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Effect of roasting on phytochemical properties of Thai soybeans

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Abstract

Soybeans are a rich source of protein and widely cultivated in Thailand, and they have various bioactive ingredients, which have antioxidant and anticancer activates. This investigation revealed the phytochemical properties of three Thai roasted soybeans (RSB), which were roasted at 230°C for 12, 15 and 18 min, including the total phenol content (TPC), total flavonoid content (TFC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant potential (FRAP) and β- carotene bleaching activity. Additionally, we measured the mutagenicity by the Ames test and anti-carcinogenic properties in the MCF-7 cell line. The RSB showed significantly higher antioxidant activity than raw soybean in TPC, TFC and all antioxidant assays. In particular, the RSB of SJ5 at 230°C for 15 min showed the highest antioxidant activity. Moreover, almost all of the RSBs did not have mutagenicity properties, in addition, no anticancer effects of RSBs were found. Therefore, the phytochemical properties of RSB depended upon the type of soybean, and the antioxidant activity can be maximized by roasting. This results shown beneficiary of increasing antioxidant activity of RSB with safe for non-mutagenicity approved.

Introduction

Soybeans *(Glycine max)* are an important foodstuff, particularly in traditional cooking by Southeast Asian people, such as tofu and soymilk (Mehmet and Fahad, 2014). Soybeans are rich in protein, fiber and oil, in addition to being a main source of minerals, vitamins and phytochemicals (Corley *et al*., 1999; Kim and Kim, 2005). The key phytochemicals consist of isoflavone, oligosaccharides, saponins, phytates and phenolic compounds, which have various health benefits on several chronic diseases, including the prevention of cancer, cardiovascular disease and multiple conditions ameliorated by antioxidants (Anderson and Major, 2002; Diaz-Batalla *et al.*, 2006; Zhang *et al.*, 2011).

Processing methods not only affect changes in the physical characteristics and flavor, but also in the chemical composition of food. Some researchers have shown that processing methods can preserve the health benefit effects by enhancing antioxidant activity (Dewanto *et al.*, 2002; Jaramillo-Flores *et al*., 2003). Roasting is a heat processing method that uses a dry heat treatment and causes a Maillard reaction (Oliviero *et al.*, 2009). It has been reported that naturally occurring antioxidant activity was lost as a consequence of roasting, while antioxidant Maillard reaction products were produced (Anese *et al*., 1999; Yen and Chung, 1999). The Maillard reaction has been especially linked with the increased oxidative stability of different roasted seed oils (Cai *et al*., 2013). In particular, Hyo *et al*. (2011) demonstrated that roasted small black soybeans showed significantly higher antioxidant activity than unroasted small black soybeans. The antioxidant activity increased following the roast processing. In addition, Yamabe *et al.* (2012) found that ginsenosides were generated by the Maillard reaction of Panax ginseng. They revealed that the anti-carcinogen activity of the ginsenosides increased upon heat processing.

In Thailand, there are various soybean cultivars that are cultivated in the central and northern parts, and these have different properties, such as physical, chemical and nutritional values. SOR JOR 5 (SJ5) and Chiang Mai 60 (CM60) are popular soybean cultivars in Thailand, and Chiang Mai 84-2 (CM84-2) is a new cultivars released in 2012. Although there are data regarding the antioxidant and anticancer properties of each cultivar, there are no data for after soybean processing, especially the effect of roasting on the nutritional and phytochemical properties. Generally,

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most studies of soybean processing are related to boiling (soymilk) and steaming (tofu). Therefore, the aim of this study was to investigate the phytochemical properties due to different roasting conditions among three popular soybeans in Thailand. Furthermore, we compared the phytochemical properties of the unroasted and roasted soybeans (RSB).

Materials and Methods

Plant material and chemicals

Soybean *(G. max)* seeds were acquired from the Crop Research Center in Chiang Mai, Thailand. Three soybeans were selected, including SOR JOR 5 (SJ5), Chiang Mai 60 (CM60) and Chiang Mai 84-2 (CM84-2), which is a new soybean in Thailand. The following analytical grade chemicals were purchased: 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-Striazine (TPTZ), Folin–Ciocalteu reagent and gallic acid from Sigma Chemical Co. (St.Louis, MO, USA) and Catechin from Fluka (Neu-Ulm, Germany).

Sample preparation

The three soybeans were placed in the drum of a coffee roaster (Gene Café, Genesis Co. Ltd.; Gyeonggi, Korea) and roasted with high-temperature air. They were subjected to three different treatments: roasting at 230°C for 12, 15 and 18 min. The roasting temperature and times were modified from those used in a previous study (Ramalakshmi *et al*., 2008).

Nutrient analysis

Proximate analysis of the RSB (including percentage fat, moisture, protein, ash and carbohydrate) was determined using the AOAC standard method (1990) and all samples are shown in Table 1.

Extraction

The extraction was performed according to Ramalakshmi *et al.* (2008). The RSBs were powdered and defatted with hexane (1:6, w/v) for 8 h in a soxhlet. The defatted powder was extracted in a soxhlet with ethanol (8 h) while maintaining a sample to solvent ratio in the range of 1:8 (w/v). The extracts were desolventised in a rotavapour at 50°C under reduced pressure and stored in a desiccator until further use.

Determination of total phenolic contents

The amount of total phenolic contents (TPC) in the RSB samples were determined according to the Folin-Ciocalteu procedure using the method of Yawadio *et al.* (2008). Gallic acid was used as the reference standard, and the results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g dry weight).

Determination of total flavonoid content

Total flavonoid content (TFC) was determined using the colorimetric method described by Abu Bakar *et al.* (2009) as modified from Dewanto *et al.* (2002). Results were expressed as mg catechin equivalents in 1 g of dried sample (mg CA/g dry weight).

DPPH radical scavenging activity

Radical scavenging activity was determined using a modification of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) method of Brand-Williams *et al.* (1995). 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 x [1- (AE/AD)], where AE is the absorbance of the DPPH solution with an extract added and AD is the absorbance of the DPPH solution with nothing added (Amarowicz *et al.*, 2004).

Ferric reducing antioxidant potential (FRAP) assay

The FRAP assay was done according to Benzie and Strain (1996) with some modifications. 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

The determination of the antioxidant activity as the ability to delay the bleaching of β-carotene in a water/linoleic acid emulsion was performed according to Nsimba *et al.* (2008). BHT was used as the positive control. The percentage inhibition was calculated as $[1 - (A_0 - A_t)/ (A^0_0 - A^0_t)] \times 100)$, 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^0_t are the absorbance values of the sample and control respectively at t minutes.

Determination of mutagenicity activity

The Ames test was performed as a standard plate incorporation assay with Salmonella typhimurium strains TA98 and TA100 for evaluating the mutagenic activity. For all experiments in this study, the pre-incubation method of Yahagi *et al*. (1975) was used with each Ames test sample and positive control to

Table 1 - Nutrient content of three roasted soybeans (RBS)

Type	Time	Nutrient				
		Moist. (%)	Fat (%)	Crude Protein (%)	Ash (%)	Carbo. (%)
SJ5	0 min	11.10 ± 0.47	9.90 ± 1.04	37.50 ± 0.67	3.07 ± 0.54	34.61 ± 1.25
	12 min	10.04 ± 0.49	11.28 ± 1.21	34.52 ± 0.31	5.65 ± 0.07	36.12 ± 0.90
	15 min	9.36 ± 0.81	13.48 ± 1.19	33.80 ± 0.70	6.31 ± 0.04	38.00 ± 0.69
	18 min	7.30 ± 0.19	14.58 ± 1.53	32.38 ± 1.21	6.40 ± 0.02	38.36 ± 1.48
CM60	0 min	14.83 ± 0.34	12.07 ± 0.84	38.12 ± 1.01	4.91 ± 0.51	25.12 ± 0.45
	12 min	11.41 ± 0.16	14.08 ± 0.73	35.79 ± 0.79	5.63 ± 0.01	29.96 ± 0.24
	15 min	11.13 ± 0.12	16.93 ± 0.53	34.06 ± 1.04	5.73 ± 0.11	31.98 ± 1.08
	18 min	10.96 ± 0.23	17.06 ± 1.11	30.13 ± 1.07	6.07 ± 0.09	36.11 ± 2.03
CM84-2	0 min	13.81 + 0.56	8.12 + 0.25	37.89 + 0.87	4.26 ± 0.65	32.60 ± 0.64
	12 min	10.77 ± 0.25	13.43 ± 1.35	35.17 ± 0.81	5.48 ± 0.03	35.48 ± 0.86
	15 min	10.39 ± 0.21	13.45 ± 1.28	34.54 ± 1.89	5.67 ± 0.06	35.54 ±2.13
	18 min	8.86 ± 0.31	13.48 ± 0.23	34.49 ± 0.97	5.70 ± 0.05	37.54 ± 1.95

Values expressed are means + S.D. of three replicate experiments.

identify the mutagenicity.

Determination of anticancer activity

The anticancer activity of the extracted samples in breast adenocarcinoma (MCF-7 cell line) was determined using the method of Brien *et al*. (2000). However, the positive control was 8.42 µg/ml tamoxifen and 7.24 µg/ml doxorubicine, while DMSO was used as a negative control.

Statistical analysis

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

Results and Discussion

Nutritional evaluation of RSB

Table 1 shows the nutritional parameters of soybeans under the three conditions of roasting as well as raw beans. Raw CM60 was higher in all nutrients, except carbohydrate, than raw SJ5 and CM84-2. The RSBs were observed to have decreased moisture content (7.30-11.41%), which followed the conditions of roasting. The highest moisture content was found in CM60 at 12 min (11.41±0.16). The fat content of the RSBs was found to have increased following the roasting, which was related with the time increase. The fat content of RSBs was found to be 11.28 –17.06 %. The high fat content of the RSBs was not surprising as soybeans are known as oil seeds. The protein content was lower in the RSBs than in the raw soybeans, and the range was 30.13 – 35.17%. However, the protein content of the RSBs decreased as the roasting time increased, which corresponds with Fobe *et al*. (1968) who reported the chemical composition of Arabica coffee roasted at 230°C for different times. In this, as the roasting time increased, the proteins decreased because roasting causes a degradation of polysaccharide sugars and amino acids, which resulted in the formation of Maillard reaction and condensation products. Overall, there was an increase in the organic acids and lipids (Buffo and Cardelli-Freire, 2004). The RSB's ash and carbohydrate content were analyzed and found to be significantly related to an increasing roasting period. Thus, a long roasting period reduced the nutritional quality in RSB, but it was the highest in CM60.

Total phenolic contents (TPC)

Phenolic compounds exhibit considerable free-radical scavenging activity, which is due to their reactivity as hydrogen- or electron- donating agents to free radicals, as well as the stability of the resulting antioxidant-derived radicals (Ang-Lee *et al*., 2001; Wojdylo *et al*., 2007). TPC values, as milligrams of gallic acid equivalents (GAE) per gram of dry weight, are shown in Figure 1.

Less TPC was in the raw soybeans than in the RSBs in all investigated strains with SJ5 giving the highest properties. There were significant differences among the different varieties tested [type of RSB ($p< 0.05$) and roasting time ($p< 0.05$)]. The TPC of RSB extracts ranged from 6.76 to 48.50 mg GAE/g dry weight. SJ5 at 15 min had the highest TPC and CM84-2 at 12 min had the lowest TPC. The TPC in RSBs at 230°C for 12, 15 and 18 min increased with roasting time. Interestingly, the total contents of SJ4 were highest when roasted at 230°C for 15 min (45.27+ 3.23 mg GAE/g dry weight), while the contents decreased when roasted at 18 min. Soybeans

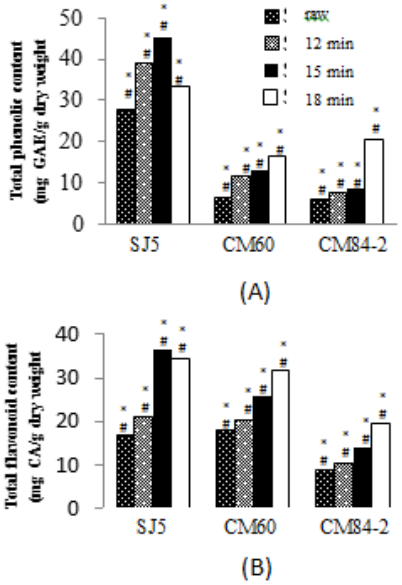

Figure 1. Effect of roasting process on roasted soybeans (RSB) at 230°C for 12, 15 and 18 min.(A) TPC (B) TFC

Values are expressed as mean ± SD of triplicate measurement.
*significant at $\alpha < 0.05$ for comparing different varieties tested
#significant at $\alpha < 0.05$ for comparing different roasting time

include a high concentration of phenolic compounds (Lee *et al.*, 2011). Hyo *et al*. (2011) reported that the phenolic content of roasted small black soybean was greater than small black soybean, and these increasing patterns were consistent with the radical scavenging assays. They assumed that the increase in phenolic contents was primarily due to the increased release of phytochemicals as phenolic acids. The heat disrupts the cell membranes and cell walls, which releases the soluble phenolic contents from the insoluble ester bonds (Dewanto *et al*., 2002). Therefore, this study the increased amount of phenolic contents caused an increase in RSB antioxidant activity during roasting.

Total flavonoid content (TFC)

TFC values are presented as milligrams of catechin equivalents (CA) per gram of dry weight. The highest TFC in raw soybeans was in CM60, in comparison with the other strains, but this was still less than in the RSB. Significant differences were found among the different varieties tested ($p< 0.05$) in the case of roasting. SJ5 at 15 min shown the maximum and CM84-2 at 12 min shown the minimum TFC among the extracts (Figure 1). TFC varied widely among the extracts and ranged from 9.06 to 39.57 mg CA/g dry weight. In addition, the extracts with higher flavonoid contents also had higher phenolic contents, as was evident for SJ5 at 15 min, which had a higher TPC and TFC. The increase of the TFC in all RSBs could result from the release of bound polyphenols

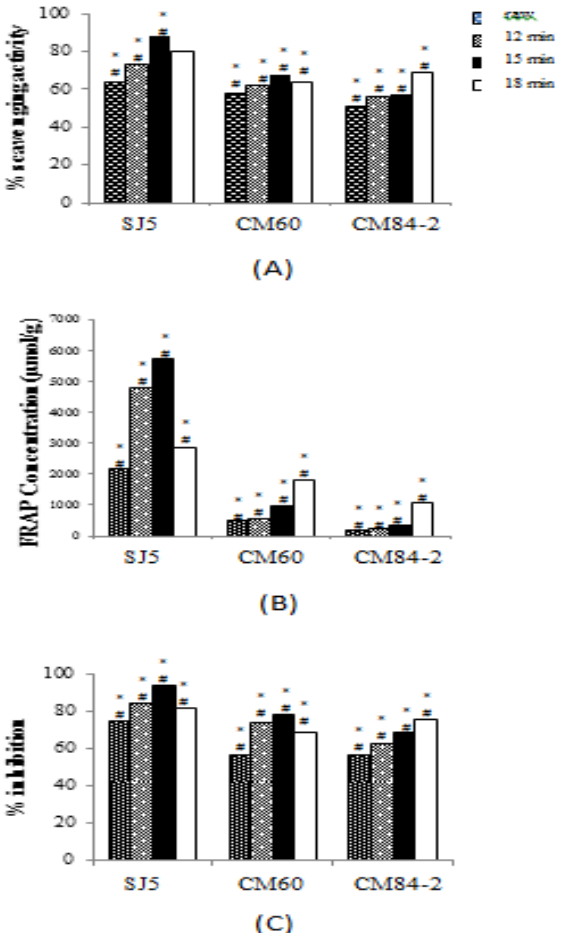

Figure 2. Antioxidant capacity of roasted soybeans (RSB) (A) on DPPH assay shown as % scavenging activity, (B) on FRAP assay shown as FRAP concentration (µmol/g) and (C) on β-carotene bleaching activity shown as percentage inhibition.

Values are expressed as mean ± SD of triplicate measurement.
*significant at $\alpha < 0.05$ for comparing different varieties tested
#significant at $\alpha < 0.05$ for comparing different roasting time

or from Maillard reaction products forming during roasting, which then exhibited scavenging activity on the reactive oxygen species (Hayase *et al*., 1990). In our results, we found a higher browning index (data not shown) and TFC in SJ5 at 15 min. Jokic *et al.* (2004) reported that sugars, amino acids and polyphenols were precursors of the Maillard reaction via the inhibition of polyphenol oxidase.

Antioxidant potential of RSB

The extracts were allowed to react with the DPPH solution to evaluate the free radical scavenging activity, which determines the hydrogen-donating ability of the extracts to DPPH° (stable free radical). In the present study the extracts possessed hydrogen donating capabilities to act as antioxidants, and showed 73.03, 88.27 and 79.64% of scavenging activity for the roasted SJ5, roasted CM60 showed

61.99, 67.85 and 66.94%, whereas roasted CM84-2 showed 56.12, 57.22 and 68.60% at 1000 µg/ml after roasting at 230°C at 12, 15 and 18 min, respectively (Figure 2). While for raw SJ5, CM60 and CM84-2 our results showed 62.71, 59.02 and 55.40% scavenging activity, respectively. The extract of SJ5 at 18 min showed the maximum activity and CM84-2 at 12 min showed the minimum activity. The different percentages of scavenging activity were statistically significant between types of RSB ($p< 0.05$) and roasting time ($p< 0.05$).

The antioxidant activity was analyzed by its ability to reduce Fe^{3+}–TPTZ to a blue colored Fe^{2+}–TPTZ (Butsat and Siriamornpun, 2010). The extracts showed the same trend as with the DPPH radical scavenging activity method. The extract of SJ5 at 18 min showed the maximum activity (5750.33 + 27.51 µmol/g) and of CM84-2 at 12 min showed the minimum activity (232.00 + 1.74 µmol/g) (Figure 2).

The β-carotene bleaching activity at 1000 µg/ml is presented in Figure 2. This assay's results are similar to the data obtained from the DPPH and FRAP assays, but the antioxidant activity is slightly higher. This implied that this assay had greater sensitivity to our sample contents than the DPPH assay. The higher sensitivity could be due to the fact that a high level of phenolic content is responsible for blocking lipid oxidation (Conforti *et al*., 2007).

Our results show that elevated roasting temperatures increase the involvement of Maillard-type antioxidants. As phenolic antioxidants that naturally occur in RSBs are increased during roasting, the formation of other antioxidants from Maillard reactions during roasting can enhance the antioxidant activity of RSBs. Compared to a low roasting temperature, a medium roasting temperature indicated lower radical scavenging activity due to the occurrence of Maillard reactions of polyphenol, and thus the antioxidant activity also depends on the roasting temperature and type of RSB (Krings *et al.*, 2000; Giampiero *et al*., 2009).Many article revealed that, other processing can increase antioxidant compounds and antioxidant activity with corresponding with Dajanta *et al.* (2013) revealed that Thai fermented soybeans (thua ednao) in black and yellow soybeans are enhanced TPC TFC and antioxidant capacities. They explained that β-glucosidase is produced by *B. subtilis* catalyzing the release TPC and TFC from the soybean during fermentation. Then other making way of cell membranes and cell walls broken down a side from roasting process shall be effected antioxidant compounds and activity is increased.

Table 2. Mutagenicity of sample extracts towards *S. typhimurium*

Sample		Amount (µg/plate)	Number of His^+ revertants/plate	
			TA 98	TA 100
Negative		-	19 ± 2	91 ± 13
SJ 5	12 min	6.25	20 ± 3	94 ± 8
		12.50	19 ± 2	92 ± 7
		25.00	22 ± 3	90 ± 12
	15 min	6.25	16 ± 2	83 ± 4
		12.50	16 ± 3	80 ± 10
		25.00	20 ± 4	61 ± 12
	18 min	6.25	23 ± 2	70 ± 4
		12.50	17 ± 2	80 ± 15
		25.00	20 ± 4	102 ± 14
CM60	12 min	6.25	22 ± 3	87 ± 3
		12.50	18 ± 2	87 ± 5
		25.00	20 ± 4	76 ± 4
	15 min	6.25	23 ± 1	83 ± 6
		12.50	21 ± 2	67 ± 6
		25.00	16 ± 1	68 ± 4
	18 min	6.25	18 ± 4	63 ± 3
		12.50	15 ± 3	64 ± 7
		25.00	13 ± 3	65 ± 5
CM84-2	12 min	6.25	17 ± 2	122 ± 14
		12.50	17 ± 1	85 ± 34
		25.00	23 ± 3	90 ± 11
	15 min	6.25	14 ± 2	68 ± 25
		12.50	15 ± 1	67 ± 21
		25.00	16 ± 3	72 ± 14
	18 min	6.25	15 ± 1	70 ± 8
		12.50	17 ± 4	63 ± 7
		25.00	23 ± 5	71 ± 19
Positive		-	2050 ± 68	907 ± 35

Numbers of colonies were showed as Mean + S

Mutagenicity of RSB

The results of the bacterial reverse mutation assay (Ames test) with the RSBs are shown in Table 2. The results showed that almost all of the extracts were not mutagenic to *S. tymphimurium* TA98 and TA100. Therefore, RSB is a non-mutagenic substance and safe. The result as Albertini *et al.* (1985) reported that the mutagenicity of roasted coffee beans using *S. typhimurium* TA100 were formed at temperatures below 220°C, but roasted coffee the beans must approved a temperature of 220°C. Then our studies RSB are roasted at 230°C, therefore RSB are not mutagenicity.

Anticancer activity of RSB

The effects of RSBs on the MCF-7 cell line (ATCC HTB-22), derived from breast adenocarcinoma, and were examined. All RSBs not showed respond on anti-cancer activity (if RSBs have % Inhibition < 50% with rang 5.09 - 20.99% and interpret as inactive, we will not any indicate). Thus, the phenolic phytochemicals of the RSBs are not associated with anti-cancer causing activities.

Conclusion

The data from three Thai soybeans in this study provides evidence that RSBs are a source of phytochemicals, which have high levels of

phenolic compounds, antioxidant activities and are not mutagenic. The extracts from RSBs showed strong antioxidant activity, and phenolic compounds in substantial quantities makes RSBs suitable as sources of natural antioxidant phenolics. Maillard reaction products, in addition to the extracts of RSBs, are thought to be active. Each cultivars had different properties, with SJ5 having the strongest antioxidant activity and greatest amount of phenolic compounds.Thus, this study showed properties of RSB for increasing of antioxidant activity with safety for non-mutation which is cancer initialization factor. For further study RSB was knew properties of non-mutagenicity. The cytotoxity for cell line, oral cavity need serious for further developing RSB as instead of coffee used.

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