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Daidzein and genestein contents in tempeh and selected soy products

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ABSTRACT

The total isoflavones in tempeh and selected local soy products was determined. Raw tempeh contained 26 ± 6 mg daidzein (Da) and 28 ± 11 mg genestein (Ge) while fried tempeh contained 35 ± 11 mg Da and 31 ± 11 mg Ge in 100 g (wet basis). Total isoflavone content in 100 g of raw tempeh, based on a dry weight, was 205 ± 56 mg and significantly reduced to 113 ± 41 mg in 100 g of fried tempeh. Tempeh in batter was deep-fried for 30 min which reduced 45% of the total isoflavone content compared to the raw one. Raw tempeh contained the highest total content of isoflavone among the studied local soy products. Total isoflavone content in processed soy foods like egg tofu and home made soybean drink were significantly lower than other soy products studied.

1. Introduction

Soy isoflavones are diphenolic compounds that are similar to oestrogen and bind to the oestrogen receptor. These isoflavones appear to compete with endogenous oestrogen for receptor binding and act both as oestrogen agonists and antagonists (Kris-Etherton & Hecker, 2002). Scientific studies have shown that women can benefit from dietary manipulation, including increase intakes of phytoestrogens in order to reduce the symptoms experienced during premenopause or menopausal state (Jefferson, 2003). Previous studies (Izumi et al., 2000; Setchell et al., 2001; Zubik & Meydani, 2003) have reported the differences in the absorption rates between glycosylated and aglycone isoflavones. Isoflavone glycosides require initial hydrolysis of the sugar moiety by intestinal β-glucosidases before uptake to the peripheral circulation (Setchell et al., 2002) while aglycone form may be more bioavailable since they do not require hydrolysis in the intestine before absorption. Fermentation process increases the concentration of aglycone due to the microbial activities and may increase the bioavailability of aglycone isoflavones when ingested by human.

Tempeh is a traditional fermented soybean product, which is normally produced by cottage industry in Malaysia. It is normally consumed as fried, boiled, steamed or roasted. Fermentation process of tempeh increases the nutritional values of some nutrients, development of vitamins, phytochemicals and antioxidative constituents (Astuti & Dalais, 2000). Studies have reported that isoflavone levels determined in tempeh are relatively high compared to other soybean products such as tofu and soy beverages (Hutabarat, Greenfield, & Mulholland, 2001; Wang & Murphy, 1994). Other beneficial constituents of soy can also be consumed beside isoflavone, for example calcium. In Malaysian diet, tempeh is also one of the calcium rich foods besides milk and dairy products (Tee, Ismail, Mohd Nasir, & Khatijah, 1997). Fermentation process of tempeh decreases the phytic acid and enhances the bioavailability of minerals such as calcium, zinc and iron (Astuti & Dalais, 2000).

Based on an unpublished data of Malaysian Food Nutrition Survey 2002/2003 carried out by the Ministry of Health Malaysia, it was found that tofu and soybean drink intakes of Malaysian adults are once a week while tempeh intake is three times a month. It is necessary to estimate the isoflavone intake of the locals in order to study the protective effects of isoflavone against the risk of chronic diseases. However, database for isoflavone content of Malaysian local soy foods is not available. Thus, this study was initiated to determine the total isoflavone (daidzein and genestein) content in tempeh and selected local soy products commonly consumed by Malaysians. Data on total isoflavone contents of soy products such as egg tofu, tofufah and tempeh fried in batter have not been reported previously. Tempeh fried in batter is the common form of tempeh being served in most food stalls in Malaysia. The total isoflavone was based on total contents of daidzein and genestein since isoflavone glycosides were hydrolysed to their aglycones due to acid hydrolysis method used during extraction of isoflavone from the soy products.

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2. Materials and methods

2.1. Food sampling

Tempeh was bought from Syukor Tempeh Production, a small cottage industry located at Bukit Dukung, Kajang, Selangor, Malaysia. Other soy products namely fujook (skin tofu), fried firm tofu, firm tofu, soft tofu, egg tofu and boxed soybean drink were purchased at open markets and local supermarkets located at Cheras, Selangor. Homemade soybean drink and tofufah (soft tofu in syrup) were purchased from night markets which also located at Cheras, Selangor. The descriptions and ingredients of the studied soy products are presented in Table 1.

2.2. Preparation of fried tempeh

Each packet of tempeh was thinly sliced to about 1 cm thickness. Ingredients used for batter preparation consisted of plain flour, cornstarch, rice flour, turmeric powder, spices (fennel and coriander seeds) and salt. All ingredients were mixed well with water. The sliced tempeh were dipped into the batter and deep-fried in palm oil for 15 min at medium heat (150 °C) until the tempeh turned golden brown. Electrical deep fryer was used for frying tempeh.

2.3. Determination of isoflavone content in soy products

All soy products were analysed in their raw form except for soybean drink and tofufah. The samples were freeze-dried and ground into fine particles before analysed. Only soybean drink and tofufah were analysed in their wet form since these products contained high amounts of sugar and are difficult to freeze-dry. All freeze-dried samples were kept in airtight containers and stored for 2 weeks at −20 °C until further analysis. Extraction of isoflavones was carried out based on method by Hutabarat, Greenfield, and Mulholland (2000). About 1 g of ground sample was added to 40 mL of 96% ethanol (containing 60 ppm of flavone) and 10 mL of (2 M) hydrochloric acid. The sample mixture was then placed in a sonicator for 20 min prior to the hydrolysis process, then heated in a water bath (Memmert, Schwabach, Germany) at 103 °C and refluxed for 4 h. The mixture was made up to 50 mL with aqueous ethanol (96%) and adjusted to pH 4 with sodium hydroxide followed by centrifugation (Universal 30RF, Tuttlingen, Germany) at 800*g* for 20 min. The clear supernatant was passed through a Whatman filter paper (No 4) and 0.20 m polytetrafluoroethylene microfilter before injected into a reverse phase high performance liquid chromatography (HPLC). Preparations of daidzein and genestein as external standards were performed as reported previously (Hutabarat, Mulholland, & Greenfield, 1998). Each 10 mg of daidzein, genestein and flavone was weighed and dissolved in 20 μl dimethylsulphoxide (DMSO) followed by addition of 96% ethanol to make 1 M of stock standard. Flavone standard was used to determine the recovery of the analytes. Each standard solution was diluted with mobile phase to a range of 10–150 μM. HPLC (Agilent 1100, Palo Alto, CA, USA) with degasser, quaternary pump, autosampler and diode array detector (DAD) was used for this analysis. Reversed-phase column phenyl Nova-Pak (150 mm × 4 mm, I.D:5 μm) used was from Waters (Milford, MA, USA). Isocratic mobile phase, acetonitrile–water (33:67, v/v) was used as an eluent at flow rate of 0.8 mL/min. About 20 μL of sample were injected into the column set at room temperature (25 °C). Analytes were monitored by diode array detection (DAD) at 200–400 nm.

2.4. Proximate analyses in raw and fried tempeh

Determinations of moisture, crude fat, crude protein and total ash in raw and fried tempeh were carried out using AOAC (1990) method.

2.5. Calcium analysis in raw and fried tempeh

Tempeh was digested using a microwave digestor (Milestone, Shelton, CT, USA) based on a close system acid digestion. The sample was weighed to less than 0.5 g in thermoplastic vessel. Nitric acid (2.5 ml) and hydrogen peroxide (2.5 ml) were added into the vessel-containing sample. Vessels were then fitted into a rotor and placed in the microwave to be digested for 15 min. This was followed by cooling down the vessels under running water for half an hour. The mixture was poured into a 100 ml volumetric flask and topped up with deionized water. Determination of calcium was carried out using an atomic absorption spectrophotometer (GBC 908A, VIC, Australia), using flame method as described by Osborne and Voogt (1978). Calcium standards ranging from 1 to 5 ppm were prepared from a 100 ppm of calcium stock solution. Asean foods reference materials (AS-FRM 2) consisting of cereal soy, obtained from Institute of Nutrition, Mahidol University was used for accuracy test.

2.6. Statistical analysis

Data were expressed as mean ± standard deviation of triplicate measurements. Data were analysed using statistical software, SPSS version 15.0 for windows (SPSS Inc., Chicago, IL, USA). *T*-tests and one-way ANOVA were used to determine the differences for all

Table 1
The descriptions and ingredients of the soy products analysed in this study.

Soy products	Description	Ingredients
Boxed soy bean drink	Boxed SB drink is produced at beverages manufacturing and available in any small shops to supermarkets in Malaysia	Soy bean extract, cane sugar, permitted flavourings
Homemade soy bean drink	Home made SB drink is usually prepared in small scale by the street hawkers and normally available at night markets in Malaysia	Soy bean extract, sugar
Egg tofu	Egg tofu also known as Japanese tofu to the locals, is a processed tofu that is yellow in colour, which may due to addition of egg. It is usually added into dishes of soup and vegetables	Water, eggs, soy bean, hydrogenated vegetable protein, seasoning
Firm tofu	Firm tofu are pressed tofu that has pattern of muslin cloth on its surface while fried tofu is the fried form of firm tofu. Both types of tofu are widely available in any local market or shop in Malaysia	Soy milk, calcium sulphate
Soft tofu	Tofu which has fine texture and has highest moisture content among all fresh tofu	Soy milk, calcium sulphate, glucono delta lactone
Tofufah	Tofufah is soft tofu served in syrup, normally taken as dessert by Malaysians	Soy milk, calcium sulphate, glucono delta lactone
Fujook	Tofu skin or also known as fujook is made by boiling soy milk in pan until it become film/skin and then dried into yellowish sheet	Soy milk

nutrient contents in all samples. Level of significance was set at $p < 0.05$.

3. Results and discussion

3.1. Isoflavone contents in raw and fried tempeh

Isoflavone analysis used in this study was based on a validated rapid isocratic HPLC technique by Hutabarat et al. (2000), in quantifying the daidzein (Da) and genestein (Ge) contents. Da and Ge standards eluted at 3.2 and 5.5 min, respectively, as shown in Fig. 1 (a and b). Fig. 1C shows Da and Ge peaks eluted at 3.3 and 5.4 min in soy products. Da content (based on wet weight) in studied tempeh was similar to Indonesian tempeh (Hutabarat et al., 2001) for both raw (26% vs 22%) and fried tempeh (35% vs 33%). Ge content (based on wet weight) in this study were also similar to the Indonesian tempeh, both in raw (28% vs 25%) and fried tempeh (31% vs 40%), as shown in Fig. 2A. When reported as a ratio of Da to Ge, raw tempeh from Malaysia and Indonesia contained Da:Ge in the same ratio of 1:1.1, while the cooked tempeh contained Da:Ge in ratio of 1.1:1–1:1.2, respectively. The studied raw tempeh contained more isoflavone compared to the tempeh purchased from Australia (5% Da and 9% Ge) as reported by Hutabarat et al. (2001). Differences of Da and Ge contents in soybeans may have been influenced by genetic differences, climate and growing conditions (Dalais, Wahlqvist, & Rice, 1997; Wang & Murphy, 1994).

The comparison of total isoflavone content in raw and cooked tempeh was on a dry weight basis since different water content contributed to variability in isoflavone content (Franke et al., 1999). Da and Ge contents in fried tempeh had significantly decreased ($p < 0.05$) as much as 21% and 58%, respectively, compared to raw tempeh as shown in Fig. 2B. Hutabarat et al. (2001) reported a decrease of 25% Da and 42% Ge in fried tempeh. Total isoflavone content in studied raw tempeh (205 ± 56 mg) was significantly reduced ($p < 0.05$) to 113 ± 41 mg in fried tempeh. This study showed 45% of reduction in total isoflavone content in fried tempeh compared to 30% decrement reported by Hutabarat et al. (2001). The latter study used 3–4 min for deep-frying the tempeh while a longer frying time (30 min) was applied for studied tempeh since they were fried in batter. This may cause the decrement of the isolflavone content. Heat applied during tempeh frying caused the decarboxylation of the compounds (Hutabarat et al., 2001). Cooking alters soy products glucoside conjugates where dry heat results in the formation of 6″-O-acetyl-β-glucoside. A decrease of total isoflavone in burned foods was observed by Coward, Smith, Kirk and Barnes (1998). However, Murphy et al. (1999) reported no difference in isoflavone content between raw and cooked tempeh, in which the product was sautéed on nonstick skillet for 3 min per side. Thus, there was little difference in the loss of isoflavones compared to deep-frying. Average recovery of flavone standard obtained in the present analysis was 94 ± 8%, ranging from 83% to 106%. The high percentage of recovery indicates that 4 h reflux time used during extraction of isoflavone was sufficient to give the highest yield of Da and Ge from tempeh.

3.2. Proximate and calcium contents in tempeh

Crude fat, total carbohydrate and total ash in fried tempeh were significantly higher ($p < 0.05$) while moisture content was significantly lower ($p < 0.05$) than the raw tempeh (Table 2). Crude protein content was similar between raw and fried tempeh. Proximate composition and calcium content in tempeh of this study were also compared with Malaysian Food Composition Table, on line USDA – Iowa State University database and that from Indonesia (Karyadi & Lukito, 1996). All nutrients in studied raw tempeh were comparable to the values in the Malaysian Food Table. Total ash content in raw tempeh of USDA database was twice higher, while the rest of the nutrient contents were similar compared to the studied tempeh. Nutrient contents in raw tempeh from Indonesia (Karyadi & Lukito, 1996) were higher in total ash and carbohydrates contents but with lower fat content than the studied tempeh. Moisture and protein contents in Indonesian tempeh were similar with the studied tempeh in this work.

The Malaysian Food Composition Table does not have nutrient contents for fried/cooked tempeh. Thus, nutrients in fried tempeh were compared with USDA database. Crude fat and carbohydrate contents in the studied fried tempeh were much higher compared to the reported cooked tempeh (USDA). This could be due to the deep-frying method and the use of batter containing flour in the preparation of fried tempeh. The cooking method for cooked tempeh in USDA food table was not mentioned but increment of fat content (0.58%) in the cooked tempeh compared to the raw one may indicate use of oil to cook the tempeh. Other nutrient contents in raw and cooked tempeh from USDA were similar to this study.

Ash content represents the total mineral content in food (Harbers, 1994). It is reflected in the studied raw tempeh which contained lower ash and calcium contents compared to the one in Malaysia, USDA and those reported by Karyadi et al. (1996). Standard Reference Material (SRM) used consisting of cereal soy, was analysed together with tempeh samples for accuracy test. SRM analysed in this study was found to contain 485 ± 14 mg Ca/100 g and was within the range of the consensus value for the SRM (491 ± 32 mg Ca/100 g).

3.3. Isoflavone contents in other selected soy products

Total isoflavone contents in other soy products were expressed per 100 g wet samples as shown in Fig. 3. Raw tempeh had the highest content of total isoflavone (54 mg) but not significantly different with the one in fujook (43 mg), fried firm tofu (43 mg), soft tofu (42 mg) and firm tofu (41 mg). Egg tofu and homemade SB (soybean) drink contained total isoflavone contents which were significantly lower ($p < 0.05$) compared to the studied soy products like tempeh, fujook, fried firm tofu, firm tofu and soft tofu. In this study, raw tempeh contained comparatively high concentrations of Da and Ge as in other fermented foods like miso (bean paste) and natto (fermented soybeans), resulting from the action of β-glucosidases of the fermentation organisms (Fukutake et al., 1996). Total isoflavone content in the studied raw tempeh (54 mg) was slightly higher than 46–52 mg in previous studies (Hutabarat et al., 2001; Wang et al., 1994).

The studied SB drink contained low total isoflavone content among soy products, and the amount was similar ($p > 0.05$) whether in boxed or homemade SB drinks. Both types of SB drinks contained higher Da (19–24 mg) compared to Ge content (1–4 mg). Total isoflavone level in SB drink of this study, however, was higher compared to other studies (Hutabarat et al., 2001; Murphy et al., 1999). The SB drinks had Da:Ge ratio of 6:2–13:1, while other studied soy products had Da:Ge ratio of 1:1–1:1.5. Variations of Da and Ge contents in soymilk could have been contributed by the variety of soybeans and techniques to produce soymilk (Hutabarat et al., 2001). Processing method caused the conversion of glycoside forms of isoflavone to their corresponding aglycones, thus increasing the concentration of Da and Ge. Some of the isoflavones may also be lost through the by-products such as okara and whey (Jackson et al., 2002).

Da and Ge contents in the studied firm tofu were in the range with other studies (Franke et al., 1999; Hui, Henning, Park, Heber, & V.L.W., 2001) which reported varied amount of Da (7–34 mg)

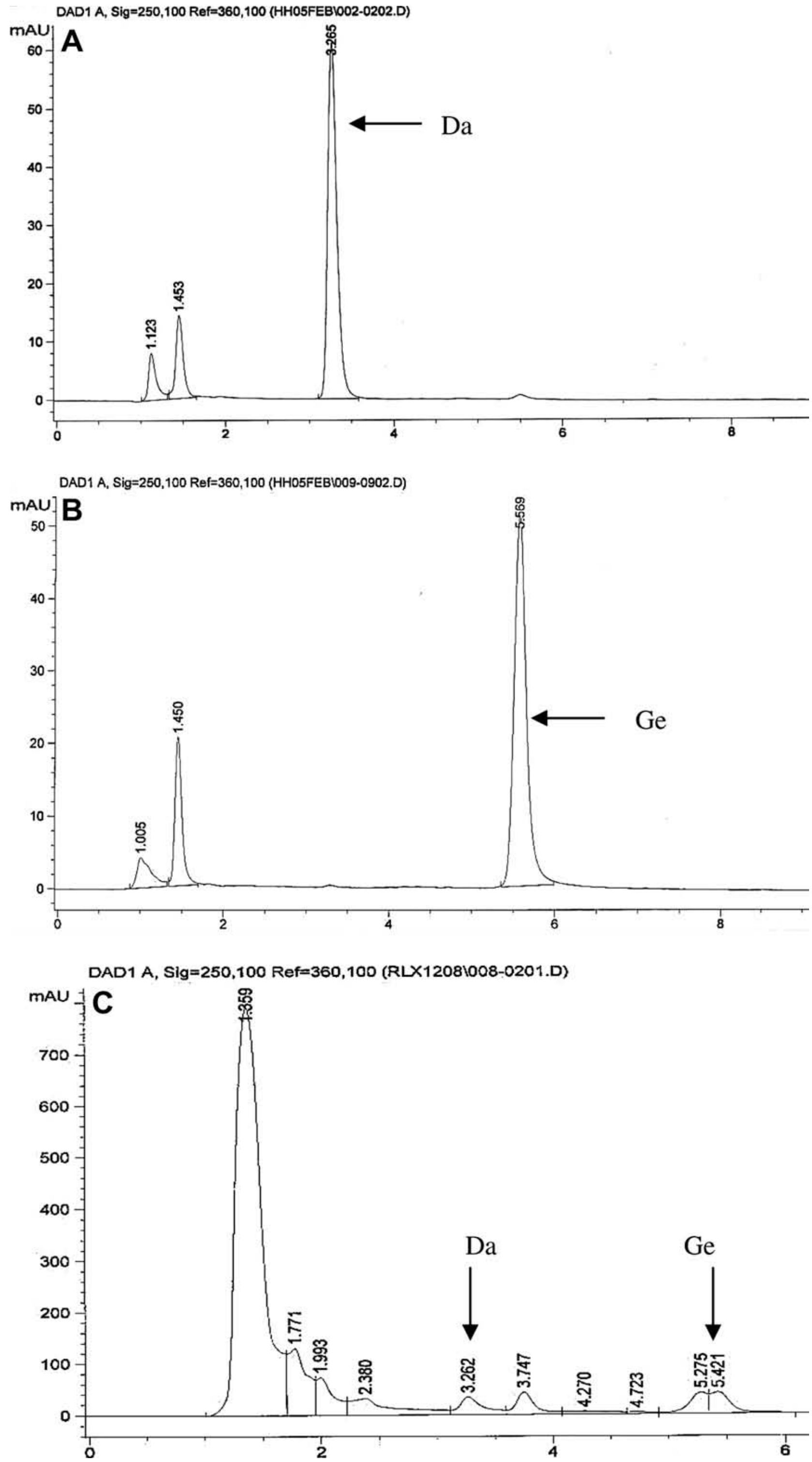

Fig. 1. HPLC chromatograms of (A) daidzein standard peak eluted at 3.2 min; (B) genestein standard peak eluted at 5.5 min; and (C) daidzein and genestein peak eluted at 3.3 and 5.4 min in sample.

and Ge (10–24 mg). Franke et al. (1999) reported that fried tofu from Hawaii contained 24 mg Da and 25 mg Ge, which was comparable to this study. In this study, fujook was immersed in water (usual preparation before cooking) until it became soft before being freeze-dried. The studied fujook (43 mg) was lower in Da and Ge contents than Indonesia's fujook which contained 39–43 mg Da and 42–43 mg Ge (Hutabarat et al., 2001), but these were comparable to Singapore's boiled fujook which contained 23 mg Da and 29 mg Ge (Franke et al., 1999).

Da content in the studied soft tofu was higher than in previous studies that reported to contain 11–17 mg Da while Ge content was comparable to other studies, ranging from 10 to 14 mg Ge (Hui et al., 2001; Hutabarat et al., 2000). To the best of our knowledge, no data has been published on isoflavone content in egg tofu

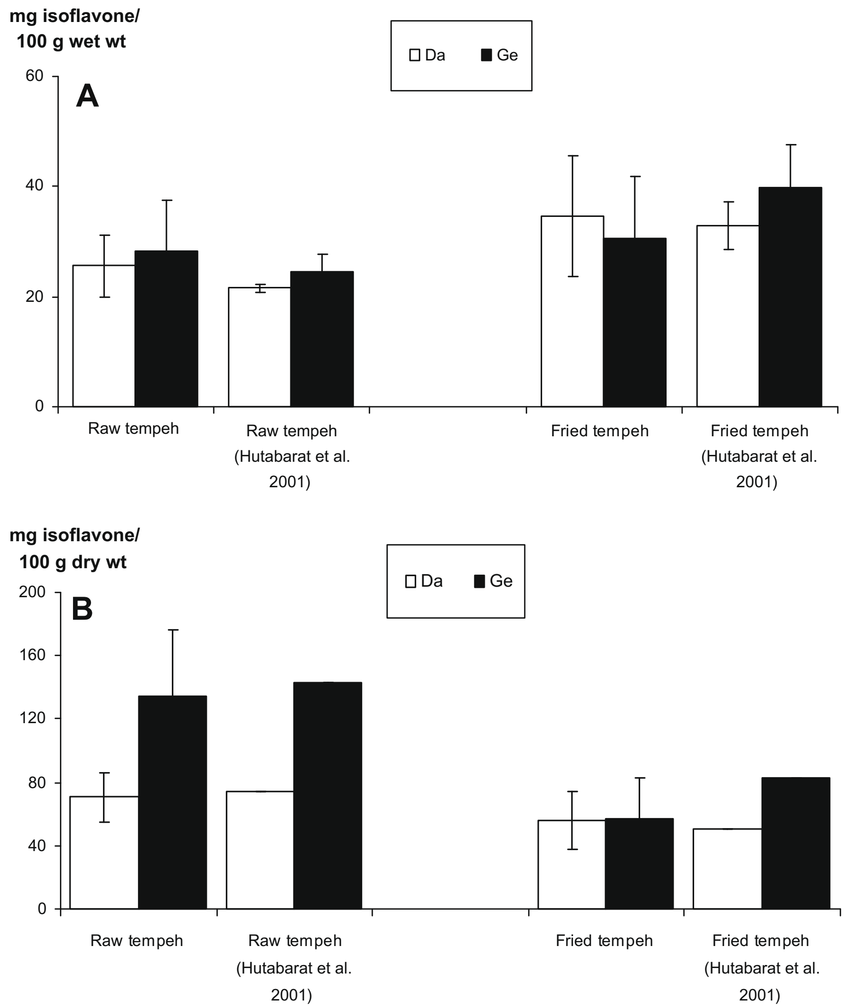

Fig. 2. Daidzein (Da) and genestein (Ge) contents in raw and fried tempeh obtained from this study compared to the study by Hutabarat et al. (2001): (A) Da and Ge contents based on a wet weight basis and (B) Da and Ge contents on a dry weight basis.

Table 2
Proximate and calcium contents[A] in raw and fried tempeh (based on wet weight) in this study compared to tempeh in other food composition tables.

Type of nutrient	Nutrient contents			
	Present study	Malaysia Food Table (Tee et al., 1997)	Indonesia (Karyadi and Lukito, 1996)	USDA online database
Moisture (%)				
Raw	65.1 ± 1.4^a	66.0	64.0	59.6
Fried	41.8 ± 5.1^b			59.5
Protein (%)				
Raw	17.5 ± 0.8^a	15.9	18.3	18.5
Fried	18.6 ± 1.2^a			18.2
Fat (%)				
Raw	9.2 ± 2.9^a	7.5	4.0	10.8
Fried	18.8 ± 7.2^b			11.4
Carbohydrate (%)				
Raw	7.6 ± 3.1^a	6.8	12.7	9.4
Fried	19.9 ± 3.4^b			9.4
Ash (%)				
Raw	0.6 ± 0.1^a	0.9	1.0	1.6
Fried	0.8 ± 0.2^b			1.5
Calcium (mg/100 g)				
Raw	56.8 ± 1.9^a	69	129	111
Fried	63.3 ± 2.7^b			96

Values with no common letter are significantly different ($p < 0.05$), as assessed by *T*-test.
[A] Means ± standard deviation of nutrient contents in raw and fried tempeh.

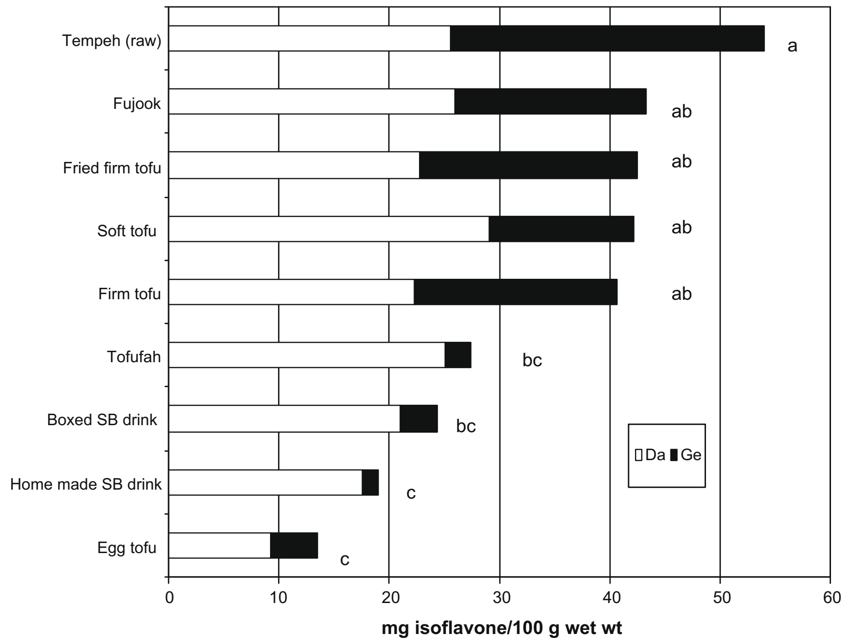

Fig. 3. Daidzein and genestein contents in selected soy products. Values with no common letter are significantly different ($p < 0.05$), as assessed by ANOVA.

and tofufah. Egg tofu had the lowest isoflavone content compared to other soy products. Soy products like tofufah, SB drink and egg tofu may have undergone high heat treatments that result in a total loss of 15–21% Da and Ge (Franke et al., 1999). Apart from heating, other processing techniques like grinding and various fractionations involved in manufacturing of soy beverage and tofu may also affect the loss of isoflavones from soy products (Jackson et al., 2002). Factors such as different analytical techniques applied in different laboratories could also result in varying isoflavone contents even if it was reported from the same food group (Song, Barua, Buseman, & Murphy, 1998). Recovery for flavone standard during analysis of isoflavone contents in soy products was 93.2 + 9.6%, ranging from 80% to 110% indicated that 4 h reflux time used during extraction of isoflavone from soy products was sufficient to give the highest yield of Da and Ge.

4. Conclusions

Tempeh was found to contain the highest amount of total isoflavone (daidzein and genestein) compared to other soy products. Deep-fried tempeh in batter for 30 min has decreased the total isoflavone contents by as much as 45% compared to the raw one. Total isoflavone in processed soy foods like egg tofu and home made soybean drink were significantly lower compared to the other studied soy products in this study. On the whole, isoflavone content in studied soy products was comparable to previous studies when such data were available.

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References

AOAC (1990). *Association of Official Analytical Chemists. Official Methods of Analysis* (15th ed.). USA: AOAC Inc.

Astuti, M., & Dalais, F. S. (2000). Tempe, a nutritious and healthy food from Indonesia. *Asia Pacific Journal of Clinical Nutrition, 9*, 322–325.

Coward, L., Smith, M., Kirk, M., & Barnes, S. (1998). Chemical modification of isoflavones in soyfoods during cooking and processing. *American journal of Clinical Nutrition, 68*(6), 1486S–14915S.

Dalais, F. M., Wahlqvist, M. L., & Rice, G. E. (1997). Variation in isoflavonoid phytoestrogen content in soybeans grown in Australia. *Proceeding of the Nutrition Society of Australia, 21*, 16.

Franke, A., Hankin, J. H., Yu, M. C., Maskarinec, G., Low, S. H., & Custer, L. J. (1999). Isoflavone levels in soy foods consumed by multiethnic populations in Singapore and Hawaii. *J. Agric. Food Chem., 47*, 977–986.

Fukutake, M., Takahashi, M., Ishida, K., Kawamura, H., Sugimura, T., & Wakabayashi, K. (1996). Quantification of genestein and genistin in soybeans and soybean products. *Food and Chemical Toxicology, 34*, 457–461.

Harbers, L. H. (1994). Ash analysis. In S. S. Nielsen (Ed.), *Introduction to the chemical analysis of foods* (pp. 115). London, England: Jones and Bartlett Publishers.

Hui, E., Henning, S.M., Park, N., Heber, & Vay Liang W.G. (2001). Genistein and daidzein/glycitein content in tofu. *Journal of Food Composition and Analysis, 14*, 199–206.

Hutabarat, L. S., Greenfield, H., & Mulholland, H. (2000). Quantitative determination of isoflavones and coumestrol in soybean by column liquid chromatography. *Journal of Chromatography A, 886*, 55–63.

Hutabarat, L. S., Greenfield, H., & Mulholland, H. (2001). Isoflavones and coumestrol in soybeans and soybean products from Australia and Indonesia. *Journal of Food Composition and Analyses, 14*, 43–58.

Hutabarat, L. S., Mulholland, H., & Greenfield, H. (1998). Development and validation of an isocratic high performance liquid chromatographic method for quantitative determination of phytoestrogens in soy bean. *Journal of Chromatography A, 795*, 377–382.

Izumi, T., Piskula, M. K., Osawa, S., Obata, A., Tobe, K., Saito, M., et al. (2000). Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J. Nutr., 130*(7), 1695–1699.

Jackson, C. J. C., Dini, J. P., Lavandier, C., Rupasinghe, H. P. V., Faulkner, H., Poysa, V., et al. (2002). Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. *Process Biochemistry, 37*, 1117–1123.

Jefferson, A. (2003). Dietary phytoestrogens – A role in women's health. *Nutrition & Food Science, 33*, 16–22.

Karyadi, D., & Lukito, W. (1996). Beneficial effects of tempeh in disease prevention and treatment. *Nutrition Reviews, 54*, S94–S98.

Kris-Etherton, P. M., & Hecker, K. D. (2002). Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *American Journal of Medicine, 113*, 71–88.

Murphy, P. A., Song, T., Buseman, G., Barua, K., Beecher, G. R., Trainer, D., et al. (1999). Isoflavones in retail and institutional soy foods. *Journal of Agricultural and Food Chemistry, 47*, 2697–2704.

Osborne, D. R., & Voogt, V. (1978). *The Analysis of Nutrients in Foods*. London: Academic Press Inc.

Setchell, K. D. R., Brown, N. M., Desai, P., Zimmer-Nechemias, L., Wolfe, B. E., Brashear, W. T., et al. (2001). Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. *Journal of Nutrition, 131*, 1362S–1375S.

Setchell, K. D. R., Brown, N. M., Zimmer-Nechemias, L., Brashear, W. T., Wolfe, B. E., Kirschner, A. S., et al. (2002). Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. *American Journal of Clinical Nutrition, 76*(2), 447–453.

Song, T. T., Barua, K. K., Buseman, G., & Murphy, P. A. (1998). Soy isoflavone analysis: Quality control and new internal standard. *American Journal of Clinical Nutrition, 68*, 1474S–1479S.

Tee, E. S., Ismail, M. N., Mohd Nasir, A., & Khatijah, I. (1997). *Nutrient composition of Malaysian Foods* (4th ed.). Institute for Medical Research, Kuala Lumpur: Malaysian Food Composition Database Programme.

USDA – Iowa State University database. <http://www.nal.usda.gov/fnic/foodcomp/search/index.html>.

Wang, H. J., & Murphy, P. A. (1994). Isoflavone content in commercial soybean foods. *Journal of Agricultural and Food Chemistry, 42*, 1666–1667.

Zubik, L., & Meydani, M. (2003). Bioavailability of soybean isoflavones from aglycone and glucoside forms in American women. *American Journal of Clinical Nutrition, 77*(6), 1459–1465.