

## Total phenolic content and antioxidant capacity of beans: organic vs inorganic

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### Abstract

The purpose of this study was to evaluate and compare the effect of different cooking procedures on the total phenolic content and antioxidant capacity of organic and inorganic beans based on the increasing demand of organic food products. The total phenolic content and antioxidant capacities of eight types of beans matched to the organic and inorganic samples was analyzed based on three different conditions namely raw (R), cooked without soaking (CWS) and cooked after soaking (CAS). Changes in these variables before and after processing were compared between organic and inorganic beans. CAS caused significant ( $p < 0.05$ ) losses of total phenolic content and antioxidant capacity than CWS. Although cooking caused reduction in total phenolic content and antioxidant capacity, no prevalence losses from either type of organic or inorganic bean was found. In general, black bean, red bean, green bean, red kidney bean and soybean from both organic and inorganic types of beans possessed higher total phenolic content and antioxidant capacity, whereas red dhal, yellow dhal and chickpea possessed lower levels of both parameter assessed. All antioxidant capacity assays showed positive and significant correlation ( $p < 0.001$ ) with total phenolic content. This paper provides new information on effect of cooking procedures on the health relevant functionality of organic beans. Knowing that the price of organic beans can be doubled of inorganic beans, this study provides an insight on the importance to balance out the cost and benefits of organic beans.

### Keywords

Chia

Chemical composition

Natural antioxidant

DPPH

FRAP

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### Introduction

Organically grown food products without synthetic insecticides and fertilizers are perceived as more healthier and safer than the inorganic food products (Dardak *et al.*, 2009; Menga *et al.*, 2014). The availability of organic food products in supermarket, health awareness on safe food consumption and environmental friendly behavior has led to the selection of organic foods by the consumers (de Maya *et al.*, 2011; Hjelmar, 2011). The organic food industry has expanded and contributed to high revenue for the traders especially in large market such as the United Kingdom (Soil Association, 2002) and China (Li *et al.*, 2013). The same trend was observed in Malaysia, reflected by growth of organic agriculture with the increment of total land use for organic agriculture from 130 hectares in year 2001 to 2400 hectares in year 2007 (DOA, 2009; Shaharudin *et al.*, 2010).

Beans are one of the food produced in organic farming. This legume has provided the invaluable nutrition resource for both human and animal (Sasipriya and Siddhuraju, 2012). Among different types of beans, the common bean (*Phaseolus vulgaris*

L.) has been widely consumed worldwide as the source of calorie and nutrient intake especially in developing countries (Reyes-Moreno *et al.*, 1993; Talukder *et al.*, 2010). Beans including soybean and green bean were listed as among the top 50 commodities production and exported food source for Asian regions (FAO, 2013). This implies the importance of beans as one of the food that contribute to the economic growth of a country. Protein content in beans was approximately three times higher than other cereal crops (Zhao *et al.*, 2014). It has been recognized as an important source of protein especially for vegetarian and contain complex carbohydrates, dietary fiber, protein with good amino acid profile (high lysine), important vitamins (B vitamins) and minerals (iron and folate), as well as antioxidants and polyphenols (Derbyshire *et al.*, 2011). Bioactive compounds, especially polyphenols in beans include flavonoids, phenolic acids and procyanidins which function as free radical scavengers, reducing agents and metal chelators and possess hypocholesterolemic, anti-atherogenic, anti-carcinogenic and hypoglycemic characteristics (Cardador-Martínez *et al.*, 2002; Djordjevic *et al.*, 2011).

Beans are usually consumed after cooking in

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order to increase the palatability and bioavailability of nutrients (Akanke *et al.*, 2010; Xu and Chang, 2008). Different processing techniques applied on beans such as soaking, boiling, roasting and fermentation may significantly affect the total phenolic content and antioxidant capacity of the beans (Açar *et al.*, 2009). However, total phenolic content and antioxidant capacity of beans may vary according to different cultivation system (Balisteiro *et al.*, 2013). The literature evaluating polyphenol content and antioxidant capacity among organic and inorganic vegetables after domestic cooking has showed mixed results according to the type of polyphenols measured and vegetable cultivar (Faller and Fialho, 2009). Moreover, there is a paucity in the study of comparing the effect of cooking on health-promoting total phenolic content and antioxidant capacity between organic and inorganic beans. Therefore, this study was conducted with the aim to determine and compare the total phenolic content and antioxidant capacity in eight types of organic and inorganic beans.

## Materials and Methods

### Chemical and reagents

Gallic acid, Folin-Ciocalteu, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) and 4,6-Tris(2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma Chemical Co. St Louis, MO, USA. Ethanol, sodium carbonate, potassium persulfate, acetic acid and ferric chloride were purchased from Merck Co. Ltd. All chemicals used were of analytical quality grade.

### Samples preparation

Samples of eight types of beans, i.e. adzuki bean, black bean, soybean, red kidney bean, chickpea, mung bean, red dhal and yellow dhal from each organic and inorganic type of beans were purchased from two different supermarkets in Kuala Lumpur, Malaysia. The studied cultivars were based on the availability of the organic beans that matched to the inorganic samples, since organic production is limited. Approximately 300 g of each type of bean from each type of organic and inorganic was randomly sampled from the market shelves.

The samples were prepared in three different ways: raw (R), cooked without soaking (CWS) and cooked after soaking (CAS). Each selected beans from two locations were mixed together prior to processing. The raw samples were dried at  $60 \pm 2^\circ\text{C}$

in the convection oven until fully dried, followed by grinding to obtain homogenous sample and stored at  $4^\circ\text{C}$  until further analysis. The processing procedure for CWS and CAS samples was adapted from previous study with slight modifications (Kaur and Sadana, 2013). The CWS sample was prepared by boiling 100 g of each type of bean in 500 ml of water for 45 minutes; the CAS sample was soaked in 500 ml of water for 10 hours, the soaking water was then discarded and the soaked beans were cooked using same procedure as the CWS sample. The broth was removed after cooking followed by drying the cooked beans at  $60 \pm 2^\circ\text{C}$  in the ventilated oven until the beans were completely dried. This was followed by grinding to obtain homogenous sample and stored at  $4^\circ\text{C}$  until further analysis.

### Extract preparation

The extraction method was determined according to method by Velioglu *et al.* (1998). About 2 g of grounded sample was mixed with 50 ml of 70% ethanol (ethanol:water, 70:30. v/v) and incubated in shaking water bath (Model BS-06/11/21/31, JEIO Tech Co. Ltd., Korea) set at 150 rpm for 2 hours at  $70^\circ\text{C}$ . Following the incubation, the supernatants were filtered with Whatman No.1 filter paper. The supernatants, considered to be polyphenol extracts, were stored at  $-20^\circ\text{C}$  until analysis for their total phenolic content and antioxidant capacity.

### Estimation of total phenolic content

The total phenolic content in extracts was determined in triplicates by using Folin-Ciocalteu colorimetric method as described by Xu and Chang (2007). About 50  $\mu\text{l}$  of sample extract, 3 ml of distilled water, 250  $\mu\text{l}$  Folin-Ciocalteu reagent and 750  $\mu\text{l}$  of 7% sodium carbonate were mixed and incubated at room temperature for 8 minutes. About 950  $\mu\text{l}$  of distilled water was then added to the mixture and allowed to stand for 2 hours at room temperature. The absorbance was measured at 765 nm using a spectrophotometer (Secomman) against the distilled water blank. Standard curve was constructed using 0 – 500 mg/L gallic acid. Total phenolic contents were expressed as mg of gallic acid equivalent (GAE)/100 g extract.

### Estimation of DPPH radical scavenging activity

The DPPH radical scavenging activity of extracts was determined in triplicates using method described by Brand-Williams *et al.* (1995). Ethanol solution of DPPH radical was prepared at concentration of 0.025 g/L by mixing 25 mg of DPPH with 100 ml ethanol. About 0.5 ml of sample extracts was mixed with 3.5

ml ethanol solution of DPPH radical. This mixture was vortexed for 1 minute and incubated in dark for 30 minutes at room temperature. The absorbance was measured at 515 nm using a spectrophotometer (Secomman) against 70% ethanol blank. The trolox standard was prepared in the range of 0 – 150  $\mu$ M. The concentration of DPPH was calculated from trolox standard curve and expressed as  $\mu$ mol trolox equivalents/100 g extract.

#### *Estimation of ABTS radical scavenging activity*

ABTS radical scavenging activity of extracts was determined in triplicates according to Re *et al.* (1999) with slight modification by Siddhuraju and Becker (2003). The ABTS<sup>+</sup> cation radical was produced by the reaction between 5 ml of 7 mM ABTS solution and 5 ml of 2.45 mM potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) solution, and stored in the dark at room temperature for 16 hour. Prior to usage, 1 ml of this solution was diluted with approximately 50 ml of 70% ethanol to get an absorbance of  $0.700 \pm 0.020$  at 734 nm against 70% ethanol blank. 50  $\mu$ l of sample extract was added to 5 ml of ABTS<sup>+</sup> working solution and incubated in the dark at 30°C for 30 minutes. The absorbance was measured at 734 nm using a spectrophotometer (Secomman) against 70% ethanol blank. The trolox standard were prepared in the range of 0 – 1500  $\mu$ M. The antioxidant capacity was calculated from trolox standard curve and expressed as  $\mu$ mol trolox equivalents/100 g extract.

#### *Estimation of FRAP (ferric reducing antioxidant power)*

The ferric reducing antioxidant power (FRAP) of sample extract was analyzed in triplicates using method from Benzie and Strain (1996) with slight modifications by Chaieb *et al.* (2011). The FRAP reagent was prepared by mixing 2.5 ml of 10 mM TPTZ in 10 ml of 40 mM HCl, 2.5 ml of 20 mM FeCl<sub>3</sub>·6H<sub>2</sub>O solution and 25 ml of 0.3 M acetate buffer (3.1 g of CH<sub>3</sub>COONa·3H<sub>2</sub>O and 16 ml glacial acetic acid), pH 3.6 in the ratio of 1:1:10 (v/v). 1 ml of sample extract was mixed with 4 ml of freshly prepared FRAP reagent and incubated at room temperature for 10 minutes. Following incubation, the absorbance was measured at 593 nm using a spectrophotometer (Secomman) against 70% ethanol blank. The trolox standard were prepared in the range of 0 – 1000  $\mu$ M. The antioxidant capacity was calculated from trolox standard curve and expressed as  $\mu$ mol trolox equivalents/100 g extract.

#### *Statistical analysis*

The data collected were analyzed using Statistical

Package for Social Sciences (SPSS) version 20.0. Descriptive statistics were used to determine the mean and standard deviation of all data. Independent T-test was used to compare the total phenolic content and antioxidant capacity between organic and inorganic beans of the same varieties. One-way analysis of variance (ANOVA) was used to compare total phenolic content and antioxidant capacity between raw and different processed beans, and between different varieties of beans. Pearson's correlation was used to assess the linear association between total phenolic content and antioxidant capacities. Results were expressed as mean and standard deviation and P value of < 0.05 was considered to be statistically significant.

## **Results and Discussion**

### *Total polyphenol content in raw and cooked organic and inorganic beans*

In this study total phenolic content in eight types of organic and inorganic beans were determined. Total phenolic content of organic and inorganic beans are shown in Table 1. Majority of the raw organic beans showed higher total phenolic content compared to the inorganic beans, with the exception of chickpea and green bean. Total phenolic content in raw organic beans varied from 144.0 mg GAE/100 g in chickpeas to 453.77 mg GAE/100 g in black beans. In the raw inorganic beans, adzuki beans showed the highest total phenolic content at 395.38 mg GAE/100g and red dhal had the lowest value with 135.71 mg GAE/100g.

Faller and Fialho (2009) has reported similar results to the current study which was not all the organically grown vegetables showed higher total phenolic content than the inorganic vegetables. On the other hand, Balisteiro *et al.* (2013) has reported higher total phenolic content in soybeans grown by organic cultivation. Organic cultivation may result in foods with higher total phenolic content mainly for two hypotheses. First, the usage of synthetic fertilizers in inorganic cultivation provide more bioavailable sources of nitrogen, which may accelerate the development of plant but not the production of secondary metabolites for growth. Secondly, the absence of synthetic pesticides may leads to higher exposure to stressful situations, thus initiate the production of natural defense substances in plants such as polyphenols (Winter and Davis, 2006).

Generally, all the bean samples showed a reduction of total phenolic content after cooking regardless the samples were organic or inorganic

Table 1. Total polyphenol content in raw and cooked organic and inorganic beans

Sample	Type of bean <sup>§</sup>	Raw beans (mg GAE/100 g)	TPC (mg GAE/100g)	
			CWS	CAS
Adzuki bean	OG	420.40 ± 1.60 <sup>a</sup>	237.07 ± 10.51 <sup>b*</sup>	129.66 ± 8.33 <sup>c*</sup>
	IO	395.38 ± 12.52 <sup>a</sup>	192.60 ± 8.02 <sup>b*</sup>	171.36 ± 16.66 <sup>b*</sup>
Black bean	OG	453.77 ± 13.13 <sup>a*</sup>	180.58 ± 4.24 <sup>b</sup>	169.47 ± 6.99 <sup>b*</sup>
	IO	238.04 ± 5.56 <sup>a*</sup>	189.87 ± 5.78 <sup>b</sup>	137.10 ± 8.48 <sup>c*</sup>
Soybean	OG	209.31 ± 6.41 <sup>a*</sup>	168.57 ± 8.33 <sup>b*</sup>	147.25 ± 4.24 <sup>b*</sup>
	IO	156.54 ± 5.78 <sup>a*</sup>	135.24 ± 4.81 <sup>a*</sup>	91.73 ± 8.48 <sup>b</sup>
Red kidney beans	OG	305.60 ± 6.41 <sup>a*</sup>	181.54 ± 3.21 <sup>b*</sup>	148.23 ± 5.78 <sup>c*</sup>
	IO	243.61 ± 8.33 <sup>a*</sup>	118.58 ± 7.35 <sup>b*</sup>	88.05 ± 2.78 <sup>c*</sup>
Chickpea	OG	144.00 ± 6.99 <sup>a*</sup>	131.11 ± 2.78 <sup>a*</sup>	119.98 ± 10.01 <sup>a</sup>
	IO	212.55 ± 6.99 <sup>a*</sup>	158.88 ± 9.62 <sup>b*</sup>	130.17 ± 11.56 <sup>c</sup>
Mung bean	OG	268.99 ± 10.51 <sup>a</sup>	208.84 ± 7.35 <sup>b*</sup>	183.80 ± 12.72 <sup>b</sup>
	IO	277.37 ± 9.75 <sup>a</sup>	273.70 ± 12.53 <sup>a*</sup>	188.46 ± 5.78 <sup>b</sup>
Red dhal	OG	146.82 ± 9.75 <sup>a</sup>	120.90 ± 6.99 <sup>ab</sup>	101.46 ± 1.60 <sup>b</sup>
	IO	135.71 ± 4.24 <sup>a</sup>	96.82 ± 6.99 <sup>b</sup>	97.75 ± 2.78 <sup>b</sup>
Yellow dhal	OG	154.26 ± 3.21 <sup>a</sup>	122.73 ± 7.35 <sup>b</sup>	99.58 ± 8.93 <sup>b</sup>
	IO	139.40 ± 7.35 <sup>a</sup>	118.11 ± 3.21 <sup>a</sup>	82.95 ± 3.21 <sup>b</sup>

Values are expressed as mean ± standard deviation of three replications.

<sup>§</sup>Refer to organic or inorganic bean.

\*Significant difference between type of bean within each cooking procedure ( $p < 0.05$ ) according to independent t test.

Rows with different letters are significantly different between raw and cooking procedures ( $p < 0.05$ ) based on one way ANOVA.

TPC: Total polyphenol content; GAE: Gallic acid equivalent; OG: organic; IO: inorganic; CWS: cooked without soaking; CAS: cooked after soaking.

beans (Figure 1). Significantly lower total phenolic content ( $p < 0.05$ ) were observed for all CAS samples as compared to their original raw sample with the exception of organic chickpeas. Overall, CAS seemed to be the cooking procedure that resulted in greater loss of total phenolic content in both organic and inorganic types of beans compared to CWS. Our results were in agreement with previous study, whereby cooking without soaking was the most efficient preparation method in retaining nutrient content and its characteristics (Valdes *et al.*, 2011). Possible explanation to the loss in total phenolic content can be due to leaching of the soluble phenolic into soaking water and cooking water which were discarded in this study, degradation of the polyphenol due to high temperature during cooking, chemical transformation, and formation of phenolic-protein complex within thermal and stress condition (Xu and Chang, 2008).

Interestingly, significant losses in total phenolic content were mainly observed in black beans, adzuki beans and red kidney beans from organic types, in both types of treatment. Inorganic varieties also showed a reduction in total phenolic content. This reduction was more pronounced in adzuki beans and red kidney beans. These types of bean varieties are rich in anthocyanin which is very sensitive to heat and may convert to colorless chalcone upon heating

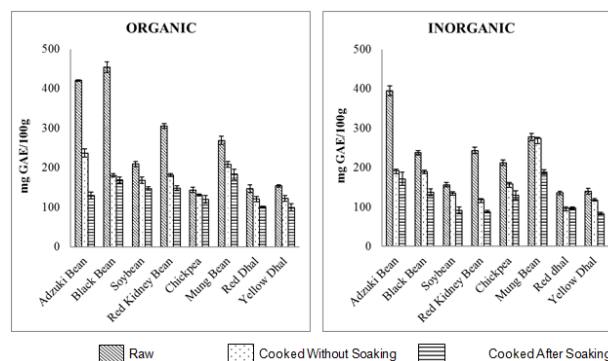


Figure 1. Comparison of total polyphenol content (mg GAE/100 g) from different processing procedures in organic (A) and inorganic (B) beans (mean ± standard deviation)

(Wrolstad *et al.*, 2005). Thus, different varieties of beans contain different types of phenolic compounds shown to possess different sensitivity towards heat. This variation can lead to different response either higher or lower cleavage of phenolic bonds in different bean varieties towards the heat applied (Jeong *et al.*, 2004).

Although beans can only be eaten after cooking, analyses of raw beans are necessary to identify the nutritional content of the beans. Heat treatment such as boiling is the most basic preparation method applied in bean processing since it begin to be used as food by human being. However, this cooking

Table 2. DPPH and ABTS radical scavenging capacity ( $\mu\text{mol TE}/100\text{ g}$ ) of raw and cooked organic and inorganic beans

Sample	Type of bean <sup>§</sup>	DPPH ( $\mu\text{mol TE}/100\text{ g}$ )			ABTS ( $\mu\text{mol TE}/100\text{ g}$ )		
		Raw	CWS	CAS	Raw	CWS	CAS
Adzuki bean	OG	1614.84 $\pm$	581.20 $\pm$	382.76 $\pm$	5254.77 $\pm$	2485.63 $\pm$	1639.68 $\pm$
		12.39 <sup>a*</sup>	19.13 <sup>b*</sup>	18.18 <sup>c*</sup>	34.69 <sup>a*</sup>	24.99 <sup>b*</sup>	19.09 <sup>c</sup>
	IO	1531.44 $\pm$	527.54 $\pm$	460.25 $\pm$	4338.02 $\pm$	1839.59 $\pm$	1661.00 $\pm$
		14.98 <sup>a*</sup>	12.39 <sup>b*</sup>	3.44 <sup>c*</sup>	41.94 <sup>a*</sup>	38.17 <sup>b*</sup>	45.06 <sup>c</sup>
Black bean	OG	1965.03 $\pm$	526.19 $\pm$	414.83 $\pm$	3923.68 $\pm$	2339.48 $\pm$	2047.93 $\pm$
		11.39 <sup>a*</sup>	1.52 <sup>b</sup>	11.47 <sup>c</sup>	12.50 <sup>a*</sup>	28.86 <sup>b*</sup>	24.99 <sup>c*</sup>
	IO	925.61 $\pm$	506.13 $\pm$	411.41 $\pm$	2740.28 $\pm$	1640.09 $\pm$	1206.84 $\pm$
		26.31 <sup>a*</sup>	5.48 <sup>b</sup>	10.63 <sup>c</sup>	38.19 <sup>a*</sup>	19.09 <sup>b*</sup>	62.90 <sup>c*</sup>
Soybean	OG	311.84 $\pm$ 2.38 <sup>a</sup>	191.22 $\pm$	201.50 $\pm$	2721.68 $\pm$	2352.19 $\pm$	2049.08 $\pm$
			5.99 <sup>b*</sup>	8.36 <sup>b</sup>	33.68 <sup>a*</sup>	20.97 <sup>b*</sup>	16.66 <sup>c*</sup>
	IO	299.15 $\pm$ 3.66 <sup>a</sup>	215.02 $\pm$	201.52 $\pm$	2124.47 $\pm$	2066.05 $\pm$	1907.67 $\pm$
			4.95 <sup>b*</sup>	4.95 <sup>b</sup>	22.04 <sup>a*</sup>	22.04 <sup>a*</sup>	30.04 <sup>b*</sup>
Red kidney bean	OG	1892.48 $\pm$	419.76 $\pm$	380.93 $\pm$ 0.0 <sup>c*</sup>	2846.80 $\pm$	1513.59 $\pm$	1483.26 $\pm$
		13.16 <sup>a*</sup>	4.96 <sup>b*</sup>		17.35 <sup>a*</sup>	12.73 <sup>b*</sup>	0.0 <sup>b*</sup>
	IO	1842.32 $\pm$	453.06 $\pm$	280.92 $\pm$	2744.44 $\pm$	1435.75 $\pm$	1097.11 $\pm$
		10.05 <sup>a*</sup>	1.37 <sup>b*</sup>	9.52 <sup>c*</sup>	4.81 <sup>b*</sup>	26.78 <sup>b*</sup>	33.68 <sup>c*</sup>
Chickpea	OG	118.19 $\pm$ 2.99 <sup>a</sup>	92.86 $\pm$	70.62 $\pm$ 3.64 <sup>b</sup>	1624.79 $\pm$	1435.28 $\pm$	1368.34 $\pm$
			3.15 <sup>ab</sup>		19.24 <sup>a</sup>	26.79 <sup>b</sup>	12.73 <sup>b*</sup>
	IO	141.64 $\pm$ 3.15 <sup>a</sup>	103.17 $\pm$	48.41 $\pm$ 5.99 <sup>c</sup>	1682.16 $\pm$	1454.65 $\pm$	1165.66 $\pm$
			3.44 <sup>b</sup>		8.33 <sup>a</sup>	19.25 <sup>b</sup>	25.00 <sup>c*</sup>
Green bean	OG	594.27 $\pm$	468.12 $\pm$	436.29 $\pm$	1712.54 $\pm$	1673.75 $\pm$	1523.41 $\pm$
		2.75 <sup>a*</sup>	3.64 <sup>b*</sup>	8.36 <sup>b*</sup>	12.73 <sup>a*</sup>	14.43 <sup>a***</sup>	14.43 <sup>b*</sup>
	IO	645.14 $\pm$	554.76 $\pm$	395.91 $\pm$	2229.39 $\pm$	1840.83 $\pm$	1420.96 $\pm$
		4.12 <sup>a*</sup>	6.30 <sup>b*</sup>	1.37 <sup>c*</sup>	17.34 <sup>a*</sup>	38.19 <sup>b***</sup>	25.45 <sup>c*</sup>
Red dhal	OG	206.12 $\pm$	99.62 $\pm$	74.99 $\pm$ 2.17 <sup>b*</sup>	998.97 $\pm$	549.06 $\pm$ 8.33 <sup>b*</sup>	485.18 $\pm$
		3.49 <sup>a*</sup>	3.82 <sup>b*</sup>		16.66 <sup>a*</sup>		31.54 <sup>b*</sup>
	IO	246.69 $\pm$	135.10 $\pm$	111.93 $\pm$	1223.92 $\pm$	801.66 $\pm$ 9.62 <sup>b*</sup>	662.90 $\pm$
		2.87 <sup>a*</sup>	2.26 <sup>b*</sup>	1.09 <sup>b*</sup>	30.04 <sup>a*</sup>		12.71 <sup>c*</sup>
Yellow dhal	OG	126.10 $\pm$ 1.03 <sup>a</sup>	92.74 $\pm$	73.99 $\pm$ 3.22 <sup>b</sup>	1143.61 $\pm$	685.04 $\pm$ 12.72 <sup>b</sup>	640.55 $\pm$
			3.89 <sup>ab</sup>		4.81 <sup>a</sup>	22.04 <sup>b</sup>	22.04 <sup>b</sup>
	IO	135.58 $\pm$ 3.22 <sup>a</sup>	98.10 $\pm$ 2.36 <sup>b</sup>	84.12 $\pm$ 1.86 <sup>b</sup>	1079.40 $\pm$	665.63 $\pm$ 28.86 <sup>b</sup>	585.19 $\pm$
					26.78 <sup>a</sup>		20.97 <sup>b</sup>

Values are expressed as mean  $\pm$  standard deviation of three replications.

§Refer to organic or inorganic bean.

\*Significant difference between type of bean within each cooking procedure ( $p < 0.05$ ) according to independent t test.

Rows with different letters are significantly different between raw and cooking procedures ( $p < 0.05$ ) within each antioxidant test based on one way ANOVA.

TE: Trolox equivalent; OG: organic; IO: inorganic; CWS: cooked without soaking; CAS: cooked after soaking

process mainly resulted in loss of total phenolic content in food (Turkmen *et al.*, 2005; Siddhuraju, 2006; Aguilera *et al.*, 2011). Thus, the actual intake of total phenolic content can be overestimated if the reference was based on the data of raw beans.

#### *Antioxidant capacity of organic and inorganic beans after cooking*

Antioxidant compounds react with different radicals or oxidants through several mechanisms of actions. Scavenging of free radicals and inhibition of lipid peroxidation by antioxidants are identified by appropriate antioxidant activities (Marathe *et al.*, 2011). Therefore, more than one test is required to increase the accuracy in measuring the antioxidant

capacities.

The radical scavenging activity of DPPH and ABTS of raw and cooked beans from both organic and inorganic types are presented in Table 2 while changes in ferric reducing antioxidant power between organic and inorganic beans after cooked are shown in Table 3. Antioxidant capacities of adzuki, black and red kidney bean were among the highest as compared to other beans. The colour pigments in these beans could potentially contribute to the antioxidant capacity of the beans.

The antioxidant capacities of the bean samples measured by all the three methods were decreased after cooking, independently of the cooking procedures and types of bean. The same pattern was observed in

Table 3. Ferric reducing antioxidant power ( $\mu\text{mol TE}/100\text{ g}$ ) of raw and cooked organic and inorganic beans

Sample	Type of bean <sup>§</sup>	FRAP ( $\mu\text{mol TE}/100\text{ g}$ )		
		Raw	CWS	CAS
Adzuki bean	OG	7717.89 $\pm$ 84.38 <sup>a*</sup>	920.96 $\pm$ 8.99 <sup>b*</sup>	744.67 $\pm$ 12.37 <sup>c*</sup>
	IO	4795.47 $\pm$ 47.24 <sup>a*</sup>	881.51 $\pm$ 5.45 <sup>b*</sup>	592.53 $\pm$ 8.99 <sup>c*</sup>
Black bean	OG	2945.09 $\pm$ 37.17 <sup>a*</sup>	662.12 $\pm$ 7.43 <sup>b*</sup>	516.94 $\pm$ 6.18 <sup>c*</sup>
	IO	943.29 $\pm$ 11.48 <sup>a*</sup>	606.31 $\pm$ 3.57 <sup>b*</sup>	457.53 $\pm$ 8.25 <sup>c*</sup>
Soybean	OG	724.14 $\pm$ 7.14 <sup>a*</sup>	490.83 $\pm$ 5.45 <sup>b*</sup>	432.42 $\pm$ 4.12 <sup>c*</sup>
	IO	503.92 $\pm$ 8.25 <sup>a*</sup>	400.36 $\pm$ 5.45 <sup>b*</sup>	340.83 $\pm$ 12.54 <sup>c*</sup>
Red kidney bean	OG	1455.88 $\pm$ 7.14 <sup>a</sup>	748.78 $\pm$ 10.71 <sup>b*</sup>	686.99 $\pm$ 10.91 <sup>c*</sup>
	IO	1508.45 $\pm$ 5.46 <sup>a</sup>	664.24 $\pm$ 5.45 <sup>b*</sup>	471.50 $\pm$ 5.45 <sup>c*</sup>
Chickpea	OG	264.52 $\pm$ 5.39 <sup>a</sup>	258.45 $\pm$ 7.43 <sup>a</sup>	201.27 $\pm$ 8.99 <sup>a</sup>
	IO	321.48 $\pm$ 8.99 <sup>a</sup>	270.34 $\pm$ 3.57 <sup>a</sup>	184.62 $\pm$ 7.14 <sup>a</sup>
Green bean	OG	676.70 $\pm$ 5.45 <sup>a*</sup>	600.56 $\pm$ 3.57 <sup>b*</sup>	507.60 $\pm$ 3.57 <sup>c</sup>
	IO	788.69 $\pm$ 8.25 <sup>a*</sup>	717.38 $\pm$ 10.31 <sup>b*</sup>	489.85 $\pm$ 7.14 <sup>c</sup>
Red dhal	OG	226.86 $\pm$ 8.99 <sup>a</sup>	144.73 $\pm$ 11.48 <sup>a</sup>	125.69 $\pm$ 0.0 <sup>a</sup>
	IO	242.33 $\pm$ 7.43 <sup>a</sup>	125.67 $\pm$ 9.45 <sup>a</sup>	122.11 $\pm$ 3.57 <sup>a</sup>
Yellow dhal	OG	458.45 $\pm$ 7.43 <sup>a</sup>	440.44 $\pm$ 12.54 <sup>b*</sup>	398.75 $\pm$ 3.57 <sup>b*</sup>
	IO	436.89 $\pm$ 2.06 <sup>a</sup>	389.29 $\pm$ 5.45 <sup>b*</sup>	369.11 $\pm$ 2.06 <sup>b*</sup>

Values are expressed as mean  $\pm$  standard deviation of three replications.

<sup>§</sup>Refer to organic or inorganic bean.

\*Significant difference between type of bean within each cooking procedure ( $p < 0.05$ ) according to independent t test.

Rows with different letters are significantly different between raw and cooking procedures ( $p < 0.05$ ) within each antioxidant test based on one way ANOVA.

TE: Trolox equivalent; OG: organic; IO: inorganic; CWS: cooked without soaking; CAS: cooked after soaking.

all the antioxidant capacities, where CAS resulted in significant ( $p < 0.05$ ) loss of antioxidant capacities in all varieties of beans samples, for both organic and inorganic types. Reduction in antioxidant capacities can partly be explained by the thermal effect during boiling as well as softening of the cell wall tissue and dissolving of bonded polyphenol into soaking water and cooking water (Xu and Chang, 2007; Boateng *et al.*, 2008). Previous study has reported the loss in both antioxidant and polyphenol content up to 40% in lentils and peas after cooking and soaking (Han and Baik 2008). Results of current study were contrasted with previous study, in which beans that cooked after soaking were reported to possess higher antioxidant activity than the beans cooked without soaking (Valdes *et al.*, 2010).

A mixed result was also observed in the antioxidant capacities assessed from ABTS and DPPH assay between organic and inorganic beans, by which not all the organic beans possess higher antioxidant capacity than inorganic beans after cooking. Nevertheless, all organic samples had shown higher FRAP activity than inorganic beans in CAS procedure. Previous study has suggested that effect of heat on antioxidant capacities in different vegetables was not significantly different between organic and inorganic types even though total phenolic content in

organic vegetables showed higher sensitivity towards heat (Faller and Fialho, 2009).

In addition, antioxidant capacities of bean samples showed inconsistent results among different tests used. Beans that showed higher DPPH scavenging activity may possess lower ABTS and FRAP activity when comparison was made with other beans varieties. The possible explanation of the disparity is due to food processing which can lead to the alteration in both the quantity of total phenolic content and the composition of polyphenols (Makris and Rossiter, 2001). Since different chemical structures of polyphenol compounds have different antioxidant activity and capacity, alteration of the quality and of polyphenol therefore could have different effect on the antioxidant capacities of the samples analyzed.

#### *Association between antioxidant capacities and total polyphenol content*

The association between total phenolic content and antioxidant capacities for all organic and inorganic beans was shown in Table 4. Interestingly, antioxidant capacities from all assays used have shown significant ( $p < 0.001$ ) and linear association with the total phenolic content. This is consistent with the outcome reported by previous study which focused

Table 4. Pearson's correlation coefficient of total polyphenol content and antioxidant capacity in organic and inorganic beans

Parameter	Pearson's correlation coefficient (r)
DPPH radical scavenging activity	0.854***
ABTS radical scavenging activity	0.867***
FRAP activity	0.774***

\*\*\*Significant correlation ( $p < 0.001$ ) between total polyphenol content and each antioxidant capacity.

on the effect of processing on several types of beans and lentils (Han and Baik, 2008). The current findings indicated the substantial contribution of polyphenol content to the related antioxidant activity of beans. In general, the total phenolic content showed strong correlations with the antioxidant capacities of the beans analyzed. Thus, total phenolic content could be used as an indicator in evaluating the antioxidant capacity of beans which may preliminarily applied as natural sources of antioxidant functional foods (Golam *et al.*, 2011).

## Conclusion

In conclusion, this study showed that there were losses of total phenolic content and antioxidant capacity in all samples of cooked beans as compared to their original raw beans, independent of the organic or inorganic types, suggesting that actual intake of total phenolic content could be overestimated if using data of raw beans. In addition, the total phenolic content and antioxidant capacity, whether in raw, CWS or CAS conditions, had showed mixed results among organic and inorganic beans with no prevalence from either type. Despite the possible benefits from organic cultivation, there were not much differences in total phenolic content between organic and inorganic beans after cooking. Despite the possible benefits from organic cultivation, the total phenolic content was reduced after the beans were cooked. Thus, further research should be carried out to assess the loss of other nutrients upon cooking in order to balance out the cost and benefits of organic beans.

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