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The effect of cooking process on antioxidant activities and total phenolic compounds of five colored beans

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Abstract

Beans are sources of many nutrients including proteins, carbohydrate, minerals, vitamins, dietary fiber and other bioactive compounds with protective and therapeutic effects towards oxidative stress and cell damage related diseases. Although many previous researches have been focused on antioxidant properties of beans, limited information on the comparison of five colored beans under cooking process regarding their antioxidant properties and total phenolic compounds (TPC) is currently available. Thus, the aim of this study was to investigate the effect of cooking process on antioxidant activities and TPC of five colored beans including mungbean, black bean, red kidney bean, white bean and soybean. Raw and beans cooked in boiling water for 20 min were freeze-dried and ground using a grinder. All samples were extracted with 70% (v/v) aqueous ethanol for 2 hours at 30 °C. The antioxidant activities were analyzed using 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, and TPC was determined using Folin–Ciocalteu reagent. As results, the antioxidant activities and TPC of all five colored beans were elevated after the cooking process (DPPH values of 200-800 µmole TE/100 g dry weight and 400-1500 µmole TE/100 g dry weight of raw and cooked samples, respectively, and TPC values of 40-300 mg GAE/100 g dry weight and 80-300 mg GAE/100 g dry weight of raw and cooked samples, respectively). Black bean exhibited the highest TPC and DPPH scavenging activities, followed by red kidney bean, mung bean, soy bean and white bean, respectively. The high antioxidant activities and TPC in black bean might be due to the content of extracted bioactive compounds such as flavonol glycosides, anthocyanins and tannins. In addition, heat treatment greatly affected antioxidant activity and TPC of beans. This is possibly because of the disruption of plant cell walls during cooking, and sequentially, more bioactive compounds are released from the plant cells.

Keywords: beans, antioxidant activity, 1-diphenyl-2-picrylhydrazyl assay, total phenolic compound, cooking process

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1. Introduction

Natural products are promising sources for novel therapeutic agents for prevention and treatment of several severe diseases. Diet rich in bioactive phytochemicals has long been recognized to support health benefits. Beans with abundant micronutrients such as anthocyanins,lecithin, and trypsin inhibitors are economical crops, which are cultivated and consumed throughout the world. Traditional Chinese medicines are believed that five colored beans (i.e. mung bean, black bean, red kidney bean, white bean and soybean) can protect different five internal organs according to their colors. Black bean, a rich source of isoflavone and anthocyanin, is reported to support improvement of the kidney to eliminate toxin, invigorate blood circulation, strengthen the muscles and bones and protect against DNA damage (Azevedo *et al.,* 2003). Red kidney bean with high dietary fibers is believed to strengthen the heart, relax bowel, control blood pressure and adjust blood sugar level (Tovar *et al.,* 1992). Mung bean (green bean) with sweet taste and cold nature can tonify the liver, decrease cholesterol level, act as anti-allergic food and exhibit anti-inflammatory property (Zhang *et al.,* 2013; Lerer-Metzger *et al.*, 1996). Soybean (yellow bean), a saponin rich source, can reinforce the spleen through secretion of cholic acid that helps digesting fat, thus promoting fat absorption (Bed and Roberts, 1995). Lastly, white bean contains saponin, urease and multiple globulin, thus supporting lung tonification, immunity improvement, lymph T cells activation and DNA synthesis (Marzo *et al.,* 1991). Interestingly, previous studies reported that high antioxidant activities and total phenolic compounds (TPC) were found in pigmented beans such as black and red beans (Amarowicz and Pegg, 2008).

Five colored beans are widely acknowledged as sources of anti-oxidative agents with protective and therapeutic effects towards oxidative stress related diseases such as cancers, inflammation and neurodegenerative diseases (Ramani *et al*., 2004; Rynoso-Camacho *et al.,* 2006; Mazza, 2007). However, these beans cannot be consumed directly and have to be cooked. Generally, heat negatively affects health promoting properties, depending on both internal and external factors including food matrices, cooking methods (i.e., temperature, time and pressure) and quantity/quality of bioactive compounds (i.e., degradation of released compounds or formation of new compounds, synergistic combinations or counteracting of degraded compounds, and solubility of antioxidant compositions). For example, heat treatment is a significant factor that causes a decrease in antioxidant activity and TPC in black soybean (Xu and Chang, 2008). The TPCs of Chinese cabbage and broccoli were decreased by 2% and 41%, respectively, after conventional cooking (Lima *et al*., 2009; Gawlik-Dziki, 2008). It was also suggested that shorter cooking time in stream cooking could preserve more anti-oxidative agents in vegetables than those in conventional cooking (Chipurura *et al*., 2010).

Interestingly, opposite results were observed with some investigated plants, in which heating process could increase antioxidant activity and TPC. For example, elevated antioxidant activity and TPC were found in streamed yellow soybean comparing to its counterpart, raw bean (Xu and Chang, 2008). Besides, previous research was reported that chick pea (*Cicer arietinum*) and green pea (*Pisum sativum*) exhibited higher DPPH values after being heated (Nithiyanantham *et al.,* 2012). Similar results were reported on tomato puree, in which the heating process (95 ^{o}C) could increase antioxidant activity (Nicoli *et al.,* 1997). However, this activity was varied according to the heating time.

From these results, it could be suggested that the antioxidants and TPC levels were seemed to be varied according to types of vegetables and not cooking methods. Despite this information, there is no report on the effect of cooking process and the comparison of five colored beans regarding their antioxidant activities and TPC. Therefore, the objective of this study was to investigate the effect of cooking process on antioxidant activities and TPC of five colored beans including mung bean, black bean, red kidney bean, white bean and soybean.

2. Materials and Methods

2.1 Sample preparation

All beans including mung bean, black bean, red kidney bean, white bean and soybean were purchased from local market under the brand names, Khaothong (Thai Food Industry (1964) Co., Ltd, Bangkok, Thailand) and Raitip (Thai cereals world (1957) Co., Ltd, Bangkok, Thailand). These beans were boiled (1:2 w/v) at 96-98 °C for 20 minutes before being freeze-dried. The samples were ground in to powder by a cyclotex sample mill (series 1903 with 200-240 V and 50/60 Hz from FOSS, Höganäs, Sweden) until the particle size was approx. 80 sieve (0.18 mm).

Bean powder (1 g) was extracted with 70% (v/v) aqueous ethanol (20 mL) for 2 hours at 30 °C. The extractant was filtered through WhatmanNo.1 filter paper and stored at 4 °C for further analysis. The moisture content of the freeze-dried samples was analyzed according to the method of Association of Official Analytical Chemists (AOAC, 2000). Moisture content of the freeze dried samples was approx. 5–8%.

2.2 DPPH free radical scavenging activity

Antioxidant activity was determined using 1,1–diphenyl–2–picrylhydrazyl (DPPH) radical scavenging assay. This assay was performed according to the method of Fukumoto and Mazza, 2000 (Fukumoto and Mazza, 2000) with some modifications as follows. The samples (22 μL) were mixed with DPPH solution (150 μM in 95% (v/v) aqueous ethanol), an indicator of free radical scavenging activity, in a 96–well flat–bottom microplate before being incubated in dark at room temperature for 30 minutes. The reaction was monitored at a wavelength of 520 nm using a microplate reader (BioTek Instruments, Inc., Winooski, VT) and Gen5 data analysis software. Trolox solution (0.08, 0.16, 0.32, 0.64 or 1.28 mM) was used as a standard (R^2=0.99). The free radical scavenging activities were presented as trolox equivalent (TE) per 100 gram dry weight of bean.

2.3 Determination of total phenolic compounds

Total phenolic compounds were determined according to the procedure of Ainsworth and Gillespie, 2007 (Ainsworth and Gillespie, 2007). The samples (25 μL) were mixed with Folin–Ciocalteu reagent (10% v/v, 50 μL) in the 96–well flat–bottom microplate. After 5 minutes of incubation, saturated sodium bicarbonate (7.5% w/v, 200 μL) was added, and the reaction was mixed well. The mixture was then incubated in dark at room temperature (25 °C) for 2 hours. The absorbance at 765 nm was measured using the microplate reader. Gallic acid (10, 20, 40, 60, 80, 100 and 200 μg/mL) was used as a standard (R^2=0.99). The TPC was expressed as gallic acid equivalents (GAE) per 100 gram dry weight.

2.4 Statistical analysis

All experiments were performed in triplicated (n=3). All data were expressed as mean of triplicate experiments ± standard deviation (SD). One way analysis of variance (ANOVA) and Tukey's multiple comparison tests were performed to determine the significant differences at p<0.05. The significant differences of the effect of cooking was determined by independent t-test group with at p<0.05. All statistical analyses were carried out using SPSS statistical analysis (version 16 for Windows, SPSS Inc., Chicago, USA).

3. Results and Discussion

Antioxidant activities of raw (uncooked) five colored beans were in the range of 260 to 817 μmole TE/100g dry weight (Table1). Black bean was found to exhibit the highest antioxidant activity (817 μmole TE/100g dry weight), followed by red kidney bean, mung bean, soybean and white bean, respectively. Similar results were observed with TPC (45-305 mg GAE/100g dry weight), in which black bean provided the highest quantity of TPC (305 mg GAE/100g dry weight), followed by mung bean, red kidney bean, soy bean and white bean, respectively (Table1). The Pearsion's correlation of TPC and DPPH scarvenging values in raw bean and cooked bean were significantly high and were showed as 0.816 and 0.910, respectively ($p<0.05$).

It was possible that black bean contains high quantity of phytochemicals such as anthocyanins, tannins and other flavonoids with antioxidant activities. Anthocyanin, a major color pigment in black bean, is belonged to flavonoids and can act as a powerful antioxidant that can effectively fight against oxidative stress environments. Previous researches suggested that anthocyanins might be responsible for protection against DNA damage that caused by highly reactive free radicals (López-Reyes *et al*., 2008; Tsuda *et al*., 1994; Lazze *et al*., 2003). In addition to anthocyanins, delphinidin, which represents approx. 56% of the total anthocyanins in black beans (Takeoka *et al*., 1997), was found to be antagonist of the mutagenic activity of a final metabolite of benzyl-α-pyrene, a strong polycyclic aromatic hydrocarbon oxidant, *in vitro* (Huang *et al*., 1983). As well, condensed tannins isolated from black beans (0.24–24 μM) were found to be ineffective toward the growth of normal cells, but they were capable of inducing cancer cell (Caco-2 colon, MCF-7 and Hs578T breast, and DU 145 prostatic cancer cells that induced by oxidative stress environments) death by apoptosis (Bawadi *et al*., 2005). All these information suggested that high quantity of anthocyanins, tannins and other flavoniods in black bean possibly contributed to high level of antioxidant activity and TPC as being observed in this study.

Interestingly, heat treatment was found to be a significant factor that increased antioxidant capacity and TPC of beans. The antioxidant activities and TPC of cooked beans were approx. 1-3 times higher than those of raw beans (Table1). It is possible that heat treatment could promotes plant cell wall disruption, leading to an increased release of bioactive compounds from beans. In addition, heating process could breakdown glycoside moiety of flavonoids to form alglycone, which exhibited higher antioxidant capacity (Xu and Chang, 2008). Interestingly, these results were also corresponded to the previous research with Beniseed (*Sesamum indicum L.*), in which the antioxidant activity and quantity of bioactive compounds especially total flavonoid contents were increased after boiling Beniseeds for 30 minutes (Adeniyan *et al*., 2013). Besides, it was previously showed that heat treatment could increase TPC in seed coats of beans (Kruawan *et al.,* 2012). It was found that darker colored seed coats of cooked bean (such as black bean and red kidney bean) possessed higher TPC than lighter colored seed coats of cooked beans (such as mung bean and soybean). Since antioxidant activities were found to be correlated with TPC, higher TPC in darker colored seed coats of cooked beans could lead to higher antioxidant activities than lighter colored seed coat of cooked beans, the results that were corresponded to this research. On the other hand, some study showed decrease antioxidant activity after heat treatment, which was correlated to TPC. It found that red kidney bean (*Phaseolus vulgaris*) exhibited decreased TPC when comparing to its raw bean (Yasmin *et al*.,2008). Thus, the antioxidant activity in cooked beans can be described as synergistic combinations or interruption of biochemical reactions, loss of water-soluble antioxidant compositions, and formation or breakdown of bioactive compounds (such as phenolics).

Table 1. Comparisons of antioxidant activity between raw and cooked beans by DPPH free radical scavenging activity assay and total phenolic compounds.

Type of bean	Antioxidant activity			
	TPC (mg GAE/100g dry weigh)		DPPH (µmoleTE/100g dry weigh)	
	Raw	Cooked	Raw	Cooked
Black bean	305 ± 6.82^{*,a}	294 ± 3.13^{*,A}	817 ± 36.09^{*,a}	1469 ± 19.71^{**,A}
Red kidney bean	215 ± 9.41^{*,b}	220 ±10.48^{*,C}	719 ± 13.24^{*,b}	1098 ± 36.7^{**,B}
Mung bean	192 ± 1.38^{*,c}	237 ± 1.61^{**,B}	493 ± 21.87^{*,c}	880 ± 34.68^{**,C}
Soy bean	162 ± 2.53^{*,d}	174 ± 1.62^{**,D}	181 ± 7.00^{*,e}	538 ± 0.85^{**,D}
White bean	45 ± 2.27^{*,e}	83 ± 7.09^{**,E}	260 ± 10.03^{*,d}	417 ± 3.53^{**,E}

.*,** Significant difference between raw and cooked beans (p<0.05).

a,b,c,… Significant difference of raw bean (p<0.05).

A,B,C,… Significant difference of cooked bean (p<0.05).

4. Conclusion

Black bean exhibited the highest antioxidant activity and TPC among all investigated beans, suggesting that black bean could be a potential source of anti-oxidative agents for further development of nutraceuticals or dietary supplement regarding protective and therapeutic effects on oxidative stress related diseases. Besides, heat treatment is a significant factor that could statistically increase antioxidant activity and TPC, thus consumption of cooked beans could be promoted as functional food with health benefits towards prevention and reducing risk of diseases caused by oxidative stress.

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