International Food Research Journal 21(5): 2019-2024 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

The effects of cooking on oxalate content in Malaysian soy-based dishes: Comparisons with raw soy products

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Article history

Received: 28 November 2013
Received in revised form: 7 March 2014
Accepted: 13 March 2014

Keywords

Calcium
Cooking
Malaysian
Oxalate
Soy products

Abstract

The advantage of cooking cannot be summarized just as the better food digestion. Some investigations showed the effect of cooking on reduction of food anti-nutrients such as oxalate. This study was aimed to determine the effect of cooking on oxalate content and its negative effects on calcium availability in eight Malaysian soy-based dishes. Since there is few data which examined the effects of cooking on food oxalate content globally, thus this study was designed as the first in Malaysia. Oxalate in this research was analyzed by using enzymatic methods, while calcium content was determined by using Atomic Absorption Spectrophotometer. The oxalate concentration was in the range of 6.43-19.40 mg/100 g for whole cooked samples, 9.03-11.90 mg/100 g for raw soy products, and 4.36-7.99 mg/100 g for cooked ones. There were 5 out of 12 samples containing oxalate, which was significantly lower ($p < 0.05$) in cooked products compared to the raw ones. The rest of the samples were also lower in oxalate but not significantly different ($p > 0.05$). Oxalate in raw/cooked fermented soy products (tempeh) was slightly lower compared to the non-fermented ones. However, there was no significant difference ($p > 0.05$) in oxalate amount between fermented and non-fermented soy products. As Oxalate/Calcium ratio was below 1, oxalate did not have an effect on availability of calcium in the studied samples. Optimal cooking and food processing might be effective in reducing oxalate content in soy products. There is a need for more investigations about the effect of cooking on soy products to confirm the present results.

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Introduction

The emergence of interest in healthier nutrition in recent years has given a trend towards use of plant-based food products with multiple functional properties. Soy as a functional food presents a significant demand for a growing segment of consumers with certain dietary and health concerns. Soy beans (*Glycine max*) belong to the legume family (Jooyandeh, 2011) and are consumed worldwide. On an average, Asians consume 20 to 80 grams of soy foods daily. These foods consist primarily of tofu, miso, and tempeh (Omoni and Aluko, 2005). Malaysians do consume soy product in their daily dietary intake, since the Food Consumption Statistics for Malaysia (Jamal Khair, 2006) indicated that intakes of tempeh and bean curd among them were 6.91 g/day and 19.40 g/day, respectively.

Legumes have been found to contain inherent anti-nutritional factors that limit their nutritive value by exerting certain deleterious effects. Consequently, raw, unprocessed soy beans contain anti-nutritional factors such as trypsin inhibitors, lectin, saponins, phytate and oxalate, which seriously limit the nutritional benefits of soybeans (Kricka *et al*., 2009; Chen *et al*., 2010).

The anti-nutritional factor that is of primary concern is oxalic acid. Oxalic acid (ethanedioic acid, $H_2C_2O_4$) is a strongly oxidized and corrosive compound with good chelating activity, synthesized by a broad range of animals, plants and microorganisms (Stewart *et al*., 2004). Oxalic acid and its salts are extensively spread in numerous plant tissues as the end products of metabolism. Oxalic acid content in foodstuffs has long been a concern in human diets, due to the negative health effects connected to a high intake of oxalic acid. Incidences of kidney stones, hypocalcemia and hyposideremi (low plasma levels of calcium and iron) that correspond strongly with the intake of oxalic acid that perform as an absorption inhibitor are common (Palaniswamy *et al*., 2002). High oxalate content in urine and blood causes several diseases such as hyperoxaluria and vitamin deficiencies (Jiang *et al*., 1996). Small doses of oxalate in the body may result in pain, headaches, and twitching in muscles and cramps. Larger doses can result in a drop in blood pressure, weak, irregular heartbeat, and signs of heart failure. Large doses of oxalate may rapidly put a person in a shock-like state, causing convulsions (because of low plasma calcium), coma, and even death. The mean fatal dose for an adult is about 15 to 30 g, but the lowest

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reported fatal dose is merely 5 g (or about 70 mg/kg) (Noonan and Savage, 1999; Tsai *et al.*, 2005). Consumption of foods high in oxalic acid in the long term can be troublesome. Healthy persons can securely consume such foods moderately, but those with gout, rheumatoid arthritis, kidney disorders, or certain forms of chronic vulvar pain (vulvodynia) are normally advised to stay away from foods high in oxalates or oxalic acid (Roy *et al.*, 2010).

Despite the potential dire health and life-impacting nutritional outcome of oxalate consumptions associated with soy beans, there is a lack of data on oxalate contents in soy beans and other legumes usually consumed by human beings. Plant food such as spinach and rhubarb and others that contain high oxalate have been well studied (Massey *et al.*, 2001). Therefore, this study aimed to determine the effect of cooking on oxalate content in eight Malaysian soy-based dishes.

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 has been described by Shimi and Hasnah (2013). They were chosen based on the most common recipes used to cook local soy products like tempeh, tofu, soft tofu and Japanese tofu/egg tofu obtained from the local wet market. Samples were divided into three groups, consisted of whole cooked dish, separated cooked soy products from the whole cooked dish and raw soy products used in preparing the whole cooked dish. All raw and cooked samples were homogenized. Then they were stored at -20°C prior to analysis

Oxalate content

Oxalate content was determined by using an oxalate kit made by Trinity Biotech. This kit was based on enzymatic method, incorporating the principal of Tsai *et al.* (2005) which is the oxidation of oxalate by oxalate oxidase, followed by detection of H_2O_2 produced during the reaction.

$$\text{Oxalate} + O_2 \xrightarrow{\text{Oxalate oxidase}} 2CO_2 + H_2O_2$$

$$H_2O_2 + \text{MBTH} + \text{DMAB} \xrightarrow{\text{Peroxidase}} \text{Indamine Dye} + H_2O$$

About 1 gram of each sample (whole cooked dish, cooked and raw soy products) was ground with 5 ml de-ionized water. All of the samples were diluted with a diluent which contained EDTA for the purpose of chelating calcium within the sample and the pH was adjusted to between 5 and 7 using either 1 N of an HCl or NaOH solution. Diluted samples were then purified with sample-purifying tubes which contained activated charcoal. Samples were centrifuged (Hermle Z 200 A) for 5 minutes at 2000 rpm in sample-purifying tubes and the supernatant was collected via filtering through filter paper. The oxalate kit was then used to measure the oxalate concentration of each sample. Oxalate reagents were warmed to assay temperature (any temperature between ambient and 37°C). Tubes were labeled for blank, control, standard, and sample. About 1 mL oxalate reagent A [DMAB (3-dimethylamino) benzoic acid and MBTH (3-methyl-2-benzothiazolinone hydrazone), pH = 3.1±0.1] was added to each tube. About 50 mL of sample (purified supernatant) was added to each sample tube and 50 mL deionized water was added to the blank. About 50 mL of ascorbic acid (1 mM) was added to control tubes to determine if it interferes with the oxalate measurement, while 50 mL of oxalate standard was added to the standard tube. About 0.1 mL of oxalate reagent B (oxalate oxidase and peroxidase) was added to all tubes and immediately mixed by gentle inversion. All tubes were then incubated at 37°C for 5 minutes and absorbances of blank, control, standard, and sample were determined at 590 nm using spectrophotometer (SECOMAM CE, France. r.). Measurements were taken two to three times to check repetitiveness of instrument. Corrected absorbances were determined by subtracting blank absorbance from absorbance readings of standard, control, and sample. The O.D. ratio of 1 mM ascorbic acid was invariably measured near zero. Thus, the food ascorbic acid did not interfere with the oxalate reading. Calculations to determine oxalate concentration in milligrams was determined as follows:

$$\text{Oxalate (mmol/L)} = \frac{\Delta A\ sample}{\Delta A\ standard} \times 0.5 \times 10$$

$$\text{Oxalate (mg/L)} = \frac{\Delta A\ sample}{\Delta A\ standard} \times 45 \times 10$$

Where:
0.5 = Concentration of oxalate in standard
10 = Dilution factor

Oxalate/calcium ratio

The content of calcium in samples was measured by using Atomic Absorption Spectrophotometer (AAS). Standard stock solutions of calcium were prepared from AAS grade chemicals (Fisher scientific, UK) with appropriate dilutions. Oxalate can decrease the availability of dietary essential minerals such as calcium. Thus, the adverse effects of oxalate in relation to calcium must be considered in terms of the oxalate/calcium (mEq/mEq) ratio.

Statistical analysis

Data were expressed as mean ± standard deviation and coefficient of variation of duplicate measurements for oxalate content and triplicate measurements for calcium. Data were analyzed using statistical software, SPSS version 19.0 for Windows. Paired t-tests were used to compare the oxalate content in raw and cooked soy-based dishes. One-way ANOVA with Tukey's HSD test was used to determine the differences for oxalate contents in all samples. Independent sample t-test was used to compare oxalate contents in fermented and non-fermented soy products. Level of significance was set at $p < 0.05$.

Results

Oxalate contents

Oxalate concentrations of studied samples were based on wet weight and were in the range of 6.43-19.40 mg/ 100 g for whole cooked dish samples, 9.03-11.90 mg/100 g for raw soy products and 4.36-7.99 mg/100 g for cooked ones. The percent CV for analyses in most samples were less than 20% which showed the high precision in the obtained results.

Oxalate content in raw firm tofu in sambal tofu, *sambal goreng jawa* and *masak lodeh* contained (11.25 mg/100 g), (9.07 mg/100 g) and (11.90 mg/100 g), respectively. Also, raw egg tofu in egg tofu soup and stir fried mustard with egg tofu contained (11.25 mg/100 g) and (11.05 mg/100 g) oxalate content, respectively. Meanwhile, raw tempeh in *tempeh goreng kicap*, *sambal tempeh*, *sambal goreng jawa* and *masak lodeh* contained 10.65 mg/100 g, 10.65 mg/100 g, 9.03 mg/100 g and 10.46 mg/100 g oxalate content, respectively. Raw *fujook* in *sambal goreng jawa* and *masak lodeh* contained 11.57 mg/100 g and 11.48 mg/100 g oxalate, respectively, as well. Therefore, it can be concluded that, oxalate content in similar raw soy products used in different dishes were fairly the same.

In the present study, the oxalate content in the whole dish was lowest for *sambal goreng jawa* (6.43 mg/100 g) and was significantly ($p < 0.05$) lower than egg tofu soup (15.91 mg/100 g) and *sambal tempeh* (19.40 mg/100 g). The oxalate content was recorded highest for *sambal tempeh* (19.40 mg/100 g) and was significantly ($p < 0.05$) higher than other whole dishes like sambal tofu (10.85 mg/100 g), stir fried mustard with egg tofu (9.49 mg/100 g), *tempeh goreng kicap* (8.83 mg/100 g), *sambal goreng jawa* (6.43 mg/100 g) and *masak lodeh* (8.20 mg/100 g). There was no significant difference ($p > 0.05$) for oxalate content in all raw soy products. However,

Table 1. Comparison of oxalate content between cooked and raw soy products

Soy products (Name of dishes)	Oxalate content (mg/100 g) Whole dish	Raw form	Cooked form	P value*	Reduction in oxalate content by cooking	Percent reduction in oxalate after cooking
Soft tofu (Steamed soft tofu)	12.14±1.06^{abc}	11.45±2.97^{NS}	7.09±2.43^{ab}	0.457	4.36	38.11
Firm tofu (Sambal tofu)	10.85±0.30^{bc}	11.25±0.57^{NS}	7.60±0.54^{ab}	0.003	3.65	32.44
Egg tofu (Stir fried mustard with egg tofu)	9.49±0.25^{bc}	11.05±0.24^{NS}	7.78±0.61^{a}	0.117	3.27	29.59
Egg tofu (Egg tofu soup)	15.91±5.62^{ab}	11.25±0.57^{NS}	7.99±0.70^{a}	0.019	3.26	28.98
Tempeh (Tempeh goreng kicap)	8.83±0.39^{bc}	10.65±0.09^{NS}	6.00±0.05^{ab}	0.070	4.65	43.66
Tempeh (Sambal tempeh)	19.40±0.49^{a}	10.65±0.83^{NS}	6.45±0.23^{ab}	0.064	4.20	39.44
Tempeh	6.43±0.15^{c}	9.03±0.18^{NS}	6.22±0.37^{ab}	0.089	2.81	31.15
Firm tofu		9.07±1.01^{NS}	4.36±0.07^{b}	0.089	4.71	51.94
Fujook (Sambal goreng jawa)		11.57±0.26^{NS}	4.97±0.00^{ab}	0.018	6.60	57.05
Tempeh	8.20±0.73^{bc}	10.46±0.09^{NS}	6.38±0.27^{ab}	0.052	4.08	38.98
Firm tofu		11.90±0.30^{NS}	5.81±0.09^{ab}	0.029	6.09	51.17
Fujook (Masak lodeh)		11.48±0.17^{NS}	5.90±0.32^{ab}	0.012	5.58	48.61

*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 ($p < 0.05$)
NS: Not Significant

Table 2. Comparison of oxalate content between fermented and non-fermented soy products

Type of food		N	Mean (mg/100 g)	P value
Non fermented	Raw	16	11.13	0.060
Fermented		8	10.20	
Non- fermented	Cooked	16	6.44	
Fermented		8	6.26	0.653

Significant mean difference between fermented and none-fermented soy products in $P < 0.05$ (Independent sample t-test)

Table 3. Oxalate (mg/100 g), Ca (mg/100 g) and oxalate/Ca (mEq) ratio of eight local soy-based dishes

Dish samples	Oxalate (mg/100g)	Ca (mg/100g)	Oxalate/Ca (mEq)
Steamed soft tofu	12.14±1.06	33.45±1.61	0.16
Sambal tofu	10.85±0.30	72.49±4.05	0.07
Stir fried mustard with egg tofu	9.49±0.25	53.53±2.42	0.08
Egg tofu soup	15.91±5.62	28.75±1.59	0.25
Tempeh goreng kicap	8.83±0.39	82.00±2.18	0.05
Sambal tempeh	19.40±0.49	60.15±4.21	0.15
Sambal goreng jawa	6.43±0.15	47.63±3.62	0.06
Masak lodeh	8.20±0.73	44.21±1.24	0.08

there was a significant difference ($p < 0.05$) among oxalate content in cooked soy products. The firm tofu used in *sambal goreng jawa* had the lowest oxalate content (4.36 mg/100 g) and was significantly ($p < 0.05$) lower than egg tofu in stir fried mustard (7.78 mg/100 g) and egg tofu in egg tofu soup (7.99 mg/100 g). In addition, egg tofu in egg tofu soup contained the highest oxalate content (7.99 mg/100 g) and was significantly higher ($p < 0.05$) than tofu in *sambal goreng jawa* (4.36 mg/100 g).

Table 1 shows oxalate content in cooked firm tofu (used in *sambal tofu*) was significantly lower ($p < 0.05$) than the raw one (32.44% reduction). There was a 28.98% oxalate reduction in egg tofu cooked in soup, *fujook* in *sambal goreng jawa* (57.05 % reduction), tofu (*masak lodeh* had 51.17% reduction) and *fujook* in *masak lodeh* (48.61% reduction). The rest of the samples were also lower in oxalate content but not significantly different ($p > 0.05$). Egg tofu soy product had the lowest oxalate reduction. In addition, since, the whole dish samples of this study include other ingredients besides soy products, some whole dish samples have much higher of oxalate content in comparison with raw and cooked forms.

As Table 2 shows no significant difference ($p > 0.05$) result existed between fermented and non-fermented soy products, oxalate content in raw and cooked fermented soy products (tempeh) was slightly lower compared to the non-fermented ones. In addition, for dishes like *masak lodeh* and *sambal goreng jawa*, oxalate reduction in tempeh was the lowest compared to tofu and *fujook.*

Oxalate/calcium ratio

Table 3 shows oxalate and calcium content as well as oxalate/calcium (mEq) ratio of the whole dish cooked samples based on wet weight. Tempeh goreng kicap contained the highest calcium (82.00 ± 2.18 mg/100 g) and egg tofu soup contained the lowest one (28.75 ± 1.59 mg/100 g). Moreover, oxalate/calcium ratios were ranged from 0.05-0.25 mEq (less than 1), being highest in egg tofu soup and lowest in *tempeh goreng kicap.*

Discussion

Oxalate contents in soy beans

Judprasong *et al*. (2006) reported the total oxalate content in raw soy bean seeds (*Glycine max* (L.) Merrill) was high (204±14 mg/100 g) while the level in the cooked seeds (by boiling) was noticeably low (5.5 mg/100 g, with moisture content of 61.1 g/100 g). In a study performed by Massey *et al.* (2001) the oxalate content of seeds from 11 cultivars of soy bean demonstrated quite elevated levels of total oxalate from 0.67 to 3.5 g/100 g of dry weight. Oxalate was found generally as calcium oxalate crystals. Thirteen commercial soy foods were reported to contain 16 to 638 mg of total oxalate per serving. These values were higher in comparison with other legume foods such as refried beans, peanut butter and lentils; containing 197, 193 and 100 mg of total oxalate per serving, respectively. The oxalate content of the two highest oxalate soybean cultivars was lower than that of spinach per gram. Besides, food products developed from soybeans contained high levels of oxalate. Foods made from soy beans tested by Massey *et al*. (2001), have varied oxalate levels. However, this research and Chai and Liebman (2005) study demonstrated much lower total oxalate content in soy beans and lentils than values reported by Massey *et al.* (2001). The differences in oxalate content of soybeans can be attributed to biological variation includes cultivar, growing conditions, and time of harvest.

In other study performed by Al-Wahsh *et al.* (2005) thirty commercial soy foods contained of 0.02-2.06 mg oxalate/g. Commercial soy foods contained 2-58 mg of total oxalate per serving. There were 18 from tofu brands and two soymilk brands contained less than 10 mg oxalate per serving, considered as a low oxalate food. Textured vegetable soy protein, soy flour, soy nuts, vegetable soybeans, soy nut butter and tempeh, contained oxalate more than 10 mg per serving.

Horner *et al.* (2005) analyzed soybean seeds from 116 cultivars for total, soluble, and insoluble oxalate. These cultivars were separated into four groups (A-D) based on the year and geographic spot where they were grown. Oxalate content ranged from about 82 to 285 mg/100 g of dry seed.

References to Al-Wahsh *et al.* (2005) revealed that one serving (85 gram) of different kinds of tofu such as firm tofu, Japanese and soft tofu contained 1.7-11.1 mg/serving oxalate content and one serving of tempeh (85 gram) also contained 40-55.3 mg/serving oxalate content. According to Judprasong *et al.* (2006), oxalate levels less than 100 mg/100 g are considered small amounts. Thus, based on the results of present study, samples contained small amounts of total oxalate. Moreover, American Dietetic Association's Nutrition Care Manual (2005) recommends that patients with kidney stones are to restrict dietary oxalate to less than 40 to 50 mg per day. Furthermore, foods containing greater than 10 mg of oxalate per serving are considered high oxalate foods by the American Dietetic Association (2000). Therefore, such patients should avoid foods highest in oxalate such as: spinach, beets (roots and leaves), rhubarb, chocolate, some tree nuts, bran concentrates and cereals and legumes (beans, peanuts, soybeans and some soyfoods) (Massey and Sutton, 1993). However, the Food Consumption Statistics for Malaysia (Jamal Khair, 2006) reported that intakes of tempeh and bean curd among the Malaysian population were 6.91 and 19.40 g/day, respectively. Since oxalate levels less than 100 mg/100 g are considered small amounts, thus, patients with kidney stones are safe to consume local soy-based dishes considered in this study as all of them contained low amounts of oxalate.

Cooking effect on oxalate

Not much is known about the effects of cooking on the oxalate content of soybeans. In the current study oxalate content in cooked soy products was significantly lower than raw products ($p < 0.05$) for some samples and for the rest were lower but was not significant. Chai and Liebman (2005) discovered that boiling vegetables reduced total oxalate concentration 30% to 87%, mainly by loss of soluble oxalate. In carrots and spinach the reduction in total oxalate corresponded to the amount of oxalate found in the cooking water. Jaworska (2005), Savage *et al*. (2000) and Judprasong *et al.* (2006) discovered similar results in boiled vegetables from Thailand and New Zealand. Even though the amount of oxalate in raw soy bean (*Glycine max*) is quite low, germination and soaking of the seed decreased the oxalate content. Cooking germinated soybeans decreased their oxalate content lower than that of uncooked germinated soy beans. Soaking after cooking proved to be useful as well, even though not as useful as germination (Noonan and Savage, 1999). Al-wahsh *et al.* (2005) indicated that, different cooking methods have different effects on food oxalate. As Bhandari and Kawabata (2006) pointed out; cooking treatments were found to be an effective measurement to reduce oxalate content in wild yam tubers. Decreased in oxalate content was highest in boiling compared to pressure cooking and baking. Additionally, Linda and Massey (2007) reported that boiling vegetables may be a choice to decrease soluble oxalate, if the cooking water is not consumed, but baking potatoes or roasting peanuts or sesame seeds does not affect oxalate content. It was Noonan and Savage (1999) who reported that cooking has proved to be effective in terms of the reduction of total oxalate and thus high oxalate foods should be cooked to reduce the oxalate content.

Effect of fermentation on oxalate content

The first advantage of soy fermentation is the decrease of its chalkiness and beany flavor (Jooyandeh, 2011). Noonan and Savage (1999) has drawn attention to the fact that fermentation that is frequently used in Asian countries has been reported to decrease the oxalate content of foods. Jooyaneh (2011) supported the fact that fermentation can decrease anti-nutritional factors, flatulence and unflavored sugars like stachyose and raffinose, however, the data presented in table 2 shows insignificant ($p > 0.05$) results between fermented and non-fermented soy products, although fermented soy products (tempeh) had slightly lower oxalate content compared to the non-fermented ones.

Oxalate/calcium ratio

Noonan and Savage (1999) reported that oxalate/calcium ratio varies widely and can be classified into three groups: plants with a ratio greater than 2 such as spinach and rhubarb; plants with a ratio about 1, like potatoes and currants and plants with a ratio of less than 1 such as lettuce, cabbage and cauliflower. Morrison and Savage (2003) stated foods with an oxalate-calcium ratio higher than 2 have no usable calcium and contain excess oxalate which can combine calcium in other foods eaten at the same time. Foodstuffs with a ratio of about 1 provide little calcium but do not inhibit the utilization of calcium provided by other products. Since, the studied samples had ratios below 1, it is concluded that, oxalate does not have an effect on availability of calcium in these samples as far as other calcium sources are concerned.

As a limitation, it is noteworthy to mention that 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 ratios. The different inhibitors in a food may have synergistic effects or antagonistic effects and consequently figuring out ratios based on a few micronutrients may not give a good prediction of the bioavailability.

Conclusions

The results of this study indicated these eight soy-based dishes contained small amounts of oxalate and oxalate content in cooked soy products was significantly lower than raw products ($p < 0.05$) for some samples and for others were also lower in oxalate content but was not significant. Although no significant different ($p > 0.05$) result existed between fermented and non-fermented soy products, oxalate content in raw and cooked fermented soy products (tempeh) was slightly lower than non-fermented ones. In addition, oxalate did not have an effect on availability of calcium in the studied samples. Thus, cooking and optimal food processing might be effective in reducing oxalate content in soy bean products.

Acknowledgement

The authors wish to acknowledge the financial support received from Universiti Kebangsaan Malaysia Research Grant, INDUSTRI 2012-2008.

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