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Nutritional Quality of Fermented Soy Foods in Thailand

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Abstract

Soybean has been favored by many Thai people and it has been prepared by numerous different methods. Collected samples are as follows: Thua-nao paste from Chiangrai province, dried Thua-nao for Jatujak Market, Bangkok, 3 types of commercial soybean paste, soybean sauce and 2 types of fermented soybean curd cakes with other ingredients. Moisture contents of fresh and dried Thua-nao were 68.5 and 7.6%, respectively; therefore the shelf-life of dried Thua-nao can be extended to 1 year with proper packaging. The remainder of the soy foods had moisture contents of 55.4 to 64.4%. Fat contents of fresh and dried Thua-nao were 7.4 and 19.7%, respectively, whereas other samples contained less than 3%. Dried Thua-nao had the highest CHO (carbohydrates) content (37.4%); in contrast, soybean sauce contained only 4.5%. Calcium content was highest in dried Thua-nao followed by fresh Thua-nao; the other fermented soy foods had less than 44.7 mg/100 g. Salt was added to samples other than Thua-nao resulting in high Na contents. Free and total daidzein contents of dried Thua-nao were 355 and 676 ug/g; similarly free and total genistein contents were 293 and 616.5 μg/g, respectively.

Key words: fermented soy foods, daidzein, genistein, Thailand

INTRODUCTION

Soybeans and processed soy products are well established as health-promoting functional foods, and have been used for centuries in the orient. The carbohydrates in soybean are largely polysaccharides and indigestible fiber, which protect against diseases of the lower gastrointestinal tract. Soybeans are also rich in proteins composed of essential amino acids (1). Several clinical studies have shown that soybean consumption can decrease the risk of esophageal, breast, prostate, skin, liver, and colon cancers. These effects may be medicated by the antioxidative properties of soybean and their ability to block tumor promotion (2).

Phytoestrogens are plant components (diphenols) that have a wide range of biological effects, many related to the close structural relationships between phytoestrogens and endogenous reproductive hormones such as estrogens. Isoflavones are found in legumes and are most highly concentrated in soybeans and soy products. Traditional soy foods, such as soy beverages, tofu, tempeh, and miso, are consumed frequently in Asian countries and increasingly in Western countries. Soybeans contain three types of isoflavones, as four chemical forms (aglycone form, β-glucoside form, malonylglucoside form, and acetylglucoside form): the aglycones being daidzein, genistein, and glycitein (3-5). Daidzein and genistein, the predominant isoflavones in soybeans, are usually found as glycosides. There have been reports on the content of genistein and daidzein and their glycosides in a few soybean varieties and soy foods; moreover, the effects of processing on these compounds were evaluated (6). Some studies have suggested that consumption of 1.5~2.0 mg/kg/d of isoflavones is needed to reduce cancer risk (7).

To evaluate the potential of the isoflavones as dietary anticarcinogens, the amounts available in typical soy foods and soybeans must be quantified. Especially, the fermented soybean products, soybean paste (*Chongkukjang*) had the highest isoflavone content (920 mg/kg). *Doenjang*, *Chunjang*, and *Kanjang* contained lesser amounts of isoflavones and their contents were 627, 291, and 10 mg/kg, respectively (8).

However, less information has been published on the physicochemical properties of fermented soy foods products in Southeast Asia. The present study was conducted to analyze physicochemical properties and phytoestrogen contents of fermented soy foods: 3 types of soybean pastes: Thua-nao paste, dried Thua-nao, and commercial soybean paste, as well as soybean sauce, and fermented

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soybean curd cake with other ingredients (2 types) from Thailand.

MATERIALS AND METHODS

Materials

All soy ingredients and processed soybean products were purchased locally. Traditional soybean paste, Thua-nao, was from Chiangrai province, and dried Thua-nao from Jatujak Market, Bangkok. The ingredients in the commercially produced soybean pastes, U brand, Y brand, and N brand are shown in Table 1. Soybean sauce and the 2 types of fermented soybean curd cake with other ingredients are also shown the Table 1. Thua-nao, a fermented soy food from northern Thailand is prepared as follows: clean whole soybean are soaked overnight, boiled for 3~4 hours, transferred to a bamboo basket lined with banana leaves, covered with additional banana leaves, exposed to sunlight, and fermented at 40~42°C for 2~3 days. The fermentation is activated by naturally occuring *Bacillus subtilis*. The raw thua-nao is mixed lightly with salt and, in most cases, garlic, onion, and red chillies to make raw thua-nao pastes (9).

Proximate composition and mineral analysis

Chemical compositions of the fermented soy foods were analyzed using the AOAC methods (10). Moisture content was measured by 105°C drying method, ash content by 550°C ashing method, crude protein content by the Micro-Kjeldahl method, and crude fat content by Soxhlet extraction. Pre-treatment of samples for mineral analysis was done following the AOAC method. Samples were resolved in acid, and Ca and Fe contents were measured through by $KMnO_4$ method using an atomic absorption spectrophotometer (Perkin Elmer, 4110ZL) at 477 nm.

Measurement of water soluble vitamin B contents

The water-soluble vitamins were analyzed through JASCO's method (11). HPLC system consisted of a Waters 2690, Waters 474 & 996 UV detector, and Waters Nova-pak C18 column. Injection volume was 10 μl, and the flow rate was 0.5 mL/min. The mobile phase was 5 mM hexanesufonate:MeOH=6:4, and eluted carbendazim was detected at 254 nm. A calibration curve was prepared by plotting the peak area versus the concentration of the standard.

Measurement of color

Color was measured in each sample by placing it on a standard white plate to measure L (lightness), a (green to red), and b (blue to yellow) using a chroma meter (Minolta CR-210). The standard white plate used at this time had the L value of 97.23, a value of -1.02, and b value of 2.16. Since the factors such as the container holding the sample, the amount of sample, and the color of the container, amount of sample measured (8.6 g) and thickness (2 mm) were maintained constant to minimize errors.

Measurement of isoflavone contents

Isoflavone standards were used for quantifying the amounts isoflavones in food samples. Authentic stan-

Table 1. Ingredients of soy foods in Thailand (Unit: %)

Soybean paste for industrial style							
Products	U brand		Y brand		N brand		
Ingredients	soybean salt sugar wheat flour citric acid MSG preservative	50 20 10 1 0.16 0.08	soybean wheat flour salt solution sugar preservative	62 22 11 4.9	soybean soybean sauce formula sugar preservative	84 13 2.9	
Soybean sauce							
Ingredients	soybean extract wheat flour sugar cane salt solution preservative						
Curd cake fermented							
Products	House brand		Y brand				
Ingredients	soybean red rice wheat flour salt sugar cane water	65 15 8 8 4	soybean red rice wheat flour salt water	50 7 20 8 20			

dards for daidzein and genistein were obtained from commercial sources (ICN Pharmaceuticals, Plainview, NY, and Calbiochem Corp., San Diego, CA).

One gram of dry ground sample was suspended in 3 mL of 1 M HCl and heated at 98~100°C for 1 hr in heating block (Thermolyne, USA). At the end of the digestion, methanol was added to completely dissolve the isoflavones. The mixture was settled for a few minutes, and the supernatant was filtered through a Corning syringe filter (nylon, 0.2 μM) and used for HPLC analysis (1,12) by Wang's method (1), with slight modification. The 20 μL filtrate was injected into the HPLC equipped with a Bondapack C18 column after the system had been equilibrated at ambient temperature, and the UV detector was stabilized with mobile phase (methanol-1 mM ammonium acetate, 6:4) at a flow rate of 1 mL/min for 30 min. Eluent was detected at 254 nm and the chromatogram was recorded for 20 min. Isoflavones were identified by their retention times or standard addition, and their contents were calculated by comparing their peak areas with those of standards.

Statistical analysis

The experiments were triplicated, and the data presented as mean±SD. Statistical significances were analysed using SAS software (13).

RESULTS AND DISCUSSION

Proximate composition

Results of the chemical composition analysis for the soy foods are shown in Table 2. The water content was 55~70% except for dried form. Moisture contents of dried Thua-nao was 7.6%, therefore shelf-life of it can be extended to 1 year when using proper packaging. Calories per 100 g were 98~134 kcal for all of the soy foods except for dried. The crude protein contents were highest in the soybean sauce. Fat contents of fresh and dried Thua-nao were 7.4 and 19.7% respectively, whereas other samples contained less than 3%.

Mineral contents and vitamin B contents

Mineral contents are shown in Table 3. Calcium content was highest in dried Thua-nao followed by fresh Thua-nao, with concentrations less than 44.7 mg/100 g in the others. Salt was added to samples other than Thua-nao resulting in high Na contents. Interestingly Na contents of fresh and dried Thua-nao were 0.45 and 0.48%, respectively. Since dried Thua-nao (Jatujak market) is not prepared from the Thua-nao paste (Chiangrai), there is no point of comparing their compositions. K contents were in the range of 89.0~1887.5 mg/100 g. Fresh and dried Thua-nao were high in K, while soybean

Table 2. Proximate composition of soy foods in Thailand (Unit: %)

Sample[1)]	Calorie (kcal)	Moisture	Crude protein	Crude fat	CHO	Ash
1	134.0±0.83[2)]	68.5±0.01	3.0±0.02	7.4±0.03	13.9±0.12	7.2±0.06
2	349.3±4.5	7.6±0.09	5.6±0.08	19.7±0.06	37.4±0.91	29.7±0.68
3	100.2±2.57	58.2±0.04	11.4±0.10	3.0±0.09	6.9±0.34	20.5±0.11
4	111.4±9.04	55.4±0.02	15.5±0.94	1.4±0.04	9.2±1.23	18.5±0.23
5	99.1±9.19	64.4±0.63	13.4±0.32	1.9±0.07	7.1±1.82	13.2±0.80
6	101.2±6.73	64.0±0.45	19.9±0.30	0.4±0.05	4.5±1.27	11.2±0.47
7	112.5±4.83	58.6±0.13	15.4±0.46	0.5±0.03	11.6±0.68	13.9±0.06
8	98.1±1.1	61.4±0.09	12.4±0.02	0.5±0.02	11.0±0.21	14.7±0.08

[1)]1: Thua-nao paste, 2: Dried Thua-nao, 3: Soybean paste of U brand, 4: Soybean paste of Y brand, 5: Soybean paste of N brand, 6: Soybean sauce, 7: Curd cake fermented (House brand), 8: Curd cake fermented (Y brand).
[2)]Values are mean±SD.

Table 3. Mineral and vitamin contents of soy foods in Thailand (Unit: mg/100 g)

Sample[1)]	Ca	Na	K	P	Vit. B_1	Vit. B_2
1	126.2±1.03[2)]	451.4±3.42	523.3±3.67	185.8±1.13	0.4±0.02	0.1±0.02
2	192.5±2.5	475.0±3.47	1887.5±3.78	477.8±2.86	0.2±0.01	0.1±0.01
3	34.8±0.26	6087.6±10.53	353.8±2.51	102.6±1.02	1.0±0.02	0.0±0.01
4	44.7±0.84	7556.34±10.26	292.9±2.23	87.9±0.98	0.6±0.03	0.1±0.01
5	33.0±0.23	6390.0±14.31	370.0±1.25	79.0±0.27	0.6±0.01	0.1±0.02
6	19.4±0.04	9200.0±16.45	89.0±0.20	17.9±0.02	0.6±0.21	0.0±0.02
7	39.6±0.60	6970.0±17.31	91.0±0.17	82.9±0.23	0.8±0.04	0.1±0.01
8	43.0±0.03	5670.0±18.92	92.0±0.11	110.2±1.04	0.1±0.01	0.0±0.01

[1)]Samples are the same as in Table 2.
[2)]Values are mean±SD.

Table 4. Hunter color-values of soy foods in Thailand

Sample[1)]	L	a	b
1	35.98±1.62[2)]	-1.93±1.58	9.15±1.97
2	57.90±1.02	1.06±1.10	21.93±1.66
3	41.45±1.25	4.81±1.82	21.58±0.87
4	36.84±1.21	5.97±1.25	21.8±1.60
5	38.66±1.13	5.44±0.57	21.99±0.18
6	11.70±0.08	35.4±0.05	20.0±0.03
7	32.67±0.13	15.7±0.07	12.2±0.23
8	31.61±0.65	13.93±0.22	12.95±0.24

[1)]Samples are the same as in Table 2.
[2)]Values are mean±SD.

sauce and fermented curd cakes were low in K. These results were almost same for P, with P concentrations of 17.9 mg/100 g for soybean sauce, 185.8 mg/100 g for Thua-nao paste, and 477.8 mg/100 g for dried Thua-nao.

Vitamin B concentrations are shown in Table 3. The commercial soybean paste contained a large amount of thiamin; however, riboflavin content was low. Other products were also low in riboflavin.

Physical property

The Hunter's color value of soy foods in Thailand are shown in the Table 4. The lightness L value of dried Thua-nao was 57.90, the green-red, a value was 1.06, the blue-yellow, b value was 21.93; thus L value was the highest one. On the other hand, soybean sauce had the lowest L value (11.70) and highest a value (35.44). Soybean paste of N brand showed the highest b value as 21.99, while reporting the lowest 9.15 in Thua-nao paste.

On the other hand, physical appearance of fermented soy foods in Thailand are presented in Fig. 1.

Isoflavone contents

The content of isoflavone in soy foods from Thailand are shown in Table 5. The content of daidzein in soy foods varied widely ranging from 1.5~676.0 μg/g. Dried Thua-nao had the highest daidzein concentrations with free and total daidzein of 355.0 and 676.0 μg/g, respectively, whereas soybean sauce was lowest at ND (no detection) and 1.5 μg/g.

Genistein in the soy foods also varied widely, ranging from 8.5~616.5 μg/g, exhibiting similar patterns as daidzein. Dried Thua-nao had the highest content at 616.5 μg/g. N brand of soybean paste, when adjusted for moisture content, contained a greater amount of genistein than Y and U brand, has similar effects to the female hormone estrogen.

According the study reported by Wang and Murphy (14), total isoflavone contents of soybean varieties range from 1200 to 4300 μg/g, with the glucoside form of the

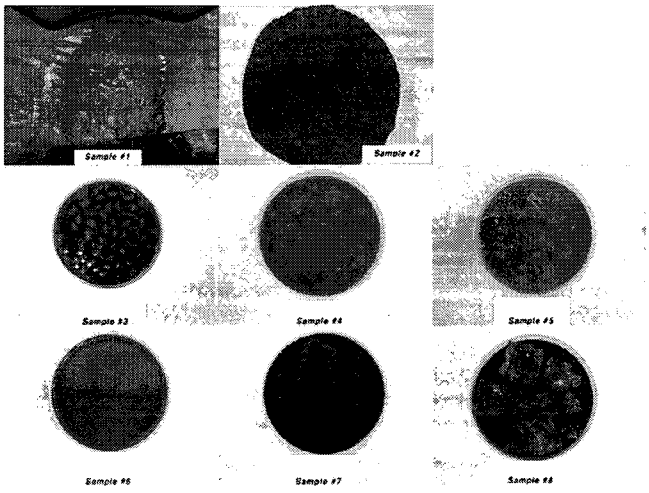

Fig. 1. Physical appearance of fermented soy foods in Thailand. Sample #1: Thua-nao paste, #2: Dried Thua-nao, #3: Curd cake fermented (House brand), #4 : Curd cake fermented (Y brand), #5: Soy sauce, #6: Soybean paste of U brand, #7: Soybean paste of Y brand, #8: Soybean paste of N brand.

Table 5. Isoflavone contents of soy foods in Thailand (Unit: μg/g)

	Daidzein		Genistein	
	free	Total	free	Total
1[1)]	89.0±12.7[2)]	119.5±7.8	53.5±12.0	68.5±12.0
2	355.0±29.7	676.0±22.6	293.0±19.8	616.5±12.0
3	87.5±0.7	95.0±0.1	49.5±2.1	69.0±0.1
4	71.0±14.1	107.0±7.1	49.5±10.6	72.5±6.4
5	43.5±5.0	48.0±0.1	56.5±0.7	73.5±14.9
6	ND	1.5±0.7	ND	8.5±2.1
7	48.0±1.4	50.0±2.8	50.5±3.5	56.5±6.4
8	40.0±4.2	51.0±4.2	46.0±5.7	56.0±5.7

[1)]Samples are the same as in Table 2.
[2)]Values are mean±SD.

isoflavone predominating. Levels of isoflavones could be modified greatly during soybean processing (6). Traditional nonfermented soy foods, roasted soybeans (1625 μg/g), and instant soy beverage powder (1001~1183 μg/g), have 2~3 times the total amount of isoflavone as compared with fermented soy foods, tempeh (625 μg/g), bean paste (593 μg/g), miso (294 μg/g), and fermented bean curd (390 μg/g) (3). The distribution of isoflavone isomers in nonfermented foods was greater in the glucosides, genistin and daidzin, whereas the fermented soy foods retained low amounts of glucosides. The aglycone isomers were the major isoforms in these fermented soy foods.

Protein isolates and tofu were reported to contain reduced amounts of isoflavones as compared with soybeans and flours, as a result of the aqueous treatment used during manufacture.

Genistein and daidzein are biphenolic compounds, usually present in the soybean as glycosides. According

to the study by Eldridge et al. (15,16), about 98% of soybean isoflavones are present as genistin and daidzin. Accordingly, genistein was reported to be an anticancer substance, with efficacies against breast cancer and prostate cancer demonstrated by vitro testing, and is known to prevent osteoporosis (17,18)

This study investigated a wide variety of soy products and provides basic information for future human studies. Proposed anticarcinogenic doses of soybean isoflavones range from 1.5 to 2.0 mg/kg/d (7). There are a number of soy food choices that will meet this dose requirement.

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