Sains Malaysiana 45(9)(2016): 1329–1335

Effects of Different Cooking Methods on Isoflavone Content in Malaysian Soy-Based Dishes

(Kesan Pelbagai Kaedah Memasak terhadap Kandungan Isoflavon dalam Hidangan Berasaskan Soya di Malaysia)

HASNAH HARON*, TASNEEM SHAARI & CHAN BOON KENG

ABSTRACT

Soy-based products are one of famous raw ingredients used in the preparation of Asian cuisines. These soy-based products are good source of isoflavones. This study was carried out to observe the effects of different cooking methods on isoflavone content in soy-based products. A total of eight Malaysian dishes prepared using soy-based products with different cooking methods was selected as samples for this study. Daidzein and genestein contents in raw and cooked soy-based products were both quantified using high performance liquid chromatography (HPLC). The results showed that dishes containing tempe *as ingredients had significantly higher (*p*<0.05) amount of isoflavone content, in both raw and cooked forms, as compared to those prepared from other types of soy-based products when based on dry and wet. In conclusion, the isoflavone content was significantly reduced (*p*<0.05) in some cooked soy-based products (*tempe *(TSS and ML), fu jook (ML) and firm tofu (FTC)) based on dry weight as compared with the raw ones. However, the correlations between isoflavone content with cooking methods, durations and temperatures were not significant (*p*>0.05) in this study.*

Keywords: Cooking; effect; isoflavone; soy-based products; tempe

ABSTRAK

*Produk berasaskan soya merupakan bahan mentah yang sering digunakan dalam penyediaan masakan Asia. Produk berasaskan soya adalah sumber yang sangat baik bagi isoflavon. Kajian ini dijalankan untuk melihat kesan pelbagai kaedah memasak pada kandungan isoflavon dalam produk berasaskan soya. Sebanyak lapan hidangan Malaysia disediakan dengan produk berasaskan soya menggunakan kaedah memasak yang berbeza telah dipilih sebagai sampel kajian. Kandungan daidzein dan genestein dalam produk berasaskan soya ditentukan dengan menggunakan kromatografi cecair berprestasi tinggi (HPLC). Hasil kajian menunjukkan hidangan yang mengandungi tempe sebagai bahan mentah mempunyai kandungan isoflavon yang lebih tinggi secara signifikans (*p*<0.05) dalam kedua-dua keadaan mentah dan masak, berbanding dengan hidangan yang disediakan daripada produk berasaskan soya yang lain berdasarkan berat kering dan basah. Kesimpulannya, kandungan isoflavon telah berkurangan dengan ketara (*p*<0.05) dalam beberapa produk berasaskan soya (tempe (TSS dan ML), fucuk (ML) dan tauhu keras (FTC)) yang dimasak berdasarkan berat kering berbanding dengan yang mentah. Walau bagaimanapun, hubung kait antara kandungan isoflavon dengan kaedah, masa dan suhu memasak adalah tidak signifikan (*p*>0.05).*

Kata kunci: Isoflavon; kesan; memasak; produk berasaskan soya; tempe

INTRODUCTION

Soy bean (*Glycine max*) is one of the legumes widely planted all over the world and used to produce commercial soy-based products. Soy-based products can be produced using various traditional and modern processing techniques. Modern processing techniques yield soy isolates, concentrates and flours (Penas et al. 2011), whereas traditional soy products can be divided into two categories namely fermented soy foods such as *tempe*, thua nao, miso, soy sauce and natto as well as non-fermented soy foods including fresh and dried soy bean sprouts, soy bean shoots or buds, soy milk, tofu, okara and fu jook (Jooyandeh 2011).

Soy bean is also a raw materials incorporated in Asian diet preparation including Japan, Korea, China, Thailand, Indonesia and Malaysia. In Malaysia, common soy-based products available in market comprises of *tempe*, firm tofu, soft tofu, egg tofu and fu jook. A local study (Hasnah et al. 2010) has reported that calcium absorption from *tempe* was comparable to milk among the Malay postmenopausal women. *Tempe* has also been reported to contain high amount of isoflavone compared to other selected local soy based products in Malaysia (Hasnah et al. 2009). This type of fermented soy product can serve as a source of high isoflavones and calcium among the elderly as well. Soy-based products received substantial attention due to its increasing reported health benefits (Wong et al. 2008). A case-control study reported that high soy intakes in adolescents are associated with low risk for breast cancer in adulthood (Shu et al. 2001).

Extensive research has been conducted to examine the soy intake and isoflavone content of diet in Asian populations such as Japan, Korea and China. Omoni and Aluko (2005) reported that soy food intake in Asian population is relatively high (20-80 g/day) as compared to Western population (America: 1-3 g/day). Maskarinec (2005) also reported the daily intake of soy in Japan is between 7-11 g for soy protein and 30-50 mg for soy isoflavone. Nagata et al. (2007) reported that dietary isoflavone decreased the risk of prostate cancer in Japanese men. Daily supplementation of soy (25 g) and aglycone isoflavone (101 mg) has been proven to reduce low density lipoprotein (LDL) cholesterol concentration by 11% and apolipoprotein B level by 8%. A reduced systolic and diastolic blood pressure was observed in hypertensive women (Welty et al. 2007).

Researchers attempted to unravel the effects of heat treatment to the isoflavone content in soy-based products. Lee and Lee (2009) found that roasting process involving higher temperature caused high amount of isoflavone loss as compared to oven drying and explosive puffing processes. Additionally, Chung et al. (2011) reported that isoflavone profile changes during soy food production depending on the processing condition and methods used (heating and fermentation). However, isoflavone content does not change if there is no mass loss during soy food production. During manufacturing of soy products, heat treatment is usually involved in drying, roasting, boiling and frying. Aguiar et al. (2012) reported that daidzein (Da) and genestein (Ge) contents in defatted soy flour were reduced when treated at 100°C for 20 to 60 min.

Heat-involving processes, enzymatic hydrolysis and fermentation were reported as factors that cause changes in the distribution of isoflavone components in soy-based foods (Jackson et al. 2002). The study also reported that processes such as boiling, grinding and protein coagulation in firm tofu production did not significantly destroy Da or Ge. However, other process such as roasting which requires higher temperature may cause up to 21% loss of Da and Ge. Albertazzi and Purdie (2008) reported that despite the profile of isoflavone conjugates in soy can be influenced by heat, but it will not change the total content of isoflavone, showing isoflavone is stable at normal cooking temperature. Malaysian soy-based dishes involved cooking techniques like boiling, frying and steaming. Thus, this study aimed to determine the effect of cooking on isoflavone content in five types of soy products used in eight types of Malaysian soy-based dishes.

Materials and Methods

Dishes Preparation

Eight soy-based Malaysian dishes were selected as the samples of this study, namely *masak lodeh* (ML), *tempe* cooked in chili (TC), *sambal goreng jawa* (SJ), *tempe* fried in soy sauce (TSS), firm tofu cooked in chili (FTC), egg tofu soup (ETS), egg tofu stir-fried with mustard (ETM) and soft tofu steamed with soy paste (STS). The cooking methods involved in preparation of the dishes included frying, boiling and steaming. Soy products were purchased from the same locations in order to avoid variations. Temperature, method and period of cooking time for each type of dishes were taken into account.

Determination of Isoflavone Content

Each dish was separated into two different groups for analysis of isoflavone contents which were consisted of raw soy products (RSP) and cooked soy products (CSP). The CSP was analyzed for dry and wet weight basis. All ingredients in the dishes were included for determination of isoflavone content at wet weight whilst the analysis of isoflavone content at dry weight only included the soy-based products alone. About 60 g of each group sample was homogenized and kept at -20ºC. The frozen sample was then freeze-dried and ground into fine particles. The freeze dried samples were kept at -20°C until further analysis.

Extraction method of isoflavone from food samples was carried out based on method by Hutabarat et al. (1998). About 40 mL of 96% ethanol containing 60 ppm flavone was added to 1 g of sample followed by addition of 10 mL of 2M hydrochloric acid into the mixture. The sample mixture was placed in a container within a sonicator for 20 min, before being hydrolyzed and refluxed in water bath (103ºC) for 4 h. Aqueous ethanol (96%) was added to the sample mixture to make up the volume to 50 mL. The pH of the mixture was then adjusted to pH4 with sodium hydroxide and centrifuged at 3800 rpm for 20 min (Universal 30RF, Tuttlingen, Germany). Supernatant was filtered twice using Whatman No. 4 and micro filter polytetrafluoro-ethylene (PTFE) 0.20 mm.

Daidzein, genestein and flavone external standard solutions were prepared as reported by Hutabarat et al. (1998). About 25 mg of each daidzein (Da), genestein (Ge) and flavones (Fl) was dissolved into 5 mL of dimethylsulfoxide (DMSO). Each standard was then diluted with acetonitrile:water mobile phase (33:67, v/v) into a series of concentrations ranged 5-30 μM. Flavone standard was used to determine the recovery of analytes during extraction. HPLC (Agilent 1100, Palo Alto, CA, USA) equipped with degasser, quartener pump, autosampler and diode array detector was used in this study. Reverse phase phenyl column (Nova-Pak 150 × 4 mm, I.D:5 μm) from Waters (Milford, MA, USA) was used. Eluent used was acetonitrile:water (33:67, v/v) isocratic mobile phase at flow rate 0.8 mL/min. 20 μL of sample was injected into the column set at temperature 25°C. Analyte was detected at wave length from 200-400 nm. The isoflavone content determination was carried out in duplicates.

Statistical Analysis

Data obtained was analyzed using SPSS software for windows version 19.0. ANOVA was used to compare the isoflavone content of various soy-based products and paired t-test was employed to compare the isoflavone between raw and cooked soy-based products. All measurements were performed in duplicates and the results were expressed as mean ± standard deviation. Pearson correlation was used to determine the correlations of isoflavone contents in dishes with cooking methods, durations and temperatures.

Results and Discussion

Detailed descriptions of preparing the dishes are shown in Table 1. Figure 1 shows five types of soy products including *tempe*, firm tofu, soft tofu, egg tofu and fu jook (soy milk skin) were used in the preparation of all these dishes. Two dishes such as *masak lodeh* (ML) and *sambal goreng jawa* (SJ) contained a combination of three different soy products (*tempe*, firm tofu and fu jook) while the other six dishes contained only one type of soy product. In preparation of these dishes, the soy products were cooked within the range of 5 - 17 min at temperature ranging from 50-94°C (Table 2).

Isoflavone Content

Based on HPLC chromatogram, Da, Ge and Fl peaks were observed based on their respective retention time and wavelength. Peak for Da had earliest retention time which was 3.29 ± 0.03 min followed by Ge (5.58 ± 0.11 min) and Fl had retention time of 14.43 ± 0.20 min. Percentage of recovery for Fl was 74.04 ± 4.55 %, similar to the value (74.66 ± 4.58%) reported by Tan (2011), but Hasnah et al. (2009) reported a higher value (93.2 ± 9.6%).

Table 3 shows that among all the raw soy products, *tempe* had the highest content of Da and Ge based on wet weight. This can be observed in dishes such as *tempe* fried in soy sauce (TSS), *tempe* cooked in chilly (TC) and *masak lodeh* (ML), where *tempe* is the main ingredient. The isoflavone content of *tempe* dishes were TSS (41.00 ± 0.49 mg Da/100 g; 70.36 ± 1.25 mg Ge/100 g), TC (37.90 ± 1.20 mg Da/100 g; 65.05 ± 2.02 mg Ge/ 100 g) and ML (34.93 ± 0.76 mg Da/100 g; 60.73 ± 1.45 mg Ge/100 g). The Da and Ge contents obtained in this study were higher compared to that of previous study (Nakajima et al. 2005), which contained only 7 to 8 and 7 to 10 mg Ge/100 g, respectively. Ferreira et al. (2011) also documented that aglycone in *tempe* increased significantly over the period of fermentation and was found to increase up to 58% after 6 h of fermentation compared to the aglycone content in soybean itself.

On a contrary, raw egg tofu was found to have the lowest Da and Ge contents in both egg tofu stir-fried with mustard (ETM) (7.01 ± 0.57, 4.86 ± 0.21 mg Ge/100 g) and egg tofu soup (ETS) (6.23 ± 0.47, 4.35 ± 0.33 mg Ge/100 g) dishes. Meanwhile, other soy products including fu jook used in ML and SJ; firm tofu in FTC, SJ and STS; and soft tofu in STS showed the Da and Ge contents ranged from 11 to 18 and 8 to 33 mg /100g, respectively. These values were similar to that of reported by Hui et al. (2001), indicating 10 to 24 and 7 to 28 mg Ge/100 g, respectively, in various firm tofu and soft tofu products.

Additionally, cooked *tempe* in TSS still contain the highest content of Ge (73.17 ± 0.80 mg Ge/100g) and Da (61.45 ± 1.38 mg Da/100g), followed by *tempe* in TC (66.89 ± 0.43 46 mg Ge/100g, 38 ± 0.12 mg Da/100g), whereas, cooked egg tofu has the lowest Ge and Da contents in ETS (3.98 ± 0.56 mg Ge/100 g, 7.62 ± 1.68 mg Da/100 g), followed by egg tofu in ETM (5.74 ± 0.34 mg Ge/100 g, 12.98 ± 1.59 mg Da/100 g). This was in fair agreement with the previous study (Hasnah et al. 2009) that reported egg tofu has the least amount of Da and Ge, ranging from 5 to 10 mg.

The low isoflavone content in egg tofu is ascribed to the ingredients used in making it such as soy milk, egg and coagulant. *Tempe* is made directly from the whole soy beans, unlike other soy products such as firm tofu, soft tofu and fu jook are made from soy milk and coagulant as

TABLE 1. Descriptions of dishes prepared

Dishes	Soy product used	Description
Tempe fried in soy sauce (TSS)	*Tempe*	*Tempe* fried in oil, then sautéed with anchovies and soy sauce
Tempe cooked in chili (TC)	*Tempe*	*Tempe* fried in oil, then cooked in chili together with anchovies and ground nuts
Firm tofu fried in chili (FTC)	Firm tofu	Firm tofu fried in oil, then cooked in chili
Sambal goreng Jawa (SJ)	*Tempe*, firm tofu, fu jook	*Tempe* and firm tofu fried in oil, then sautéed with fu jook, *soo-hoon*, vegetables and potato
Masak lodeh (ML)	*Tempe*, firm tofu, fu jook	*Tempe*, firm tofu and fu jook boiled in coconut milk together with vegetables
Soft tofu steamed with soy paste (STS)	Soft tofu	Soft tofu steamed with carrot in soy paste sauce
Egg tofu stir-fried with mustard (ETM)	Egg tofu	Egg tofu fried in oil, then stir-fried with green mustard
Egg tofu soup (ETS)	Egg tofu	Egg tofu fried in oil, then cooked in soup together with vegetables

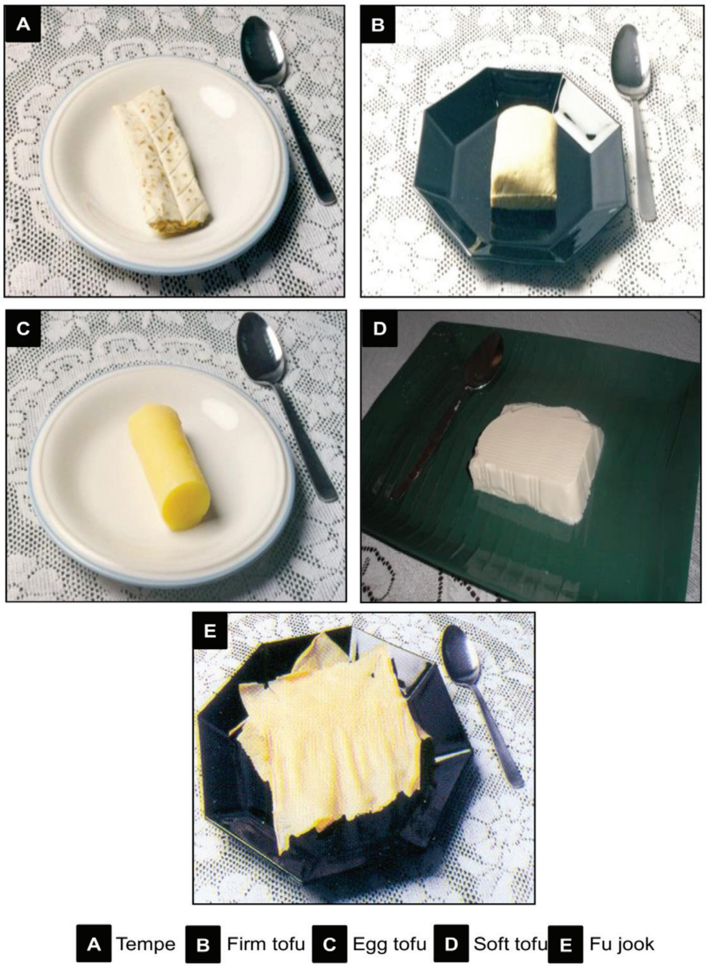

FIGURE 1. Soy-based products used in preparation of local dishes

TABLE 2. Cooking methods, time and temperature of dishes preparations

Dish (Soy product)	Cooking method	Cooking period (min)	Cooking temperature (°C)
ETS (Egg *tofu*)	Fried	10	88
ETM (Egg *tofu*)	Fried	10	90
FTC (Firm *tofu*)	Fried	12	94
TC (*Tempe*)	Fried	14	70
TSS (*Tempe*)	Fried	15	80
SJ (*Tempe*)	Fried	17	75
SJ (Firm tofu)	Fried	16	80
SJ (Fu jook)	Fried	5	50
ML (*Tempe*)	Boiled	10	80
ML (Firm tofu)	Boiled	10	80
ML (Fu jook)	Boiled	10	80
STS (Soft tofu)	Steamed	15	60

main ingredients. The increasing amount of aglycone and glycoside isofavones content in fermented soy products may be caused by the change of malonyl glucoside compound to glucoside during boiling and steaming processes (Yamabe et al. 2007). Glucoside compound can be hydrolyzed into aglycone by β-glukosidase during fermentation or senescence. This explained the higher isoflavone contents in *tempe* compared to other soy products in this study.

The total isoflavone contents were the sum of Da and Ge content. Total isoflavone content in raw *tempe* in three dishes were among the highest based on wet weight. The isoflavone content of *tempe* in these dishes were TSS (111.36 ± 1.75 mg/100 g), TC (102.95 ± 3.22 mg/100 g) and

TABLE 3. Daidzien (Da), Genistein (Ge) and total isoflavone contents in raw and cooked soy products in different soy-based dishes (based on wet weight)

Soy product sample (dishes)	Isoflavone contents (mg/100 g)					
	Raw soy product			Cooked soy product		
	Da	Ge	Total	Da	Ge	Total
Tempe (TC)	37.90 ± 1.20	65.05 ± 2.02	102.95 ± 3.22[a]	46.38 ± 0.12	66.89 ± 0.43	113.26 ± 0.55[b]
Tempe (TSS)	41.00 ± 0.49	70.36 ± 1.25	111.36 ± 1.75[a,b]	61.45 ± 1.38	73.17 ± 0.80	134.66 ± 2.18[a]
Tempe (SJ)	21.51 ± 0.54	39.33 ± 1.04	60.84 ± 1.58[c]	31.17 ± 0.21	37.30 ± 0.00	68.47 ± 0.21[c]
Firm tofu (SJ)	18.17 ± 0.48	32.59 ± 0.94	50.76 ± 1.42[d]	32.94 ± 2.40	39.17 ± 1.39	72.11 ± 3.79[c]
Fu jook (SJ)	14.71 ± 0.57	32.38 ± 1.48	47.08 ± 2.05[d,e]	42.22 ± 6.10	33.44 ± 1.42	75.65 ± 7.52[c]
Tempe (ML)	34.93 ± 0.76	60.73 ± 1.45	95.66 ± 2.21[b]	29.33 ± 0.64	40.53 ± 0.78	69.85 ± 1.43[c]
Firm tofu (ML)	15.01 ± 0.46	25.69 ± 0.88	40.70 ± 1.34[e,f]	21.28 ± 1.45	24.39 ± 0.99	45.67 ± 2.44[d]
Fu jook (ML)	14.43 ± 0.23	32.27 ± 0.64	46.70 ± 0.86[d,e]	18.78 ± 1.31	20.90 ± 0.38	39.66 ± 0.93[d]
Firm tofu (FTC)	13.52 ± 0.08	21.76 ± 0.15	35.28 ± 0.23[f]	23.40 ± 0.35	25.96 ± 0.25	49.36 ± 0.60[d]
Soft tofu (STS)	11.51 ± 1.75	8.00 ± 0.73	19.51 ± 2.48[g]	13.29 ± 3.34	7.47 ± 1.15	20.75 ± 4.50[e]
Egg tofu (ETS)	6.23 ± 0.47	4.35 ± 0.33	10.58 ± 0.80[g]	7.62 ± 1.68	3.98 ± 0.56	11.59 ± 2.23[e]
Egg tofu (ETM)	7.01 ± 0.57	4.86 ± 0.21	11.86 ± 0.78[g]	12.98 ± 1.59	5.74 ± 0.34	18.72 ± 1.93[e]

Superscripts of different letters in the same column represent significant difference (p<0.05) using Tukey post-hoc test

ML (95.66 ± 2.21 mg/100 g). The lowest total isoflavone content was demonstrated in egg tofu in ETS (10.58 ± 0.80 mg/100 g) and ETM (11.86 ± 0.78 mg/100 g). There was no significant difference (p>0.05) between the isoflavone content in both egg tofu in ETS and ETM. However, there were significant differences (p<0.05) between egg tofu (in ETS and ETM) as compared to other soy-based products. The most remarkable difference was observed in *tempe* used in SJ, ML, TC and TSS dishes, with isoflavone content ranged 60.84 - 111.36 mg/100 g as compared to egg tofu. As for cooked soy-based products, *tempe* from TSS dish still has the highest isoflavone content (134.66 ± 2.18 mg/100 g) based on wet weight, followed by isoflavone content in *tempe* from TC (113.26 ± 0.55 mg/100 g). Whilst, the lowest isoflavone content was found in egg tofu in ETS (11.59 ± 2.23 mg/100 g) and ETM (18.72 ± 1.93 mg/100 g) based on wet weight.

DIFFERENCES OF ISOFLAVONE CONTENT IN RAW AND COOKED SOY PRODUCTS

The effect of cooking on isoflavone content in the studied soy-based products can be seen using differences of isoflavone contents before and after cooking. The differences of isoflavone content was calculated based on dry weight since water content may differ from one dish to another, thus affecting the isoflavone content. Table 4 shows the percentage of the isoflavone content differences in soy-based products according to their respective dishes. There were 9 out of 12 samples that showed isoflavone content was found to reduce from 0.32 to 37.56% after cooking. The percentage of reduction was most apparent in *tempe* samples in TSS (37.56%) and TC (37.03%). Besides, fu jook from ML, firm tofu from FTC and *tempe* from SJ also exhibited relative reductions of 31.88%, 28.88% and 27.98%, respectively. However, the reductions of isoflavone content were only significant (p<0.05) in *tempe* (TSS and ML), fu jook (ML) and firm tofu (FTC). The percentage of reduction in total isoflavone content of *tempe* in this study was lower than that deep fried *tempe* (45%) as reported by Hasnah et al. (2009) but higher than the value in another study (Hutabarat et al. 2001) with 30% reduction in pan fried tempe.

There were three samples which showed increament of isoflavone content; soft tofu in STS (-3.47%), egg tofu in ETS (-3.74%) and egg tofu in ETM (-27.57%) dishes. The elevation of isoflavone content after cooking process was probably due to other ingredients used in preparation of dishes such as soy paste in soft tofu steamed with soy paste (STS). Soy paste is made from soy bean preserved in high concentration of salt, thus it also contains isoflavone.

Apart from cooking, raw ingredients used during formulation, method and processing techniques, product storage and distribution also contribute to the compositional differences of isoflavone in soy-based products. Shimoni (2004) reported the changes of isoflavone content might happen at stage of raw materials storage. Kim et al. (2005) reported that reduction of isoflavone content in soy bean happens for prolonged storage at room temperature. Besides processing method, heat treatment, enzymatic action, growth climate and condition as well as genetic factor of soy bean also have impacts on the isoflavone content in soy bean and ultimately the soy-based products (Barbosa et al. 2006; Jackson et al. 2002).

Non significant (p>0.05) positive correlations between isoflavone content and cooking method (r=0.268) as well as cooking period (r=0.143). There was also non-significant (p>0.05) negative correlation established between isoflavone content and cooking temperature (r=-0.263). A significant (p<0.05) loss of isoflavone content was observed specifically in *tempe* of TSS and TC dishes which involved frying method.

TABLE 4. Percentage of differences of total isoflavone content in soy-based products due to cooking processes (based on dry weight)

Soy-based product sample (Dishes)	Total isoflavone in raw soy-based products (mg/100 g)	Total isoflavone in cooked soy-based products (mg/100 g)	Difference in total isoflavone content (mg/100 g)	Percentage of difference (%)
Tempe (TC)	287.56 ± 9.01	181.07± 0.88	106.49	37.03
Tempe (TSS)	310.27 ± 4.88	193.73 ± 3.14**	116.54	37.56
Tempe (SJ)	175.35 ± 4.54	126.29 ± 0.38	49.06	27.98
Firm tofu (SJ)	216.89 ± 6.11	185.89 ± 9.77	31.00	14.29
Fu jook (SJ)	144.59 ± 6.29	144.12 ± 14.32	0.47	0.32
Tempe (ML)	278.56 ± 6.45	232.46 ± 4.75*	46.10	16.55
Firm tofu (ML)	177.94 ± 5.83	159.23 ± 8.52	18.71	10.52
Fu jook (ML)	161.45 ± 2.98	109.97 ± 2.58**	51.47	31.88
Firm tofu (FTC)	150.89 ± 1.00	107.31 ± 1.32**	43.58	28.88
Soft tofu (STS)	122.68 ± 15.58	126.93 ± 27.51	-4.26	-3.47
Egg tofu (ETS)	60.24 ± 4.54	62.49 ± 12.02	-2.25	-3.74
Egg tofu (ETM)	63.15 ± 4.18	80.56 ± 8.31	-17.41	-27.57

Significant difference with *$p<0.05$ and **$p<0.01$ between raw and cooked soy-based products using paired t test

CONCLUSION

Tempe is an excellent source of isoflavone compared to other local soy-based products studied. A considerable loss of isoflavone content in dishes that involved frying method. A low cooking temperature with optimum cooking period might be suitable for retention of isoflavone in soy-based products. An in-depth study in looking for the best cooking method to reduce isoflavone loss should be carried out in future.

ACKNOWLEDGEMENTS

The authors would like to thank Nutritional Sciences Programme at Faculty of Health Sciences, Universiti Kebangsaan Malaysia for the use of Food Analysis Laboratory for this study. The authors would like to acknowledge Universiti Kebangsaan Malaysia for the INDUSTRI-2012-008 grant used to support this study.

REFERENCES

Aguiar, C.R., Haddad, R., Eberlin, M.N., Carrão-Panizzi, M.C., Tsai, S. & Park, Y.K. 2012. Thermal behavior of malonylglucoside isoflavones in soybean flour analyzed by RPHPLC/DAD and eletrospray ionization mass spectrometry. *LWT - Food Science and Technology* 48: 114-119.

Albertazzi, P. & Purdie, D.W. 2008. Reprint of the nature and utility of the phytoestrogens: a review of the evidence. *Maturitas* 61(1-2): 214-229.

Barbosa, A.C.L., Lajolo, F.M. & Genovese, M.I. 2006. Influence of temperature, pH and ionic strength on the production of isoflavone-rich soy protein isolates. *Food Chemistry* 98: 757-766.

Chung, I.M., Seo, S.H., Ahn, J.K. & Kim, S.H. 2011. Effect of processing, fermentation, and aging treatment to content and profile of phenolic compounds in soybean seed, soy curd and soy paste. *Food Chemistry* 127: 960-967.

Ferreira, M.P., de Oliveira, M.C.N., Mandarino, J.M.G., da Silva, J.B., Ida, E.I. & Panizzi, M.C.C. 2011. Changes in the isoflavone profile and in the chemical composition of tempeh during processing and refrigeration. *Pesq. Agropec. Bras., Brasília* 46(11): 1555-1561.

Hasnah, H., Suzana, S., Kimberley, O.B., Amin, I., Norazmi, K. & Suriah, A.R. 2010. Absorption of calcium from milk and tempeh consumed by postmenopausal Malay women using the dual stable isotope technique. *International Journal of Food Sciences and Nutrition* 61(2): 125-137.

Hasnah, H., Amin, I., Azrina, A., Suzana, S. & Loh, S.P. 2009. Daidzein and genestein contents in tempeh and selected soy products. *Food Chemistry* 115: 1350-1356.

Hui, E., Henning, S.M., Park, N., Heber, D. & Go, V.L.W. 2001. Genistein and daidzein/glycitein content in tofu. *Journal of Food Composition and Analysis* 14: 199-206.

Hutabarat, L.S., Greenfield, H. & Mulholland, M. 2001. Isoflavones and coumestrol in soybeans and soybean products from Australia and Indonesia. *Journal of Food Composition and Analysis* 14: 43-58.

Hutabarat, L.S., Mulholland, M. & Greenfield, H. 1998. Development and validation of an isocratic high-performance liquid chromatography method for quantitative determination of phytoestrogens in soya beans. *Journal of Chromatography A* 795: 377-382.

Jackson, C.J.C., Dini, J.P., Lavandier, C., Rupasingh, H.P.V., Faulkner, H., Poysa, V., Buzzell, D. & DeGrandis, S. 2002. Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. *Process Biochemistry* 37: 1117-1123.

Jooyandeh, H. 2011. Soy products as healthy and functional foods. *Middle-east Journal of Scientific Research* 7(1): 71-80.

Kim, J.J., Kim, S.H., Hahn, S.J. & Chung, I.M. 2005. Changing soybean isoflavone composition and concentrations under two different storage conditions over three years. *Food Research International* 38: 435-444.

Lee, S.W & Lee, J.H. 2009. Effects of oven-drying, roasting, and explosive puffing process on isoflavone distributions in soybeans. *Food Chemistry* 112: 316-320.

Maskarinec, G. 2005. Soy foods for breast cancer survivors and women at high risk for breast cancer. *Journal of the American Dietetic Association* 105(10): 1524-1528.

Nagata, Y., Sonoda, T., Mori, M., Miyanaga, N., Okumura, K., Goto, K., Naito, S., Fujimoto, K., Hirao, Y., Takahashi, A., Tsukamoto, T. & Akaza, H. 2007. Dietary isoflavones may protect against prostate cancer in Japanese men. *The Journal of Nutrition* 137(8): 1974-1979.

Nakajima, N., Nozaki, N., Ishihara, K., Ishikawa, A. & Tsuji, H. 2005. Analysis of isoflavone content in tempeh, a fermented soybean, and preparation of a new isoflavone-enriched tempeh. *Journal of Bioscience and Bioengineering* 100(6): 685-687.

Omoni, A.O. & Aluko, R.E. 2005. Soybean foods and their benefits: Potential mechanisms of action. *Nutrition Reviews* 63(8): 272-283.

Penas, E., Gomez, R., Frias, J., Baeza, M.L. & Vidal-Valverde, C. 2011. High hydrostatic pressure effects on immunoreactivity and nutritional quality of soybean products. *Food Chemistry* 125: 423-429.

Shimoni, E. 2004. Stability and shelf life of bioactive compounds during food processing and storage: soy isoflavones. *Journal of Food Science* 69(6): 160-166.

Shu, X.O., Jin, F., Dai, Q., Wen, W., Potter, J.D., Kushi, L.H., Ruan, Z., Gao, Y.T. & Zheng, W. 2001. Soyfood intake during adolescence and subsequent risk of breast cancer among Chinese women. *Cancer Epidemiology Biomarkers & Prevention* 10(5): 483-488.

Tan, M.C. 2011. Penentuan kandungan isoflavon dan perencatan fitat terhadap bioavailabiliti kalsium, besi dan zink dalam produk kacang soya tempatan terpilih. Tesis Sarjana Muda Sains Pemakanan, Universiti Kebangsaan Malaysia (Unpublished).

Welty, F.K., Lee, K.S., Lew, N.S. & Zhou, J.R. 2007. Effect of soy nuts on blood pressure and lipid levels in hypertensive, prehypertensive, and normotensive postmenopausal women. *Archives of Internal Medicine* 167(10): 1060-1067.

Wong, M.C.Y., Emery, P.W., Preedy, V.R. & Wiseman, H. 2008. Health benefits of isoflavones in functional foods? Proteomic and metabonomic advances. *Inflammopharmacology* 16: 235-239.

Yamabe, S., Kobayashi-Hattori, K., Kaneko, K., Endo, H. & Takita, T. 2007. Effect of soybean varieties on the content and composition of isoflavone in rice-koji miso. *Food Chemistry* 100: 369-374.

Nutritional Sciences Programme
School of Healthcare Sciences
Faculty of Health Sciences
Universiti Kebangsaan Malaysia
Jalan Raja Muda Abdul Aziz
50300 Kuala Lumpur, Federal Territory
Malaysia

*Corresponding author; email: hasnaharon@ukm.edu.my

Received: 8 December 2015
Accepted: 27 April 2016