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Contributed Paper

Polyphenol Contents, Antioxidant and Anticancer Activity (MCF-7) of Soybean Products in Thailand

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ABSTRACT

This investigation aimed to assess the phytochemical properties of processed soybeans (steamed soybean, soymilk and tofu) from three Thai cultivars, which included the total phenol content (TPC), total flavonoid content (TFC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and β- carotene bleaching activity, the anti-carcinogenic property in the MCF-7 cell line and genistin content were also measured. The three Thai soybean cultivars were Chiang Mai 6 (CM6), Chiang Mai 60 (CM60) and new cultivar Chiang Mai 84-2 (CM 84-2). The levels of TPC and TFC were in the ranges of 60.02 - 70.47 mg GAE/100g dry weight and 21.90 - 39.84 mg CE/100g dry weight, respectively. The tofu produced from all cultivars exhibited significantly higher antioxidant activity in the DPPH assay than the other products. The soymilk of all cultivars had the greatest FRAP values, while 3 products of CM 84-2 had the highest percentage inhibition in the β-carotene bleaching assay regardless of the soybean product type. Moreover, almost soybean products showed low anticancer activity, which was more significant in tofu and steamed soybean CM6 samples and inactive in tofu from CM 60 and CM 84-2. The genistin levels in soybean products were estimated to be between 3.43 and 32.02 µg/g. Therefore, the phytochemical properties of soybean depended upon the different Thai cultivars and type of postharvest processing.

Keywords: Soybean products, Antioxidant activity, Anticancer, Genistin

1. INTRODUCTION

Plant phenolic compounds are a class of phytochemicals that have health-promoting properties. Phenolic compounds may be antioxidants, which help to decrease atherosclerosis, coronary heart disease and especially some forms of cancer [1]. A number of epidemiological studies have suggested that cancer is inversely correlated to the consumption of soybean products [2]. Some studies reported that certain ingredients of soybean, such as saponin, phytate and isoflavone, are protective against oxidative stress [3].

Soybean (*Glycine max* L. Merrill) is an important foodstuff, particularly in traditional cooking by Southeast Asian people, widely

consumed as tofu and soy milk [4]. In Thailand soybean is widely cultivated with an area of 25, 257, 600 square meters; there are about 14 soybean cultivars that have different properties, such as physical, chemical and nutritional values [5]. Chiang Mai 6 (CM6) and Chiang Mai (CM60) are popular soybean cultivars in Thailand, while Chiang Mai 84-2 (CM84-2) is a new cultivar released in 2012. In Thailand, soybean is used to prepare several soy products, such as soymilk and tofu, and the consumption of soy foods has been associated with beneficial health effects [5]. Soy isoflavones (such as daidzein, genistein and its β-gluoside conjugate, genistin) are important phytochemicals for the prevention of cancers [6].

When soybeans are heated during processing, their functional properties can be altered. In previous studies, the naturally occurring antioxidant activity and isoflavone content were lost as a consequence of heating [7, 8]. While on the contrary, some research showed that heat treatment increased the antioxidant capacity and total phenolic content, including the isoflavone content sometimes [9, 10]. Thus, the objective of this work was to evaluate the phenolic compound content, antioxidant activity, anticancer activity and isoflavone content (genistin) of selected soy products, including steamed soybean, soymilk and tofu, made from three different Thai soybean cultivars. According to the literature, this is probably the first study where the antioxidant activity and antioxidant compound genistin were measured from different soybean cultivars produced in Thailand, as most of the previous research focused on genistein.

2. MATERIALS AND METHODS

2.1 Plant Material and Chemicals

Soybean (*G. max*) seeds were acquired from the Crop Research Center in Chiang Mai, Thailand. Three soybeans were selected, CM6, CM60 and CM84-2 (which is a new soybean in Thailand).

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-Striazine (TPTZ), Folin-Ciocalteu reagent and gallic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA), while Genistin and Catechin came from Fluka (Neu-Ulm, Germany). All chemicals and reagents used in the study were of analytical grade.

2.2 Sample Preparation

The steamed soybean, soymilk and tofu processing were performed according to Cai et al. [11], with some modification, and each cultivar's processing was performed in two replicates. All soybeans were soaked in water (six times the soybeans w/w) for nine hours at 20-22°C, and then divided into three portions (300g/part: two replicates). Portion 1: *Steamed soybean* - this was steamed at 100°C for 30 min. Portion 2: *Soymilk* - these soaked beans were ground with water (12:1 w/w) at high speed in a blender for 4 min. After grinding, the slurry was filtered with a white cloth might not be suitable as cheese cloth and squeezed manually to obtain the filtrate, and then it was heated at 94 - 96°C for 1 h. Portion 3: *Tofu* - in this the soaked beans were ground with a blender at high speed for 4 min, and water was added at a ratio of 6:1 (w/w). The product was then filtered to separate the soy cake from the soymilk; after which the soymilk was cooked and maintained at 94 - 96°C for 5 min. Once the soymilk had cooled to 87°C, calcium sulfate ($CaSO_4.2H_2O$ at 2% of raw soybean weight) dissolved in 20 ml of water was added. The mixture was left to stand for 8 min, after which the tofu was cooled and stored at 4°C. All samples were freeze-dried prior to storage at -20°C.

The dried ground sample was extracted with 80% methanol (1:10, w/v) for six hours

in a dark room at room temperature with shaking at 200 rpm. The extracts were filtered using Whatman No. 1 paper and centrifuged at 3,000 rpm 15 min. The filtrate was evaporated in a water bath at 50°C to a final volume of 1 ml. The evaporated residues were used for the analysis.

2.3 Total Phenolic Content (TPC) Determination

TPC was determined by Folin-Ciocalteu with modifications described by Nsimba *et al.* [12]. Gallic acid was used as the reference standard, and the results were expressed as mg gallic acid equivalents in 100 g of dried sample (mg GAE/100 g dry weight).

2.4 Total Flavonoid Content TFC Determination

The TFC was determined using the colorimetric method described by Abu Bakar et al. [13] as modified from Dewanto et al. [14]. Results were expressed as mg catechin equivalents in 100 g of dried sample (mg CE/ 100 g dry weight).

2.5 Antioxidant Activity Determination

- DPPH radical scavenging activity

The radical scavenging activity was determined using a modification of the stable DPPH method of Brand-Williams et al. [15]. In brief, the samples (0.2 ml) were mixed with a 0.20 mM DPPH ethanol solution for 2 ml. After incubation at room temperature in the dark for 30 min, the mixture was measured at the absorbance of 517 nm using a spectrophotometer. The standard curve was linear between 0.08 and 0.64 mM Trolox. The radical scavenging activity was calculated as a percentage of DPPH scavenging activity using the equation: % scavenging activity = $100 \times [1- (A_E/A_D)]$, where A_E is the absorbance of the DPPH solution with an extract added and A_D is the absorbance of the DPPH solution with nothing added [16].

- Ferric reducing antioxidant power (FRAP) assay

FRAP assay was employed to measure the antioxidant activity of the soybean products, and according to Benzie and Strain [17], the method modified. The fresh working FRAP solution was prepared by mixing 10 ml acetate buffer, 1 ml TPTZ solution, and 1 ml $FeCl_3.6H_2O$ solution and then warming the solution to 37°C before use. The samples (0.15 ml) were allowed to react with of the FRAP solution (2.85 ml) for 30 min in the dark. Spectrophotometry readings of the coloured product (ferrous tripyridyltriazine complex) were then taken at 593 nm. In the FRAP assay, the antioxidant potential of the sample was determined from a standard curve plotted using the $FeSO_4.7H_2O$ linear regression equation to calculate the FRAP values of the sample.

- β-Carotene bleaching activity

Determination of antioxidant activity as the ability to delay the bleaching of β-carotene in a water/linoleic acid emulsion was performed according to developed by Wettasinghe and Shahidi [18] cited by Nsimba et al. [12]. Antioxidant activity is expressed as the ability to delay the bleaching of a β-carotene/linoleic acid emulsion. BHT was used as the positive control. The inhibitory percentage was calculated as $[1 - (A_0 - A_t)/(A^0_0 - A^t_0)] \times 100$), where A_0 and A^0_0 are the absorbance values measured at the initial time of the incubation for the sample and control respectively, and A_t and A_t^0 are the absorbance values of the sample and control at t (105) minutes, respectively.

2.6 Anticancer Activity Determination

The anticancer activity of the extracted samples in a breast adenocarcinoma (MCF-7 cell) was determined using the method of

O'Brien et al. [19]. This assay is performed in triplicate wells of 384-well plate. Each well is added with 5 µl of test compound and 45 µl of cell suspension, to obtain 1,500 cells/well. Plate is then incubated at 37°C in a humidified incubator with 5% CO_2 for 3 days. After that, 12.5 µl of sample or positive control is filled to each well and the plate is further incubated for 4 hours. Fluorescence is measured at 530 nm excitation and 590 nm emission wavelengths by using the bottom-reading mode of fluorometer. The positive control was 8.42 µg/ml tamoxifen and 7.24 µg/ml doxorubicine, while DMSO was used as a negative control. The percentage of growth inhibition is calculated by the following equation: % Inhibition = [1- (FUT/ FUC)] ×100, where FUT and FUC represent the fluorescence units of cells treated with test compound and negative control agent, respectively.

2.7 Soy Isoflavone Content Determination

Determination of the soy isoflavone content was performed according to the method of Niamnuy et al. [20]. A high-performance liquid chromatograph (HPLC) equipped with a photodiode array (PDA) detector (Shimadzu, SPD-M20A, Kyoto, Japan) was used. A Phenominex C_{18} column (250 × 4.6 mm; 5-µm) (GL Sciences, Inc., Tokyo, Japan) was used for the separation of isoflavone. The mobile phase was a mixture of 0.1% acetic acid in acetonitrile and 0.1% glacial acetic acid in distilled water at a total flow rate of 1.0 ml/min. The quantitation limit was 0.99 µg/g for genistin. The determination was performed in triplicate on the sample.

2.8 Statistical Analysis

The antioxidant activities data derived from this study were analyzed statistically. The results are expressed as means ± standard deviation (SD). The statistical significance was determined by one-way ANOVA, in which *p* values of less than 0.05 were assumed to be statistically significant.

3. RESULTS AND DISCUSSION

3.1 TPC & TFC

The TPC of the three soybean products from the different cultivars are presented in Table 1. In 100 grams of soybean product extract, the TPC levels were in the range of 60.02 - 70.47 mg GAE/100g dry weight. The soymilk of CM 84-2 showed the highest TPC, while the tofu of CM 84-2 had the lowest content. In particular, for the tofu from the soybeans, the cultivar variation for TPC was significant ($p < 0.05$). All the soymilk had greater TPC than the steamed soybean and tofu.

The TFC of the soybean product extracts are present in Table 1, and they ranged from 21.90 - 39.84 mg CE/100g dry weight. The soymilk of CM 6 contained the greatest TFC among all the products. Only soymilk showed significant ($p < 0.05$) differences between cultivars. Interestingly, soymilk products also showed the greatest TFC.

The results indicated that the different processing affected the TPC and TFC of soy product. Our finding correlates with result Somdee et al. [21], who showed that the TPC and TFC of the cooked edible leaves of 30 vegetables increased or decreased depend on the type of vegetable. In addition, there are many factors related to phenolic and flavonoid contents, such as the genotype, agronomic practices, maturity level at harvest, post-harvest storage and climate [22, 23]. In particular, each soybean has different properties, of which the first that can be noticed is the difference in size. Therefore, different sizes may affect the TPC and TFC values of the soybean products. Sapbamrer et al. [5] found that the raw soybeans of 13

cultivars from Thailand had different isoflavone contents (classes of phenolic compounds), but this has not been studied in detail yet.

Table 1. Antioxidant compounds and antioxidant activity of soy products from three different cultivars.

Soy product	Cultivar name	Antioxidant compound		Antioxidant activity		
		TPC (mgGAE/100g dry weight)	TFC (mgCE/100g dry weight)	DPPH (scavenging activity,%)	FRAP (mmol $FeSO_4$/100 g dry weight)	β-carotene bleaching activity (Inhibition, %)
Steamed soybean	CM 6	63.52 ± 9.97	21.90 ± 2.88	46.18 ± 3.31*	718.12 ± 3.85*	49.31 ± 7.91*
	CM 60	60.65 ± 1.42	22.43 ± 2.01	46.36 ± 1.20*	812.56 ± 5.10*	30.06 ± 7.08*
	CM 84-2	63.92 ± 4.18	27.18 ± 3.68	28.77 ± 3.82*	758.12 ± 8.39*	57.34 ± 4.80$^{*\#}$
Soymilk	CM 6	68.84 ± 4.60$^{\#}$	39.84 ± 5.11$^{*\#}$	67.62 ± 1.33*	880.34 ± 5.78$^{*\#}$	25.35 ± 1.90*
	CM 60	69.24 ± 3.80$^{\#}$	29.14 ± 0.56$^{*\#}$	63.06 ± 1.77*	1602.56 ± 8.39$^{*\#}$	38.50 ± 9.26*
	CM 84-2	70.47 ± 7.67$^{\#}$	33.44 ± 2.50$^{*\#}$	59.10 ± 0.18*	982.56 ± 3.85$^{*\#}$	46.54 ± 8.30$^{*\#}$
Tofu	CM 6	64.74 ± 1.42*	23.94 ± 1.59	72.48 ± 0.73$^{*\#}$	514.78 ± 8.39*	31.16 ± 2.31*
	CM 60	67.61 ± 2.80*	27.63 ± 4.57	70.49 ± 1.88$^{*\#}$	798.12 ± 5.10*	18.70 ± 2.08*
	CM 84-2	60.02 ± 2.64*	33.28 ± 0.60	67.94 ± 0.46$^{*\#}$	581.45 ± 9.62*	69.40 ± 9.80$^{*\#}$

*Significant differences at $p < 0.05$ for one-way ANOVA comparing cultivar values expressed as mean ± SD of triplicate measurements.

$^{\#}$ Significant differences at $p < 0.05$ for one-way ANOVA comparing soy product values expressed as mean ± SD of triplicate measurements.

3.2 Antioxidant Activity

The results showed that the DPPH scavenging activity of the soybean extracts was in a range from 28.77 - 72.48 %. The highest percentage inhibition was obtained from the tofu of CM 6, while the steamed soybean of CM 84-2 exhibited the lowest percentage inhibition (Table 1). Tofu and soymilk from CM6 and steamed soybean from CM60 showed higher antioxidant activity measured by DPPH.

The results are displayed in Table 1. FRAP values were in the range of 514.78 - 1602.56 mmol $FeSO_4$/100 g dry weight. The soymilk of CM 60 had the highest FRAP value, while the tofu of CM 6 had the lowest FRAP value. The antioxidant activities of those products were statistically significant in comparison to the other soybean products and cultivars ($p < 0.05$).

The results of β-carotene bleaching method are summarized in Table 1. Stronger activity is indicated by greater inhibitory percentage. The extract of the tofu from CM 84-2 exhibited the greatest level of antioxidant activity with an inhibition of 69.40%, followed by 57.34% of the steamed soybean from CM 84-2 . Significant differences ($p < 0.05$) existed among the different soybean products from each cultivar. Interestingly, when comparing 3 cultivars, CM 84-2 gave products that had the strongest antioxidant activity.

The result showed that all Thai soybean products displayed antioxidant activity, in which they act as antioxidants as well as scavengers of free radicals ($DPPH^{\bullet}$), transfer electrons (FRAP) or delay the bleaching of β-carotene.

3.3 Anticancer Activity

Treatment with soybean products at various concentrations had no influence on cell proliferation and caused no cell toxicity at 1 - 100 μg/ml (data not shown). However, the concentration of 250 μg/ml showed cell toxicity. Thus, almost products showed a low level of anticancer activity, except the tofu of CM 60 and CM 84-2, which were inactive (Table 2). The tofu of CM 6 was found to have the greatest inhibitory percentage inhibition (18.87%), followed by the steamed soybean of CM 6. Thus, this result showed that CM 6 has anticancer activity, but only at a low level.

Many researchers have shown that soy products had a growth-inhibitory effect on MCF-7 cells [24], which are a human breast carcinoma cell line. This study of the phenolic compounds from Thai soybean products may effectively be related to the anticancer activity, in that isoflavones, such as genistein, daidzein, genistin and daidzin, which are the phenolic components in soybean products, induce the generation of reactive oxygen species (ROS). ROS production was probably the cause of this apoptotic cell death [24].

Table 2. Anticancer activity (MCF7-breast cancer) and genistin content of soy products from three different.

Soy product	Cultivar name	Inhibition of cell growth (%)	Genistin content (μg/g)
Steamed soybean	CM 6	17.92±1.24	32.02±2.56*
	CM 60	3.89±0.46	14.07±1.04*
	CM 84-2	2.67±0.66	17.69±1.14*
Soymilk	CM 6	4.11±0.81	13.70±0.88
	CM 60	6.74±1.07	13.34±0.28
	CM 84-2	1.19±0.52	13.90±1.36
Tofu	CM 6	18.87±2.22	6.55±1.74*
	CM 60	Inactive	3.43±1.09*
	CM 84-2	Inactive	6.15 ±1.05*

*Significant differences at $\alpha < 0.05$ for one-way ANOVA comparing cultivar values expressed as mean ± SD of triplicate measurements.

3.4 Genistin Content

Genistin is a β-glucoside conjugate of genistein in soybean. The isoflavone contents, as genistin, of different soybean products and cultivars are presented in Table 2. The amounts of genistin in soybean products were estimated to be between 3.43 and 32.02 μg/g. When comparing different cultivars, the steamed soybean and tofu had significant differences ($p < 0.05$). The genistin levels were lower in soymilk and tofu. As genistin is considered to be heat stable, it is probably lost during the production of soy milk and tofu mainly in the filtration steps [25].

Coldham et al. [26] and Singh et al. [27] revealed that genistein is major soy isoflavones compounded and its biological activity was absorbed in intestine, while genistin is the natural form of genistein. Eventhough Choi et al. [28] showed genistin induced apoptosis and inhibit cell proliferation by disrupting the cancer cell cycle.

The results of this study suggested that antioxidant compounds, antioxidant activities, anticancer activity and genistin content in the soybean products of the three cultivars were affected by genetic factors and methods of soy product preparation. This finding corresponded to the studies of Sapbamrer et al [5] who showed that besides genetics, environmental conditions and geographical location during cultivation also affected to those properties of soy products [29, 30].

4. CONCLUSION

In conclusion, the results from the soybean products from the three Thai cultivars indicate that they are a sources of phytochemicals and have antioxidant activities, including anticancer activity. Soy milk exhibited greater antioxidant compound content and antioxidant activity. The anticancer activity of tofu and steamed soybean in CM 6 demonstrated greater efficiency than the

other cultivars. This study showed significant relationship between breast cancer and isoflavones is complex as other studies explained positive effects and negative. Therefore the genistin level found depending respond on not only itself but several factors still conflicting related to other isoflavones food and individual mechanismconditions.

In addition, this study offers useful recommendations for consumers select soybean products and cultivars that are sources of phytochemicals in defense of free radicals or cancer. The next focus study could be performed in human colon carcinoma cell line because soybean products have been used as foodstuff.

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