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Isoflavones and Anti-oxidant Activities of Soybeans in Thailand

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ABSTRACT

Soybeans are industrial crops and widely cultivated in central and northern Thailand. Isoflavones have considerable attention because of their beneficial health effects and anti-oxidant properties. The present study aimed to investigate isoflavone levels in form of aglycones (genistein and daidzein) and anti-oxidant activities among 13 soybean cultivars in central and northern Thailand. Three soybean cultivars in central region consisted of Sukhothai 1 (SK1), Sukhothai 2 (SK2) and Srisamrong 1 (SS1) and ten soybean cultivars in northern Thailand were Chiang Mai 1 (CM1), Chiang Mai 2 (CM2), Chiang Mai 3 (CM3), Chiang Mai 6(CM6), Chiang Mai 60 (CM60), SOR JOR 1 (SJ1), SOR JOR 2 (SJ2), SOR JOR 3 (SJ3), SOR JOR 4 (SJ4) and SOR JOR 5 (SJ5). Isoflavones were analysed by solid phase extraction method and anti-oxidant activities were determined using DPPH scavenging assay. The results were found that genistein was the predominant isoflavones in soybeans. Isoflavone levels and % DPPH scavenging activities among soybeans in northern cultivars were significantly higher than those in central cultivars (p=0.000). Soybean cultivars in northern region had an average of 104.08±45.25 μg g^{-1} for genistein, 76.78±36.91 μg g^{-1} for daidzein and 25.72±3.67% for DPPH scavenging activities. Soybean cultivars in central region had an average of 18.81±9.10 μg g^{-1} for genistein, 17.46±12.35 μg g^{-1} for daidzein and 14.07±1.10% for DPPH scavenging activities. The remarkable findings were that the correlation coefficient of % DPPH scavenging activities with genistein (r = 0.706, p = 0.000) was higher than those with daidzein (r = 0.497, p = 0.000). Present results therefore suggested that genistein showed the most potent anti-oxidant and high bio-availability as a promising candidate for the prevention of cancers and other diseases.

Key words: Daidzein, DPPH, genistein, phytoestrogen, *Glycine max*

INTRODUCTION

Soybeans (*Glycine max*) are industrial crops and widely cultivated in Thailand with 25,257,600 square meters cultivated area. They are composed of macronutrients such as protein (36-46%), carbohydrate (30%) and lipid (18%) which mainly consist of polyunsaturated fatty acids such as linoleic acid (Kim and Kim, 2005; Cederroth and Nef, 2009). They also contain micronutrients which include isoflavones, saponins, phytates, vitamins and minerals. Among soybean micronutrients, isoflavones have considerable attention because of their biological properties including estrogenic and anti-oxidant activities (Mitchell *et al*., 1998; Patel *et al*., 2001; Magee *et al*., 2004; Lee *et al*., 2005; Cederroth and Nef, 2009). Epidemiological and clinical studies have shown that dietary soy isoflavones is associated with beneficial health effects, such as

reduction of menopause symptom, prevention of coronary heart disease and prevention of breast and reproductive cancers form (Ozasa *et al.*, 2004; Hedelin *et al.*, 2006; Bandera *et al.*, 2011; Bolanos-Diaz *et al.*, 2011; Setchell *et al.*, 2011; Ollberding *et al.*, 2012). It have been suggested that some of the effect were related to the anti-oxidant activities of isoflavones.

Although, there are various soybean cultivars cultivated in Thailand for long times, there is little data regarding their isoflavone contents and anti-oxidant properties in each cultivar. Therefore, the aim of the present study was to investigate isoflavone levels in aglycone forms (genistein and daidzein) and anti-oxidant activities among 13 soybean cultivars in Central and Northern Thailand.

MATERIALS AND METHODS

Cultivars of soybeans and sampling: Between December 2011 and May 2012, thirteen soybean cultivars were took a random for analyzing isoflavones and anti-oxidant activities. Cultivar name, locality of origin and breed certification of 13 soybean cultivars are shown in Table 1. Three soybean cultivars in central Thailand were Sukhothai 1 (SK1), Sukhothai 2 (SK2) and Srisamrong 1 (SS1). Ten soy bean cultivars in northern Thailand were Chiang Mai 1(CM1), Chiang Mai 2 (CM2), Chiang Mai 3 (CM3), Chiang Mai 6(CM6), Chiang Mai 60 (CM60), SOR JOR 1 (SJ1), SOR JOR 2 (SJ2), SOR JOR 3 (SJ3), SOR JOR 4 (SJ4) and SOR JOR 5 (SJ5). One kilogram of each foundation seed was brought from Crops Research Center in Chiang Mai and Sukhothai province. Each cultivar was stepwise sampling until the final sample as 250 g and then ground into powder. The powdered were mixed and took a random 5 samples in each cultivar for analysis. All samples were stored in freezer at -20°C throughout the period of the experiment.

Analysis of isoflavones: Standards of genistein and daidzein were obtained from Sigma-Aldrich, USA. All solvents for extraction were HPLC grades (J.T. Baker, USA). Sample extraction and analysis was modified from the method of Nakamura *et al.* (2000). Recovery was 118.9% for genistein and 110% for daidzein. The quantitation limit was 0.95 $\mu g\ g^{-1}$ for genistein and 0.88 $\mu g\ g^{-1}$ for daidzein. The intrabatch coefficient of variation (% CV) was 10.68% for genistein and 10.06% for daidzein and interbatch % CV was 10.18% for genistein and 17.78% for daidzein.

Table 1: Cultivar name, locality of origin, and breed certification of 13 soybean cultivars

Cultivar name	Abbreviation	Locality of origin in Thailand	Breed certification from Department of Agriculture
Sukhothai 1	SK1	Central region	1986
Sukhothai 2	SK2	Central region	September 25, 1987
Srisamrong 1	SS1	Central region	August 31, 2007
Chiang Mai 1	CM1	Northern region	March 5, 1993
Chiang Mai 2	CM2	Northern region	March 18, 1998
Chiang Mai 3	CM3	Northern region	April 24, 2000
Chiang Mai 6	CM6	Northern region	May, 2010
Chiang Mai 60	CM60	Northern region	September 30, 1987
SOR JOR 1	SJ1	Northern region	1965
SOR JOR 2	SJ2	Northern region	1965
SOR JOR 3	SJ3	Northern region	No data
SOR JOR 4	SJ4	Northern region	October 5, 1976
SOR JOR 5	SJ5	Northern region	1980

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Determination of anti-oxidant activities: DPPH scavenging assay was measured by modified method of Katsube *et al.* (2004). One gram of the powdered were mixed with 10 mL of 70%(v/v) ethanol and extracted for 12 h. The extracts were then filtered with filter paper (Whatman no.1) and stored at 4°C until analysis. The extract was diluted with water stepwise and 10 μL of dilution was pipetted into a 96-well plate. One hundred and eighty-five microliters of DPPH (2,2-diphenyl-2-picrylhydrazyl) solution dissolved in a 50% ethanol solution was added to each well and the plate was shaken for 5 min at room temperature. The absorbance was read at 550 nm by Microplate Reader (MultiRead 400, Anthos). The experiment was carried out in triplicate:

$$\text{Scavenging activities (\%)} = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis: All results were expressed as Mean±standard deviation (SD). Data were analyzed by two independent sample test (Mann-Whitney U test) and Spearman Rank correlation coefficient (r) using SPSS 11.5. Statistical significance was set at $p<0.05$.

RESULTS

The highest level of genistein was found in CM1 with mean of 159.14±60.71 μg g^{-1}. The second highest level was that in CM2 (150.85±38.56 μg g^{-1}), followed by SJ3 (128.21±18.39 μg g^{-1}). The highest level of daidzein was found in SJ4 with mean of 97.12±10.08 μg g^{-1}. The second highest level was that in SJ1 (96.40±53.86 μg g^{-1}), followed by CM2 (89.89±12.99 μg g^{-1}). Genistein levels in most strain samples (except CM3, CM6, SJ1 and SJ5) were higher than daidzein. The mean ratio between genistein and daidzein was 1.49 (ranged from 0.31-8.96) (Table 2). Comparing with isoflavones of soybean cultivars in central and northern regions, the levels in northern cultivars (104.08±45.25 μg g^{-1} for genistein and 76.78±36.91 μg g^{-1} for daidzein) were significantly higher than those in central cultivars (18.81±9.10 μg g^{-1} for genistein and 17.46±12.35 μg g^{-1} for daidzein) ($p = 0.000$) (Table 3).

The highest % scavenging activities was found in CM1 with mean of 29.4±1.95%. The second highest scavenging activities was that in SJ2 (29.2±1.3%), followed by SJ3 and CM2 (27.6±1.82

Table 2: Isoflavone levels (μg g^{-1}) and DPPH scavenging activities in 13 soybean cultivars

Locality of origin	Cultivar	Genistein	Daidzein	DPPH scavenging activities (%)
Central region	Sukhothai 1	29.76±7.430	29.87±15.05	14.8±0.84
	Sukhothai 2	12.88±1.720	10.50±3.090	13.6±0.89
	Srisamrong 1	13.79±2.590	12.00±2.770	13.8±1.30
Northern region	CM1	159.14±60.71	40.87±12.69	29.4±1.95
	CM2	150.85±38.56	89.89±12.99	27.6±2.70
	CM3	68.84±40.08	74.12±33.15	24.2±2.17
	CM6	121.54±30.82	78.67±21.21	23.6±1.14
	CM60	90.19±35.12	39.53±17.68	23.0±3.32
	SJ1	69.46±26.49	96.40±53.86	22.8±1.10
	SJ2	96.79±10.23	56.11±11.17	29.2±1.30
	SJ3	128.21±18.39	73.06±18.25	27.6±1.82
	SJ4	102.77±16.50	97.12±10.08	26.6±1.34
	SJ5	53.01±21.32	53.66±24.01	23.2±7.09

Values are Mean±SD, n = 5

Table 3: Isoflavone levels (μg g^{-1}) and DPPH scavenging activities, compared between soybeans of central and northern regions of Thailand

	Genistein		Daidzein		DPPH scavenging activities (%)	
Locality of origin	Mean±SD	p-value	Mean±SD	p-value	Mean±SD	p-value
Central region (n = 15)	18.81±9.10	0.000**	17.46±12.35	0.000**	14.07±1.10	0.000**
Northern region (n = 50)	104.08±45.25		76.78±36.91		25.72±3.67	

**Statistically different at 0.01

Table 4: Spearman Rank correlation coefficient between isoflavones and % DPPH scavenging activities in soybeans

	Spearman correlation coefficient (R)		
	Genistein	Daidzein	DPPH scavenging activities (%)
Genistein	-	0.666**	0.706**
Daidzein	-	-	0.497**
DPPH scavenging activities (%)	-	-	-

**Statistical correlation significant at 0.01, n = 65

and 27.6±2.7), respectively (Table 2). Comparing with % scavenging activities of soybeans cultivated in central and northern regions, % scavenging activities in northern cultivars (25.72±3.67%) were significantly higher than those in central cultivars (14.07±1.10%) (p = 0.000) (Table 3).

Genistein levels were positively associated with daidzein (r = 0.666, p = 0.000) and positively associated with % scavenging activities (r = 0.706, p = 0.000). Daidzein levels were also positively associated with % scavenging activities (r = 0.497, p = 0.000) (Table 4).

DISCUSSION

In our study, genistein was the predominant isoflavones in soybeans and the results were in agreement with several studies (Franke *et al*., 1994, 1999; Shao *et al*., 2009).

The remarkable findings were that isoflavones and anti-oxidant activities in northern cultivars were significantly higher than those in central cultivars. Especially, CM1 from northern cultivar had the highest genistein and anti-oxidant activities. The results therefore suggest to cultivating CM1 for more production of isoflavones and anti-oxidant activities.

Several studies have reported that isoflavone contents were affected by genetic factor, geographical location and environmental conditions during seed development (Wang and Murphy, 1994; Tsukamoto *et al*., 1995; Seguin *et al*., 2004; Vamerali *et al*., 2012). Central and northern Thailand are differences in geographical and environmental conditions which include temperature, precipitation, soil fertility, soil moisture and light level. Temperature seems to be a major factor affecting isoflavone synthesis. In Thailand, an average temperature in northern region (26°C) is lower than in central region (28°C). Soybeans are subtropical plants that require root zone temperature in range of 25-30°C for optimal cultivation (Jones and Tisdale, 1921). Several studies have reported that low temperature increased the activity of enzymes of phenylpropanoid and flavonoid pathways and temperature higher than 24°C during seed development reduced isoflavone contents (Tsukamoto *et al*., 1995; Carrao-Panizzi *et al*., 1999; Janas *et al*., 2002; Posmyk *et al*., 2005). Water supply is also expected to be increased isoflavone contents (Caldwell *et al*., 2005). However, the study of Vamerali *et al*. (2012) reported that water supply increased protein and yield but effect on isoflavones was negligible.

Genistein and daidzein levels were positively associated with % scavenging activities (r = 0.706, r = 0.497, p = 0.000, respectively). It was due to their ability to reduce free radical formation and to scavenge free radicals (Pietta, 2000; Djuric *et al*., 2001). The remarkable findings were that the correlation coefficient of % scavenging activities with genistein was higher than those with daidzein. A number of studies have reported that genistein showed the most potent anti-oxidant (Wei *et al*., 1995; Ruiz-Larrea *et al*., 1997; Rimbach *et al*., 2003). It was also mentioned that the number and position of hydroxyl groups were factors for anti-oxidants activities and the c-4' position was crucial (Wei *et al*., 1995; Arora *et al*., 1998). Therefore, it could be hypothesized that high amounts and relatively high anti-oxidants of genistein is a promising candidate for the prevention of human cancers and other diseases.

In conclusion, the important factors affecting isoflavone contents in soybeans were geographical and environmental conditions. The high association between genistein and anti-oxidant activities in soybean indicated that genistein showed the most potent anti-oxidant and high bio-availability as a promising candidate for the prevention of cancers and other diseases.

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