/ 100 g) = Concentration of phytate (mg/ml) x dilution factor x 50 ml x 100 g

where, dilution factor = 3.

Determination of molar ratio of phytate/ minerals

Molar ratio phytate/ mineral were used to determine the bioavailability of minerals. The mole of phytate and minerals were obtained by dividing the mass of phytate and minerals by its atomic mass respectively (phytate: 660 g/ mol; Fe: 56 g/ mol; Zn: 65 g/ mol; Ca: 40 g/ mol). The molar ratio between phytate and mineral was determined by dividing the mole of phytate to the mole of mineral. To find the Ca× (phytate/Zn) molar ratio, the total amount of Ca (mmol) in 100 g of each sample was multiplied by the phytate/Zn molar ratio.

Statistical analysis

Data were expressed as mean ± standard deviation of triplicate measurements for proximate and mineral composition and duplicate for phytate and total dietary fiber content. Data were analyzed using statistical software, SPSS version 19.0 for windows. Paired t-test was used to compare the phytate content in raw and cooked soy based dishes. Also, one-way ANOVA with Tukey's HSD test was used to determine the differences for all nutrient contents in all samples. Independent sample t-test was used to compare phytate content in fermented and non-fermented soy products. Level of significance was set at $p < 0.05$.

Results and discussion

Proximate composition

All macronutrients in whole dish soy products were as shown in Table 2. The fat content was ranged from 3.51 - 34.55% and was highest in *sambal tempeh* (34.55 ± 4.94%) when based on wet weight. *Sambal tempeh* also contained highest protein content (7.27 ± 0.88%) and the range of protein content in the samples was between 1.08 - 7.27%. The range of total carbohydrate was between 6.16 - 32.81% and was highest in *tempeh goreng kicap* (32.81 ± 1.06%) and lowest in *egg tofu soup* (6.16 ± 0.26%). Total ash content was highest in *tempeh goreng kicap* (2.56 ± 0.03%) and lowest in *egg tofu soup* (1.09 ± 0.01%). The value of moisture was highest in *egg tofu soup* (87.87 ± 0.13%) and lowest in *tempeh goreng kicap* (31.63 ± 0.80%). The percent of coefficient variance (CV) for analyses in most samples were less than 20% which showed the high precision in the obtained results.

The Malaysian Food Composition Table does not have nutrient contents for most ready to eat soy based dishes. Thus, the nutrients were compared to USDA database, Asian Food Composition Tables (AFCT) and Food Composition Guide Singapore (FCGS). Fat, total carbohydrate, protein, moisture and total ash contents in the studied soy based dishes based on wet weight (fat: 3.51 - 34.55%; total carbohydrate: 6.16 - 32.81%; protein: 1.08 - 7.27%; moisture: 31.63 - 87.87%, total ash: 1.09 - 2.56%) were relatively in good agreement with the cooked/fried soy products listed in USDA, AFCT and FCGS. For examples *tempeh* with vegetables stir fried, yong tau hoo mixed,

Table 2. Proximate composition and total dietary fiber of the eight Malaysian soy based dishes, based on wet weight [a]

Sample (whole dish)	Nutrients (% Composition)											
	Total dietary fiber	CV	Moisture	CV	Crude fat	CV	Crude protein	CV	Carbohydrate	CV	Ash	CV
Egg tofu soup	2.44±0.07	2.91	87.87±0.13	0.15	3.80±0.11	2.82	1.08±0.26	23.80	6.16±0.26	4.15	1.09±0.01	1.07
Masak lodeh	5.60±0.17	3.09	76.43±0.73	0.96	7.28±0.25	3.47	2.60±0.24	9.21	12.03±0.87	7.21	1.67±0.03	1.79
Sambal goreng jawa	3.81±0.33	8.76	59.25±0.20	0.34	12.54±0.08	0.60	3.49±0.80	22.99	22.86±0.70	3.07	1.86±0.04	2.03
Sambal tempeh	15.52±0.06	0.41	33.69±1.85	5.50	34.55±4.94	14.31	7.27±0.88	12.15	22.46±4.46	19.87	2.04±0.07	3.42
Sambal tofu	10.16±0.26	2.60	56.90±0.46	0.81	21.19±0.75	3.54	3.71±0.34	9.16	16.24±0.17	1.07	1.96±0.07	3.75
Steamed soft tofu	1.65±0.21	12.50	84.27±1.14	1.36	3.51±1.30	36.94	3.95±0.98	24.83	6.80±2.66	39.03	1.47±0.01	0.89
Stir fried mustard with egg tofu	3.92±0.04	1.00	77.19±0.06	0.08	9.96±0.79	7.97	2.97±0.11	3.82	7.70±0.81	10.57	2.18±0.10	4.68
Tempeh goreng kicap	16.44±1.90	11.54	31.63±0.80	2.52	26.14±0.29	1.10	6.86±0.23	3.32	32.81±1.06	3.24	2.56±0.03	0.98

[a]Data are expressed as mean ± SD of triplicate determinations for proximate and duplicate determination for total dietary fiber.

Table 3. Comparison of phytate content between raw and cooked soy products

Soy product (Name of the dishes)	Phytate content (mg/100 g)		P value*	Reduction of phytate content by cooking (mg/100 g)	Percent of reduction
	Raw form of soy product	Cooked form of soy product			
Soft tofu **(Steamed soft tofu)**	321.43±45.46 ^{abc}	182.14±181.83^{c}	0.385	139.3	43.33
Firm tofu **(Sambal tofu)**	900.00±15.15^{a}	803.57±181.83^{a}	0.563	96.4	10.71
Egg tofu **(Stir fried mustard with egg tofu)**	878.57±45.46 ^{ab}	728.57±136.37^{ab}	0.258	150.0	17.07
Egg tofu **(Egg tofu soup)**	878.57±106.07^{ab}	353.57±90.91^{abc}	0.165	525.0	59.76
Tempeh **(Tempeh goreng kicap)**	375.00±121.22^{abc}	257.14±45.46^{c}	0.500	117.9	31.43
Tempeh **(Sambal tempeh)**	257.14±45.46^{c}	192.86±15.15^{c}	0.374	64.3	25.00
Tempeh	300.00±45.46^{bc}	278.57±15.15^{bc}	0.500	21.4	7.14
Firm tofu	771.43±15.15^{ab}	600±45.46^{abc}	0.079	171.4	22.22
Fujook **(Sambal goreng jawa)**	846.43±90.91^{abc}	739.29±60.61^{a}	0.126	107.1	12.66
Tempeh	364.29±15.15^{abc}	257.14±45.46^{c}	0.126	107.1	29.41
Firm tofu	889.29±60.61^{ab}	760.71±90.91^{a}	0.105	128.6	14.46
Fujook **(Masak lodeh)**	728.57±106.07^{abc}	610.71±212.13^{abc}	0.693	117.9	16.18

*Significant mean difference between cooked and raw soy products in $p < 0.05$ (paired sample t-test).
Different letters in the same column showed significant difference ($p < 0.05$).

cooked soy beans, fried *tempeh* and fried tofu, fried *tempeh* with *sambal*, Malay style stir fried *tempeh* with long beans and *taukwa* cooked with *sambal chili* (fat: 4.8 - 20.18%; total carbohydrate: 0.54 - 12.7%; protein: 6.1 - 18.2%; moisture: 50.52 - 79.00%; total ash: 1.5 - 2.3%). Differences may be due to different ways of cooking or recipes. The samples in this study were mainly whole dishes which included other ingredients besides soy products.

Total Dietary Fiber Content

Table 2 showed total dietary fiber content based on wet weight was highest in *tempeh goreng kicap* (16.44 ± 1.90%) and lowest in *steamed soft tofu* (1.65 ± 0.21%). Changes of total dietary fiber content in foods when analyzed using enzymatic-gravimetric methods may be due to different types of soybeans, processing method and its period of processing (Azizah and Zainon, 1997; Kutos *et al*., 2003). The percent of CV obtained for the total dietary fiber analysis was all less than 20% that showed precision in this analyses.

Mineral Contents

As shown in Table 4, *Sambal tempeh* contained the highest magnesium (42.59 ± 1.44 mg/100 g) and zinc (2.43 ± 0.16 mg/100 g) contents. *Tempeh goreng kicap* contained the highest calcium (82.00 ± 2.18 mg/100 g) and natrium (75.82 ± 13.87 mg/100 g). According to Dinesh Babu *et al.* (2009), tempeh contained high levels of minerals. Meanwhile, *egg tofu soup* contained the lowest zinc (0.53 ± 0.17 mg/100 g), magnesium (19.73 ± 2.43 mg/100 g), natrium (12.23 ± 2.79 mg/100 g) and calcium (28.75

Table 4 .The mean of phytate and minerals content between samples [a]

Sample (whole dish)		Minerals (wet weight)				
	Phytate content (mg/100 g)	Iron content (mg/100 g)	Zinc content (mg/100 g)	Calcium content (mg/100 g)	Natrium content (mg/100 g)	Magnesium content (mg/100 g)
Steamed soft tofu	332.14±30.30[cd]	0.41±0.045	0.61±0.16	33.45±1.61	19.22±2.84	30.25±2.59
Sambal tofu	910.71±30.30[a]	1.66±0.20	2.01±0.22	72.49±4.05	42.94±12.32	24.62±1.41
Stir fried mustard with egg tofu	739.29±30.30[ab]	1.15±0.13	1.02±0.19	53.53±2.42	25.04±0.71	34.83±2.74
Egg tofu soup	535.71±15.15[bcd]	0.48±0.05	0.53±0.17	28.75±1.59	12.23±2.79	19.73±2.43
Tempeh goreng kicap	289.29±30.30[d]	0.90±0.01	1.88±0.73	82.00±2.18	75.82±13.87	19.83±1.63
Sambal tempeh	600.00±45.46[abcd]	1.60±0.16	2.43±0.16	60.15±4.21	60.56±12.85	42.59±1.44
Sambal goreng jawa	610.71±212.13[abc]	1.48±0.03	1.62±0.52	47.63±3.62	45.74±8.90	31.43±0.31
Masak lodeh	610.71±30.30[abc]	0.85±0.09	0.76±0.19	44.21±1.24	28.05±1.56	30.17±1.19

[a]Data are expressed as mean ± SD on a wet weight basis.
Different letters in the same column showed significant difference ($p < 0.05$).

± 1.59 mg/100 g). The amount of iron was lowest in *steamed soft tofu* (0.41 ± 0.045 mg/100 g) and highest in *sambal tofu* (1.66 ± 0.20 mg/100 g). According to Frossard *et al.* (2000), cooking methods like boiling, grinding and frying processes can affect the amount of minerals in the dishes. The percent of coefficient variance (CV) for analyses in most samples were less than 20% which showed the high precision in the obtained results.

Mineral contents in this present study were compared with food composition tables from USDA, AFCT and FCGS. Calcium, magnesium and iron contents in this studied samples based on wet weight (calcium: 28.75 - 82.00 mg/100 g; magnesium:19.73 - 42.59 mg/100 g; iron: 0.41-1.66 mg/100 g) were lower to the values reported by USDA, AFCT and FCGS (calcium: 74.00 - 372.00 mg/100 g; magnesium: 60.00 - 86.00 mg/100 g; iron: 1.8 - 5.14 mg/100 g) for tempeh with vegetables stir fried, yong tau hoo mixed, cooked soy beans and fried tofu, fried *tempeh* with *sambal*, Malay style stir fried *tempeh* with long beans and *taukwa* cooked with *sambal chili*, but natrium content (12.23 - 75.82 mg/100 g) was similar compared to USDA, AFCT and FCGS (1.00 - 660.00 mg/100 g). The value of the zinc also was similar (0.53 - 2.43 mg/100 g) to the one in other food composition tables (0.66 - 1.99 mg/100 g). These dissimilarities could be due to the factors such as differences in soil, soybean cultivar and plantation environment (Frossard *et al.*, 2000).

Phytate contents

The calibration curve for sodium phytate standard with concentration ranging from 10-50 μg/ml has resulted with the coefficient of determination of $r^2 = 0.9982$. Calibration curve had negative gradient which explains that the greater the concentration of sodium phytate, the lower the absorbance reading at 500 nm. A strong negative relationship between absorbance and concentration of sodium phytate and therefore, the decrease in absorbance was due to the removal of iron from the pink complex by the sample phytate, which is proportional to the concentration of phytate presence (Norhaizan and Nor Faizadatul, 2011). Phytate concentration of studied samples was expressed in the unit mg/100 g based on wet weight. The range of phyate was found to be 257.14 - 900 mg/100 g for raw soy products, 182.14 - 803.57 for cooked soy products and 289.29 - 910.71 for whole dishes. These ranges were relatively in good agreement with the range of phytate (130 - 1878 mg/100 g based on wet weight) in soy products reported by Ma *et al.* (2005). However, data on the dosage of phytate for human beings eliciting either positive or negative effects is restricted and the best possible dosage for clinical therapies is required to be determined (Kumar *et al.*, 2010).

Table 4 indicated that phytate content among the whole dish samples showed significant difference ($P < 0.05$).This can be due to the contribution of different ingredients with different phytate content in a whole dish. *Sambal tofu* contained the highest phytate (910.71 ± 30.30 mg/100 g) content and was significantly higher ($P < 0.05$) than *egg tofu soup* (535.71 ± 15.15 mg/100 g), *steamed soft tofu* (332.14 ± 30.30 mg/100 g) and *tempeh goreng kicap* (289.29 ± 30.30 mg/100 g). The present study showed that phytate content was significantly ($P <0.05$) lower in raw and cooked fermented soy products (tempeh) compared to the non-fermented ones. Phytate content was lowest in *sambal tempeh* in raw soy products (257.14 ± 45.46 mg/100 g) and in *tempeh goreng kicap* in whole dish studied samples (289.29 ± 30.30 mg/100 g). In addition, for dishes like *masak lodeh* and *sambal goreng jawa*, the amount of phytate in *tempeh* used in raw and cooked soy products, was the lowest compared to tofu and fujook. Fermentation probably related to the reduction of phytate content as tempeh is produced by fermentation of soy beans with *Aspergillus oryzae* and *Rhizopus oligosporus*,

Table 5. Molar Ratios of Phytate to Iron, Zinc, Calcium and calcium×phytate/zinc of Malaysian soy based dishes

Sample (whole dish)	phytate/iron	Phytate/zinc	Phytate/calcium	calcium×phytate/zinc
Steamed soft tofu	68.71	53.78	0.60	44.98
Sambal tofu	46.45	44.53	0.76	80.71
Stir fried sawi with egg tofu	54.63	71.37	0.84	95.50
Egg tofu soup	95.52	100.40	1.13	72.16
Tempeh goreng kicap	27.18	15.16	0.21	31.07
Sambal tempeh	31.72	24.33	0.60	36.59
Sambal goreng jawa	35.05	37.08	0.78	44.15
Masak lodeh	61.11	79.44	0.84	87.80

both are moulds that produce intra- and extracellular phytate degrading activity. Most studies (Norhaizan and Nor Faizadatul (2009), Greiner *et al.* (2006) and Kumar *et al.* (2010) and Astuti and Dalais (2000) stated that fermentation process of tempeh decreases the phytic acid content.

Table 3 showed that although phytate reduction was observed in all cooked soy products, there was no significant difference ($P > 0.05$) that existed between the phytate content of cooked and raw soy products. This may due to the heat used during cooking did not significantly destruct the phytate since it is heat-stable (Ma *et al.*, 2005; Kumar *et al.*, 2010). The lowest and highest percent reduction of phytic acid was observed in tempeh used in *sambal goreng jawa* (7.14%) and egg tofu used in *egg tofu soup* (59.76%), respectively. Phytate is water soluble and when it is cooked in water, the amount of phytate will be reduced by discarding the soak water. This explained the reduction of phytate in egg tofu cooked in soup (Greiner and Konietzny, 2006; Ma *et al.*, 2005). *Egg tofu soup* (boiling method) had the highest phytate reduction (59.76%), followed by *steamed soft tofu* (43.33%) (Steaming method). It is concluded that hydrothermal process such as boiling and steaming may affect the phytate dephosphorelation in foods (Kumar *et al.*, 2010).

Generally, in this study whole cooked dishes contained higher amounts of phytate which can be due to the contribution of phytate from other ingredients in comparison with raw and cooked soy products.

Bioavailability of minerals

According to Table 5, the molar ratio of phytate/Fe, phytate/Zn, phytate/Ca and [phytate x Ca]/ Zn were ranging from 27.18 - 95.52, 15.16 - 100.40, 0.21 - 1.13 and 31.07 - 95.50, respectively. The zinc, calcium and iron contents in samples based on wet weight ranged between 0.53 - 2.43, 28.75 - 82.00 and 0.41 - 1.66 mg/100 g, respectively (Table 4). In the present study, all of the studied samples appear to be a poor source of iron with the molar ratio of phytate/Fe >1 (Rosalind *et al.*, 2010). The molar ratios of phytate/calcium and phytate/zinc of samples were above the critical levels (Ca >0.17 and Zn > 18), except for *tempeh goreng kicap* that had phyate/zn ratio of less than 18 (15.16) (Rosalind *et al.*, 2010). However, calcium×phytate/zinc of all samples was <200 (Janet and Jeannemarie, 2009).

When the four ratios are taken into account together, the phytate in soy products will inhibit the absorption of calcium and iron. However, the ratio of Ca×phytate/Zn for all samples was less than 200 which indicated that phytate did not affect the bioavailability of Zn although the phytate/Zn ratio for a total of 7 of 8 soy products was above the critical value (>18). Kwun and Kwon (2010) and Fordyce et al. (1987) reported that calcium may accentuate the Zn-inhibitory effect of phytate. Consequently, high levels of dietary calcium are expected to be a cofactor in reduced absorption of nutritional zinc. This is due to the formation of lower solubility Ca-Zn-phytate complex in the intestine as compared to the complex formed by either ion. Therefore, it has been proposed that the dietary Ca×phytate/Zn molar ratio could be a more helpful evaluation of Zn bioavailability than the phytate/ Zn molar ratio alone.

Vaishali *et al.* (1997) reported the effect of natural fermentation on in vitro Zn bioavailability is the increase in Zn solubility (2 - 28%) and Zn uptake by intestinal segment (1 - 16%) to a significant level in cereal-legume mixtures. However, Liang *et al.* (2009) stated that germination, soaking or fermentation cannot completely remove phytic acid, subsequently the residual phytate might still interfere with zinc solubility. The current study indicated that among the studied samples, fermented dishes had the lowest phytae/mineral molar ratio. Astuti and Dalais (2000) also reported that fermentation increases the bioavailability of minerals. Shamsuddin (1999) reported that only phytate in the form of inositol triphosphate (IP_3) can inhibit the absorption of minerals. However, in current study we looked out to the total phyate content and did not make different between the different classes of phytate.

As a limitation, it is noteworthy to mention that it was not easy to logically decide the best critical levels of different molar ratios reported in the literature.

Molar ratios are figured out based on previously known interactions for specific food items. In a food, the partial influences of different molecules and ingredients which finally have a cumulative effect on the bioavailability of a nutrient will be unnoticed when relying on molar ratios. The different inhibitors in a food may have synergistic effects or antagonistic effects and consequently figuring out molar ratios based on a few micronutrients may not give a good prediction of the bioavailability. In addition, the use of critical levels for various molar ratios are sometimes subjective, with no justification.

Conclusions

The results of these eight soy based dishes indicated that cooking did not reduce the phytate content in a significant way. However, hydrothermal process such as boiling and steaming resulted in the highest reduction of phyate among samples. Although the food samples contained high mineral content, molar ratios of phytate/minerals showed phytate gave inhibitory effect on the bioavailability of calcium and iron. Thus, optimal food processing and cooking methods should be chosen in order to diminish this inhibitory effect.

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