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The impact of processing on in vitro bioactive compounds bioavailability and antioxidant activities in faba bean (*Vicia faba* L.) and azuki bean (*Vigna angularis* L.)

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Abstract

Even though bean varieties are widely consumed all over the world, data related to how cooking methods and *in vitro* digestion affect bioactive compounds they contain and data related to bioavailability of polyphenols are limited. The aim of the present study was to investigate how some cooking methods and in vitro digestion influence antioxidant activity, total phenols (TP), and total flavonoids (TF) of faba bean and azuki bean. Soaking caused a significant decrease (27.60-38.15%) in the bioavailability of TP of dry faba beans (FB). Soaking in cold water resulted in a significant decrease in TP bioavailability of dry azuki beans (AB). TF content was well retained in AB cooked without soaking but was not detected in FB after *in vitro* digestion. FB soaked in hot water and cooked with the addition of $NaHCO_3$ showed the greatest inhibition effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical ($p < 0.05$) after *in vitro* digestion. *In vitro* digestion caused increase in the antioxidant activity of both FB and AB.

Introduction

Pulses are well known to be an economical source of protein, carbohydrate and fibre, and are low in fat. Pulses are also incorporated in human diets for their additional nutritional benefits, especially their microconstituents including phenolic compounds, oligosaccharides (Bouhnik *et al*., 1997), enzyme inhibitors, phytosterols and saponins (Mathers, 2002; Campos-Vega *et al*., 2010). Intake of legumes is reported to potentially lower the risk of cancer (Aune *et al*., 2009), CVD (Anderson and Major, 2002), hypertension and diabetes (Ranilla *et al*., 2010). Some of the microconstituents are currently marketed as functional foods and nutraceutical ingredients (Ferguson, 2001). Also, there have been many attempts to incorporate pulses into food products for enrichment of product quality and additional health benefits (Gomez *et al*., 2008; Patterson *et al*., 2010).

Some of the above beneficial effects can be due to antioxidant activities of polyphenols legumes contain (Jung *et al*., 2008). The antioxidant capacity of plant foods is derived from the cumulative synergistic action of a wide variety of antioxidants such as vitamins C and E and polyphenols, mainly phenolic acids and flavonoids, carotenoids, terpenoids, Maillard compounds and trace minerals (Pérez-Jiménez *et al*., 2008). Polyphenols are probably the most investigated molecules of nutritional interest. Several plant polyphenols are natural antioxidants with an interesting future in various fields such as food and medicine. Because natural antioxidants have shown a reduction in oxidative stress (Osawa, 1999), some flavonoids have been assayed in various diseases affecting the heart, brain, and other disorders, including those leading to cancer (Pryor, 2000).

Generally legumes cannot be consumed without cooking. The common domestic cooking procedure is pressure cooking. Soaking in water is usually applied prior to cooking to soften texture and reduce the cooking time (Luo *et al*., 2009). The other treatment used in some occasion is cooking legumes with the addition of $NaHCO_3$ in order to shorten cooking time. Heating was reported to result in significant decreases in polyphenols, enzyme inhibitors, phytic acid, some minerals and vitamins, but increase protein digestibility of faba beans (Alonso *et al*., 2000; Luo and Xie, 2013). Interestingly, Acar *et al*. (2009) reported that roasting at 150ºC for 60 min increased the antioxidant capacity of different types of pulses including black bean, borlotti bean, kidney bean, red soybean, yellow bean, giant lentils and chickpea, with an initial fall observed in the yellow and red soybeans after roasting for 10 min. Comparatively, in faba beans, the tannin content increased after roasting at 149ºC /20 min and 177ºC /18 min, but decreased after roasting at 204ºC /14 min and 232ºC /12 min (Anderson *et al*., 1994).

It has been reported that bean consumption exerts beneficial health effects and this is largely related

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to their antioxidant effects (Anderson *et al*., 1999; Cardador-Martinez *et al*., 2002; Fernandez-Panchon *et al*., 2008). These reports also suggest that legumes are excellent dietary antioxidant sources capable of reducing risks of chronic diseases. Although phenolic content has shown a good correlation with antioxidant activity in legumes other non-phenolic compounds including ascorbic acid, phytic acid, tocopherols, carotenoids and saponins could collectively contribute to this antioxidant activity, it is important to obtain knowledge about their bioavailability in order to evaluate their beneficial effects.

Up to the present time, little information is available in the literature regarding the changes in total phenols, total flavonoids, and antioxidant activities following food preparation methods and *in vitro* digestion process. Therefore, this study was conducted to was to investigate the effects of soaking and $NaHCO_3$ addition prior to cooking and in vitro digestion on antioxidant activity, total phenol, and total flavonoid content of widely consumed bean varieties in China.

Materials and Methods

Materials

Faba Beans (*Vicia faba* L.) (FB) and Azuki Beans (*Vigna angularis* L.) (AB) were collected from local market of the same batch in Nanjing, Jiangsu Province, P.R. China. (+)-Catechin hydrate (C-1251), gallic acid (48630), Folin-Ciocalteu phenol reagent (F-9252), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) diammonium salt, 2,2-diphenyl-1-picrylhydrazyl (DPPH), pepsin (P-7000), pancreatin (P-1750), bile extract (B-8631), piperazine-NN'-bis(2-ethane-sulfonic acid) (PIPES) disodium salt (Sigma P-3768), and dialysis tubing (D-9777) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents and solvents commercially obtained were of analytical grade. Acid-washed glassware were used throughout the study.

Soaking

Whole seeds of both FB and AB (100 g) were soaked in 200 mL of cold distilled water. They were left to stand for a night. Besides 100 g whole seeds of FB and AB were soaked in 200 mL boiled distilled water and left to stand for 3 hr. After incubation period, excess water was drained and it was stored at −40ºC until analysis of soaking water.

Cooking

Separate batches of raw or soaked beans were autoclaved at 120ºC for 50 min in distilled water in a bean:water ratio of 1:3 (w/v) or in 0.3%(w/v) sodium bicarbonate $NaHCO_3$ solution.

Extraction

In order to measure antioxidant activities, total phenols (TP), and total flavonoids (TF) of raw materials, beans were grounded into 60 mesh size with laboratory mill. Twenty g of powder was blended with 100 mL of 50% aqueous methanol for 5 min in a waring blender. Mixture was centrifuged at 2,500×*g* for 5 min. Pellet was extracted again with 100 mL solvent and centrifuged for the second time. Supernatants were collected for the analysis of antioxidant activity, TP, and TF.

Cooked samples were homogenized in waring blender with their own cooking water (extra 100 mL of distilled water was added to legumes cooked without soaking, because the legumes absorbed all cooking water). Twenty g of homogenate was extracted with 50 mL of 50% aqueous methanol for 2 times as described above. Supernatants were collected and stored at −40ºC until analysis.

In vitro bioavailability

In vitro bioavailability of the sample was determined according to procedure described by Gil-Izquierdo *et al*. (2002), with slight modifications. Briefly, 10 g of the sample homogenate obtained from cooked beans was placed into 100 mL polystyrene tube and after the addition of distilled water (10 mL), the pH was adjusted to 2.0 with 1 M HCl and mixed with 1 mL pepsin suspension (15,750 U). The mixture was incubated at 37ºC in a shaking water bath (Kelong, China) for 2 hr. At the end of the incubation period, a dialysis bag containing 20 mL PIPES buffer (0.15 N) was placed into the tube. Following 30 min incubation at 37ºC in a shaking water bath, 5 mL of the pancreatin/bile mixture (0.5 g pancreatin + 3 g bile extract/ 250 mL 1 N $NaHCO_3$) was added and the incubation continued for another 2 hr. At the end of the incubation, the dialysis bag was removed and rinsed by dipping in water. Measurements related to intestinal absorption were performed directly to dialysates obtained. Analysis of retentate which corresponds to unabsorbed portion through the intestinal wall was performed with both aqueous phase and methanolic extract. Methanolic extract of retentate was obtained as described above.

Determination of total phenols (TP)

The total phenolic contents of FB and AB were determined according to Xu and Chang (2007) with slight modifications. After adding Folin-Ciocalteau reagent and sodium carbonate to aliquots of samples,

the mixtures were set in a 40ºC water bath for 20 min. The absorbance was measured at 740 nm using a spectrophotometer (Unico, Shanghai, China) and total phenolic contents were expressed as milligrams of gallic acid equivalents (GAE) per grams of defatted sample.

Determination of total flavonoids (TF)

The total flavonoids content of samples was determined by Xu and Chang (2007) with slight modifications. Briefly, properly diluted samples (1 mL) and $NaNO_2$ (0.3 mL, 5%) were mixed. $AlCl_3$ (10%, 0.3 mL) was added at 5 min and 1 m NaOH (2 mL) was added after one additional minute. The absorbance readings of samples were taken at 510 nm. Total flavonoid contents were expressed as milligrams of (+)-catechin equivalents (CE) per gram of sample.

ABTS radical scavenging activity

Antioxidant activities of all extracts were measured according to the procedure described by Re *et al.* (1999). ABTS was dissolved in water to prepare ABTS stock solution (7 mM). ABTS radical cation ($ABTS^{\bullet+}$) was produced by adding 2.45 mM potassium persulfate (final concentration). Diluted $ABTS^{\bullet+}$ solution with an absorbance of 0.70 ± 0.02 at 734 nm was used as working solution. Absorbance readings (734 nm) were taken at 30ºC exactly 5 min after initial mixing of 1 mL of diluted $ABTS^{\bullet+}$ solution and 10 µL of sample solution. UV-visible spectrophotometer (Unico, Shanghai, China) was used to measure absorbances. Antioxidant activity (AA) was expressed as percentage inhibition of ABTS radical by using below equation;

$$AA = 100 - [100 \times (A_{sample}/A_{control})]$$

where A_{sample} is the absorbance of the sample at t = 5 min, and $A_{control}$ is the absorbance of the control.

DPPH radical scavenging activity

The antioxidant activity was determined by DPPH assay according to previous study [9] with some modifications. Aliquot of 200 mL sample mixed with 3.8 mL DPPH solution (200 mM in methanol) was incubated in dark at room temperature for 60 min, then its absorbance at 517 nm was measured by a spectrophotometer. Scavenging ability of the sample to DPPH radical was determined according to the following equation:

Antioxidant activity (AA) was expressed as percentage inhibition of DPPH radical by using below equation;

$$AA = 100 - [100 \times (A_{sample}/A_{control})]$$

where A_{sample} is the absorbance of the sample at t = 60 min, and $A_{control}$ is the absorbance of control.

Statistical analysis

Data were analysed with SPSS (Statistical Package for the Social Sciences) 13.0 for windows. The mean and standard deviation of means were calculated. The data were analysed by one-way analysis of variance (ANOVA). Duncan's multiple range test was used to separate means. Significance was accepted at a probability $p < 0.05$.

Results and Discussion

Total phenols (TP) and total flavonoids (TF) contents of faba beans and azuki beans

Soaking in water prior to cooking, addition of $NaHCO_3$ and cooking affected TP and TF contents of faba (FB) beans and azuki beans (AB) (Table 1). Soaking legumes in water prior to cooking has been used as a preliminary step to soften texture and reduce cooking time in many parts of the world including China. TP contents of raw FB and raw AB were determined as 3.24 ± 0.21 and 4.86 ± 0.32 mg GAE/g sample, respectively. TF of FB (0.24 ± 0.03 mg CE/g sample) were significantly lower ($p < 0.05$) than TF of AB (1.38 ± 0.43 mg CE/g sample). Legume seeds are consisted of 3 different parts including cotyledon, seed coat, and embryonic axe. Although flavonoids are mainly located in the seed coat, non-flavonoid phenolic compounds such as free and combined hydroxybenzoic and hydroxycinnamic acids are located in the cotyledon (Dueñas *et al*., 2006). Lin *et al.* (2008) examined polyphenol content of 24 common bean samples representing 17 varieties. The hydroxycinnamic acid derivatives constituted the main phenolic component of beans. No flavonoids were detected in the navy bean samples. However, red kidney bean group contained quercetin 3-O-glucoside and its malonyl derivatives. In the present study all treatments resulted in a significant increase in TP contents of both FB and AB ($p < 0.05$). The highest TP content was obtained for FB and AB when cooked with the addition of $NaHCO_3$.

However soaking in hot or cold water prior to cooking did not cause a significant difference ($p > 0.05$). The highest TF contents were obtained for AB cooked with the addition of $NaHCO_3$. It was observed that TF in AB cooked with the addition of $NaHCO_3$ was not dependent on soaking. On the contrary, TF was not detected in FB soaked in hot water prior to cooking and cooked without the addition of $NaHCO_3$

Table 1. Effect of soaking and $NaHCO_3$ addition on total phenol (TP) and total flavonoid (TF) contents of faba beans (FB) and azuki beans (AB)

Treatment	Cooking	TP (mg GAE/g sample)		TF (mg CE/g sample)	
		FB	AB	FB	AB
Soaking in hot water	with $NaHCO_3$	11.84 ± 1.12^{d}	15.06 ± 1.65^{d}	0.86 ± 0.11^{c}	2.78 ± 0.31^{c}
	without $NaHCO_3$	10.81 ± 1.32^{c}	13.16 ± 1.43^{c}	ND	2.08 ± 0.52^{b}
Soaking in cold water	with $NaHCO_3$	12.76 ± 1.55^{d}	17.28 ± 1.72^{e}	0.04 ± 0^{a}	2.81 ± 0.56^{c}
	without $NaHCO_3$	10.82 ± 1.45^{c}	13.54 ± 1.58^{c}	0.32 ± 0.02^{b}	1.92 ± 0.39^{b}
Not soaked	with $NaHCO_3$	9.48 ± 1.34^{b}	13.29 ± 1.65^{c}	ND	2.86 ± 0.65^{c}
	without $NaHCO_3$	8.79 ± 1.23^{b}	11.97 ± 1.23^{b}	ND	2.05 ± 0.53^{b}
Raw		3.24 ± 0.21^{a}	4.86 ± 0.32^{a}	0.24 ± 0.03^{b}	1.38 ± 0.43^{a}

Values represent means ± SD of three independent determinations;
Different letters within the same column indicate statistical significance at $p < 0.05$ level;
ND, not detected

Table 2. Effect of soaking and $NaHCO_3$ addition on total phenol (TP) and total flavonoid (TF) bioavailability in dialysate of cooked faba beans (FB) and azuki beans (AB)

Treatment	Cooking	Bioavailability of TP (%)		Bioavailability of TF (%)	
		FB	AB	FB	AB
Soaking in hot water	with $NaHCO_3$	27.93 ± 3.23^{b}	23.11 ± 3.22^{b}	ND	3.54 ± 0.87^{b}
	without $NaHCO_3$	28.41 ± 3.46^{b}	23.84 ± 3.45^{b}	ND	3.76 ± 0.74^{b}
Soaking in cold water	with $NaHCO_3$	23.86 ± 4.32^{a}	18.21 ± 4.22^{a}	ND	2.11 ± 0.54^{a}
	without $NaHCO_3$	23.78 ± 4.21^{a}	18.59 ± 3.21^{a}	ND	2.32 ± 0.49^{a}
Not soaked	with $NaHCO_3$	38.58 ± 3.23^{c}	23.87 ± 4.23^{b}	ND	5.12 ± 0.71^{c}
	without $NaHCO_3$	38.37 ± 4.44^{c}	24.24 ± 2.43^{b}	ND	5.34 ± 0.57^{c}

Values represent means ± SD of three independent determinations;
Different letters within the same column indicate statistical significance at $p < 0.05$ level;
ND, not detected

Table 3. Changes in the antioxidant activity of FB following *in vitro* digestion based on ABTS inhibition

Treatment	Cooking	ABTS inhibition (%)			
		FB	Dialysate of FB	Aqueous extract of retentate	Methanolic extract of retentate
Soaking in hot water	with $NaHCO_3$	15.42 ± 3.78^{a}	38.45 ± 5.32^{c}	46.25 ± 5.64^{d}	26.32 ± 2.54^{b}
	without $NaHCO_3$	15.68 ± 3.56^{a}	39.54 ± 4.45^{c}	42.23 ± 5.32^{c}	24.15 ± 2.63^{b}
Soaking in cold water	with $NaHCO_3$	13.26 ± 2.68^{a}	32.45 ± 3.55^{c}	43.65 ± 4.21^{d}	24.65 ± 3.12^{b}
	without $NaHCO_3$	14.21 ± 2.54^{a}	75.67 ± 6.24^{c}	72.56 ± 6.32^{c}	45.56 ± 5.36^{b}
Not soaked	with $NaHCO_3$	18.44 ± 3.22^{a}	38.15 ± 3.69^{c}	40.21 ± 5.21^{c}	28.65 ± 3.22^{b}
	without $NaHCO_3$	16.33 ± 2.14^{a}	76.55 ± 6.78^{bc}	72.14 ± 4.89^{b}	83.54 ± 6.47^{c}

Values represent means ± SD of three independent determinations;
Different letters within the same column indicate statistical significance at $p < 0.05$ level;
ND, not detected

and in not soaked samples.

The term *in vitro* bioavailability of TP represents the amount of TP released from food matrix and then the amount that has the ability to pass through the intestinal barrier. For that reason, polyphenols which can pass through the intestinal barrier can be absorbed through the intestinal mucosa and therefore can be metabolized. Various studies reported that absorption of polyphenols that can reach to the small intestine was very low (Clifford, 2004). Effect of soaking and $NaHCO_3$ addition on TP and TF bioavailability of FB and AB were shown in Table 2.

Soaking caused a significant decrease in the TP bioavailability of FB ($p < 0.05$). This decrease was about 27.60% for FB soaked in hot water with NaHCO3 addition and 38.15% for FB soaked in cold water $NaHCO_3$ addition. TP bioavailability of AB soaked in cold water was lower than that of AB soaked in hot water and cooked without soaking ($p < 0.05$). Furthermore, TF bioavailability of AB cooked without soaking was greater than those of AB cooked following soaking both in cold and hot water ($p < 0.05$). TF was not detected in FB after in vitro digestion. Saura-Calixto *et al.* (2007) found that approximately 25% of polyphenols in the legume mixture containing chickpeas (35%), beans (31%), and lentils (34%) were bioaccessible in the small intestine. This result is in agreement with our results in which TP bioavailability of beans changed between 18.21 and 38.58%. The lower bioavailability level of TP and TF after soaking may be due to the release of phenolic compounds into soaking water. Furthermore, lower bioavailability level of TP and TF of beans when soaked in cold water can be explained by the longer soaking time than those soaked in hot water.

Antioxidant activities of faba beans (FB) and azuki beans (AB)

Antioxidant potentials of beans have been reported in several studies. However, these studies were conducted on aqueous extracts of foods

Table 4. Changes in the antioxidant activity of FB following *in vitro* digestion based on DPPH inhibition

Treatment	Cooking	DPPH inhibition (%)			
		FB	Dialysate of FB	Aqueous extract of retentate	Methanolic extract of retentate
Soaking in hot water	with $NaHCO_3$	4.32 ± 0.25^{b}	13.58 ± 0.87^{d}	1.82 ± 0.23^{a}	7.42 ± 0.54^{c}
	without $NaHCO_3$	6.17 ± 0.65^{b}	7.56 ± 0.54^{c}	2.13 ± 0.34^{a}	7.48 ± 0.56^{c}
Soaking in cold water	with $NaHCO_3$	5.82 ± 0.74^{b}	7.65 ± 0.65^{c}	1.83 ± 0.25^{a}	7.88 ± 0.62^{c}
	without $NaHCO_3$	4.12 ± 0.32^{a}	6.23 ± 0.54^{b}	3.85 ± 0.38^{a}	7.92 ± 0.71^{b}
Not soaked	with $NaHCO_3$	3.65 ± 0.64^{a}	11.25 ± 0.91^{c}	ND	6.88 ± 0.56^{b}
	without $NaHCO_3$	3.87 ± 0.58^{a}	7.94 ± 0.69^{b}	ND	ND

Values represent means ± SD of three independent determinations;
Different letters within the same column indicate statistical significance at $p < 0.05$ level;
ND, not detected

Table 5. Changes in the antioxidant activity of AB following *in vitro* digestion based on ABTS inhibition

Treatment	Cooking	ABTS inhibition (%)			
		AB	Dialysate of AB	Aqueous extract of retentate	Methanolic extract of retentate
Soaking in hot water	with $NaHCO_3$	12.23 ± 2.58^{a}	32.14 ± 3.68^{b}	56.47 ± 7.45^{c}	32.56 ± 3.66^{b}
	without $NaHCO_3$	12.65 ± 2.74^{a}	35.45 ± 3.74^{c}	48.56 ± 5.32^{d}	27.46 ± 2.65^{b}
Soaking in cold water	with $NaHCO_3$	10.25 ± 2.69^{a}	37.81 ± 4.12^{b}	72.56 ± 8.15^{c}	31.26 ± 3.21^{b}
	without $NaHCO_3$	11.23 ± 3.21^{a}	38.49 ± 4.32^{c}	45.21 ± 4.33^{d}	28.56 ± 2.88^{b}
Not soaked	with $NaHCO_3$	10.56 ± 3.11^{a}	25.62 ± 2.66^{b}	54.16 ± 6.21^{c}	54.26 ± 4.58^{c}
	without $NaHCO_3$	9.56 ± 2.89^{a}	60.12 ± 5.21^{b}	68.59 ± 4.56^{c}	69.56 ± 5.32^{c}

Values represent means ± SD of three independent determinations;
Different letters within the same column indicate statistical significance at $p < 0.05$ level;
ND, not detected

Table 6. Changes in the antioxidant activity of AB following *in vitro* digestion based on DPPH inhibition

Treatment	Cooking	DPPH inhibition (%)			
		AB	Dialysate of AB	Aqueous extract of retentate	Methanolic extract of retentate
Soaking in hot water	with $NaHCO_3$	27.85 ± 5.23^{c}	16.25 ± 2.56^{a}	22.31 ± 2.56^{b}	16.55 ± 2.65^{a}
	without $NaHCO_3$	22.13 ± 4.52^{b}	13.45 ± 3.12^{a}	13.65 ± 2.14^{a}	14.12 ± 2.58^{a}
Soaking in cold water	with $NaHCO_3$	27.56 ± 4.36^{b}	12.65 ± 2.44^{a}	25.36 ± 3.21^{b}	14.21 ± 2.14^{a}
	without $NaHCO_3$	16.23 ± 3.22^{a}	15.8 ± 2.65^{a}	15.67 ± 2.68^{a}	14.23 ± 2.69^{a}
Not soaked	with $NaHCO_3$	38.65 ± 4.15^{c}	19.54 ± 3.11^{a}	24.12 ± 2.69^{b}	18.36 ± 2.29^{a}
	without $NaHCO_3$	32.14 ± 3.69^{b}	18.56 ± 2.68^{a}	20.14 ± 2.65^{a}	19.45 ± 2.66^{a}

Values represent means ± SD of three independent determinations;
Different letters within the same column indicate statistical significance at $p < 0.05$ level;
ND, not detected

whereas antioxidant activity in the residues can be significant. This condition is more appropriate for polyphenols most of which are bound to proteins and/or carbohydrates in the food matrix. Therefore, it is important to analyze antioxidant activity of food following digestion process. Water soluble antioxidants are most readily available from food matrix within the digestive tract (Fardet *et al*., 2008). However, bound polyphenols are probably released later in the colon during fermentation.

Comparison antioxidant activities before and after *in vitro* digestion process showed that antioxidant activity of FB increased after digestion (Table 3). Digestion appears to be an important factor for potentiating the antioxidant activity of faba beans. This can be due to increase in the solubility of polyphenols and digestion of proteins and starch which can lead to release of polyphenols because of the acidic conditions of the stomach and enzymatic hydrolysis in the duodenum. Similar explanation was reported elsewhere indicating that *in vitro* gastrointestinal digestion caused increase in the antioxidant capacity of whole grain cereal foods because of the possible release of polyphenols following digestion of proteins and starch (Perez-Jimenez and Saura-Calixto, 2005). Antioxidant activities of dialyzable fractions against ABTS radical were 2.45 to 4.69 times greater than those of samples before digestion and the greatest increase was observed for the dialyzable fraction of the samples that not soaked prior to cooking and cooked without the addition of $NaHCO_3$ ($p < 0.05$). Antioxidant activities of aqueous extracts of non-dialyzable fractions (retentates) against ABTS radical were also found to be greater than those of the samples before digestion ($p < 0.05$). Methanolic extracts of the non-dialyzable fractions of the samples showed inhibition effect on ABTS radical changing between 24.15 and 83.54%. This result supports the idea that digestion makes bound phenolics turned into unbound form.

Dialysate of FB soaked in hot water and cooked with the addition of $NaHCO_3$ showed the greatest inhibition effect on DPPH radical ($p < 0.05$) (Table 4). This effect was found to be similar to that of FB

cooked with the addition of $NaHCO_3$ without being soaked. On the contrary, inhibition effect on DPPH radical obtained for dialysate of FB were similar ($p > 0.05$) for all treatments except that FB soaked in cold water and cooked with the addition of $NaHCO_3$ had lower inhibition activity than that of cooked without soaking and $NaHCO_3$ addition ($p < 0.05$).

Liyana-Pathirana and Shahidi (2005) evaluated the effect of simulated gastric pH conditions on total phenol content and antioxidant activity of soft and hard wheat. Total phenol content was increased by 3.5-5.3 folds for soft wheat and was increased by 2.4-5.1 folds for hard wheat. One of the explanations was related with gastric conditions that might influence phenolic compositions since phenolics are commonly esterified to sugars or acids. The simulated gastric condition resulted in a dramatic increase on Trolox equivalent antioxidant capacity of wheat which was 2.5 and 4 fold for soft and hard wheat, respectively.

Changes in the antioxidant activity of AB following *in vitro* digestion were shown in Table 5. Inhibition of ABTS radical activity increased between 2.84-6.17 folds for the dialysates and increased between 3.27-5.87 and 1.17-6.28 folds for the aqueous and methanolic extract of retentate, respectively ($p < 0.05$). On the contrary, inhibition effect of AB on DPPH radical significantly decreased after *in vitro* digestion process ($p < 0.05$) (Table 6). This can be explained by the polyphenols which can inhibit DPPH radical oxidation are the member of insoluble indigestible fraction such as proanthocyanidins that can be fermented in the colon by microorganisms.

Insoluble and indigestible polyphenols cannot be soluble in the digesta media. Therefore, since the amount of antioxidant compounds which is effective on DPPH radical did not increase following *in vitro* digestion and they also were separated into 2 groups (dialysate and retentate) after *in vitro* digestion, antioxidant activity against DPPH radical seems to be reduced. Similarly, Saura-Calixto *et al.* (2007) reported that approximately 66% of polyphenols in legumes belonged to insoluble indigestible fraction. They proposed that, polyphenols associated with the soluble indigestible fraction would not pass through the intestinal barrier. However, they may have some antioxidant effect on small intestine because they are soluble in the digesta media. Furthermore, polyphenols associated with the insoluble indigestible fraction cannot pass through the intestinal barrier and they can reach the colon where they can be fermented by colonic microflora. They exert their antioxidant activity after colonic fermentation. The overall evaluation of data showed that antioxidant activity of beans, cooked beans, and digested beans on DPPH radical was lower than those of ABTS radical.

Conclusion

In conclusion, soaking in hot and cold water and cooking with and without the addition of $NaHCO_3$ caused a significant increase in TP contents of both FB and AB. FB and AB with the highest TP content were the samples that soaked in cold water and cooked with the addition of $NaHCO_3$. This can be the result of longer soaking duration leading to more phenolics diffuse outside and increase in the solubilisation of phenolics in alkaline media.

TP bioavailability of FB following soaking in hot with $NaHCO_3$ and cold water $NaHCO_3$ decreased by 27.60 and 38.15% respectively. Similarly, TP bioavailability of AB soaked in cold water was lower than those of AB cooked without soaking and also soaked in hot water.

TF was not detected in FB after *in vitro* digestion. TF bioavailability of AB cooked without soaking was greater than those of AB cooked after soaking both in cold and hot water. $NaHCO_3$ addition did not influence TP and TF bioavailability of faba beans and zkizu beans.

Antioxidant activity of dialysates which reflects the behaviour of the substances in the small intestine showed that dialyzable fractions of FB and AB had greater antioxidant effect on ABTS radical than DPPH radical. Comparison antioxidant activities before and after in vitro digestion processes revealed that digestion appeared to be an important factor for potentiating the antioxidant activity of beans. Antioxidant activity of retentates which represents non-dialyzable fraction displayed that digestion in the stomach caused increase in the antioxidant activity with respect to undigested samples.

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