

Research Paper / Araştırma Makalesi

## Effect of Some Domestic Cooking Methods on Antioxidant Activity, Total Phenols and Total Flavonoid Content of Common Beans

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### ABSTRACT

In this study the effect of domestic cooking methods on the antioxidant activity, total phenolic (TP) and total flavonoid contents (TF) of dry beans (DB) and pinto beans (PB) was investigated. Total phenolic contents of raw DB and PB were  $2.36 \pm 0.11$  mg gallic acid equivalents (GAE)/g fresh weight and  $3.74 \pm 0.13$  mg GAE/g fresh weight, respectively. Total flavonoid contents of raw DB and PB were  $0.14 \pm 0.02$  mg catechin equivalents (CE)/g fresh weight and  $1.27 \pm 0.14$  mg CE/g fresh weight, respectively. Soaking increased TP contents of both DB and PB. Addition of sodium bicarbonate ( $\text{NaHCO}_3$ ) increased TP and TF contents of PB. Soaking in water had a significant effect on the TP and TF contents of beans. Cooking increased Trolox equivalent antioxidant capacity (TEAC) values of common beans. This study revealed that cooking DB and PB, common ingredients of the Turkish cuisine, had substantial benefits in terms of their polyphenol contents and antioxidant activities.

**Key Words:** Antioxidant activity, Total phenols, Total flavonoids, Domestic cooking, Common beans.

### Kuru Fasulye ve Barbunya Fasulyesinin Toplam Fenol, Toplam Flavonoid İçerikleri ile Antioksidan Aktiviteleri Üzerine Çeşitli Pişirme Yöntemlerinin Etkisi

#### ÖZET

Bu çalışmada kuru fasulye (KF) ve barbunya fasulyesinin (BF) toplam fenol, toplam flavonoid içerikleri ile antioksidan aktiviteleri üzerine bazı ev tipi pişirme yöntemlerinin etkisi incelenmiştir. Çiğ kuru fasulye ve barbunya fasulyesinin toplam fenol içeriği sırasıyla  $2.36 \pm 0.11$  mg gallik asit eşdeğeri/g örnek (GAE/g) ve  $3.74 \pm 0.13$  mg gallik asit eşdeğeri/g örnek, toplam flavonoid içerikleri ise sırasıyla  $0.14 \pm 0.02$  mg kateşin eşdeğeri/g örnek (KE/g örnek) ve  $1.27 \pm 0.14$  mg kateşin eşdeğeri/g örnek olarak saptanmıştır. Pişirme işleminden önce uygulanan ıslatma işlemi her iki örneğin toplam fenol içeriğinde artışa neden olmuştur. Pişirme sırasında sodyum bikarbonat eklenmesi barbunya fasulyesinin toplam fenol ve toplam flavonoid içeriğinin artmasını sağlamıştır. ıslatma sularının önemli miktarda toplam fenol ve toplam flavonoid içerdiği belirlenmiştir. Pişirme işlemi kuru fasulyenin Troloks eşdeğeri antioksidan kapasitesinde artış sağlamıştır. Bu çalışmanın sonuçları pişmiş kuru fasulye ve barbunya fasulyesinin polifenol içerikleri ve antioksidan aktiviteleri nedeniyle sağlık üzerine olumlu ek bir fayda sağlayabileceğini göstermiştir.

**Anahtar Kelimeler:** Antioksidan aktivite, Toplam fenol, Toplam flavonoid, Ev tipi pişirme, Kuru fasulye, Barbunya fasulyesi

#### INTRODUCTION

Leguminous species have been used as dry grains for human nutrition for many years. However, their effects on human health began to be investigated only 20 – 30 years ago. Although most of the legumes are local food plants, they are the second crops following cereals in providing food crops for world agriculture. Legumes are important sources of dietary protein but low nutritional value of legume proteins due to antinutritional

compounds they contain is one of the biggest problems [1-3].

In general, legumes are sources of complex carbohydrates, protein and dietary fiber. They also provide micronutrients, vitamins, carotenoids, phenolic compounds. Protein content of legumes ranges from 17% to 40% and carbohydrate content of legumes ranges from 55% to 60% [1, 4, 5].

Health benefits of legumes were investigated in experimental, epidemiological and clinical studies. Lipid homeostasis control and hypocholesterolemic effects of soybean proteins, glycemic control of a lupin protein, anticarcinogenic effects of protease inhibitors and lectins, therapeutic effects of  $\alpha$ -amylase and protein inhibitors on obesity and diabetes were determined in these studies [2, 6]. Adebamowo et al. [7] reported that there was a relationship between bean and lentil consumption and a lower incidence of breast cancer. In a multiethnic case control study, protective effect of legumes except soybeans on prostate cancer was observed [8].

Some of the above beneficial effects can be due to antioxidant activities of polyphenols legumes contain. Antioxidants can inhibit the propagation of free radical reactions, protect the human body from diseases, and retard lipid oxidative rancidity in foods [9]. The most effective molecules are phenolic compounds, especially the flavonoids in plant originated foods. They exhibit a wide range of pharmacological and medicinal properties, including anti-inflammatory, anti-carcinogenic, vasodilatory actions; which have been mostly attributed to their free radical scavenging, metal chelating, and antioxidant activities [10-12].

Dry bean (*Phaseolus vulgaris* L.) is a traditional food in human diet. Consumption of dry beans has been linked to reduced risk of coronary heart disease, colon cancer, diabetes and obesity [13]. These effects are attributed to the presence of phytochemicals including polyphenols, which possess both anticarcinogenic and antioxidant properties [14].

Domestic cooking procedure may vary between different regions of the world. Legumes are commonly cooked by pressure boiling. In some occasion boiling process before cooking can be used as a pretreatment. Prior to cooking, generally soaking is used in order to soften texture and shorten cooking time [6]. Soaking water may be hot or cold depending upon preferences of individuals. Sometimes addition of  $\text{NaHCO}_3$  prior to cooking can be another application to obtain soft texture, and this treatment also helps to reduce cooking time.

Although common beans are widely consumed all over the world, very little information is available in the literature regarding the changes in total phenols, total flavonoids and antioxidant activities following food preparation methods including soaking and addition of  $\text{NaHCO}_3$  before cooking and pressure cooking. Therefore the present study was undertaken to investigate the effects of soaking, cooking and  $\text{NaHCO}_3$  addition before cooking on antioxidant activity, total phenols and flavonoid contents of widely consumed legume species in Turkey.

## MATERIALS and METHODS

### Materials and Chemicals

Dry beans (DB) and pinto beans (PB) (*Phaseolus vulgaris* L) were purchased from local markets in İzmir, Turkey. (+)-Catechin hydrate (C-1251), Gallic acid (48630), Folin-Ciocalteu phenol reagent (F-9252), ABTS [(2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt], and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich. All other reagents and solvents commercially obtained were of analytical grade.

### Soaking

Whole seeds of both DB and PB (100 g) were soaked in 200 mL of cold distilled water (20°C). They were left to stand for a night. Besides 100 g whole seeds of DB and PB were soaked in boiled distilled water (100°C) and left to stand for 3 hours. After incubation excess water was drained and stored at -40°C until analysis. Drained legumes were weighted for determination of water absorption.

### Cooking

Separate batches of raw or soaked beans were autoclaved at 15 psi (120°C) for 50 min. in distilled water in a bean:water ratio of 1:3 (w/v) or in 0,3% (w/v) sodium bicarbonate solution.

### Extraction

In order to measure antioxidant activities, total phenols and total flavonoids of raw materials, legumes were grounded into 60-mesh size with Brook Crompton Series 2000. 20 g of powder was blended with 100 mL of 50% aqueous methanol for 5 min in a Waring blender. Mixture was centrifuged at 4500 rpm (MSE MISTRAL 1000, UK) 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, total phenols and total flavonoids.

Autoclaved 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 gram of homogenate was extracted with 50 mL of 50% aqueous methanol for two times as described above. Supernatants were collected and stored at -40°C until analysis.

### Analysis

All measurements were carried out in two parallels and in duplicates. Results were calculated as both dry and wet weight basis.

### Determination of Total Phenols

Total phenols (TP) were determined by a Folin-Ciocalteu assay with slight modifications [15]. The

results were expressed as Gallic acid equivalents (GAE). Sample (50  $\mu$ L), distilled water (3 mL), Folin-Ciocalteu's reagents (250  $\mu$ L), and 7% NaCO<sub>3</sub> (750  $\mu$ L) were mixed and incubated for 8 min at room temperature. At the end of the incubation period, 950  $\mu$ L of distilled water was added. The mixture was allowed to stand for 2h at room temperature. The absorbance readings were taken at 765 nm using a UV-visible spectrophotometer (Varian, Cary50 Scan).

#### Determination of Total Flavonoids

Total flavonoids (TF) were determined using a method described by Xu and Chang [15]. The results were expressed as (+)-catechin equivalents (CE). Briefly, 0.25 mL of sample or (+)-catechin standard solution, 1.25 mL of distilled water and 75  $\mu$ L of 5% NaNO<sub>2</sub> solution were mixed and allowed to stand for 6 min at room temperature. Then 150  $\mu$ L of a 10% AlCl<sub>3</sub>·6H<sub>2</sub>O solution was added and allowed to stand for another 5 min before adding 0.5 mL of 1 M NaOH. The volume of the mixture was brought to 2.5 mL with distilled water. After gentle mixing, the absorbance was measured immediately at 510 nm using a UV-visible spectrophotometer (Varian, Cary50 Scan).

#### ABTS<sup>+</sup> Radical Scavenging Activity

Antioxidant activities of all extracts were measured according to the procedure described elsewhere [16]. To prepare ABTS stock solution, ABTS was dissolved in water to 7 mM concentration. ABTS radical cation (ABTS<sup>+</sup>) was produced by adding 2.45 mM potassium persulfate (final concentration). Diluted ABTS<sup>+</sup> solution to an absorbance of 0.70 ( $\pm$  0.02) at 734 nm was used as working solution. Absorbance readings (734 nm) were taken at 30°C exactly 5 min after initial mixing of 1 mL of diluted ABTS<sup>+</sup> solution and 10  $\mu$ L of sample solution. Absorbance readings were carried out by using UV-visible spectrophotometer (Varian, Cary50 Scan). Antioxidant activity (AA) was expressed as percentage inhibition of ABTS<sup>+</sup> radical by using below equation;

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

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

#### DPPH Radical Scavenging Activity

The ability of the samples to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals were determined according to the method of Llorach et al. [17] with some modifications. 0,08 mM DPPH radical solution in methanol was prepared. 950  $\mu$ L of DPPH stock solution was added to 50  $\mu$ L extract and incubated for 5 min. Exactly 5 min. later absorbance readings of mixture was performed at 515 nm (Varian, Cary50 Scan). Antioxidant activity (AA) was expressed as percentage inhibition of DPPH radical by using below equation;

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

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

#### Trolox Equivalent Antioxidant Capacity (TEAC)

The Trolox equivalent antioxidant capacity assay based on the reaction of DPPH and ABTS radicals with Trolox were performed in order to compare radical scavenging activity of sample with those of Trolox. Radical scavenging activity of Trolox was determined by using different concentrations of Trolox. TEAC value was calculated as follows:

$$TEAC_{\text{sample}} = A_{\text{sample}} / (\text{slope} \times [\text{sample}])$$

where  $A_{\text{sample}}$  is the decrease in absorbance of the sample and [sample] is the concentration of the sample in  $\mu$ M. The TEAC values were converted to  $\mu$ mol TEAC/g sample [18].

#### Statistical Analysis

The data were expressed as mean  $\pm$  standard deviation. Statistical analysis was performed using SPSS for Windows (Version 10.0). Analysis of variance (ANOVA) was conducted, and Tukey HSD multiple range test were used to determine significant differences at  $p < 0.05$ . Correlation between antiradical activities and the TEAC were evaluated by using SPSS for Windows (Version 10.0).

## RESULTS and DISCUSSION

#### Total Phenols and Total Flavonoid Contents of Common Beans

Total phenols of raw DB and raw PB were determined as  $2.36 \pm 0.11$  mg GAE/g sample (2.65 mg GAE/ g dry matter) and  $3.74 \pm 0.13$  mg GAE/g sample (4.08 mg GAE/ g dry matter), respectively. TP of DB and PB were significantly different ( $p < 0.05$ ). TF of DB ( $0.14 \pm 0.02$  mg CE/g sample) were significantly lower ( $p < 0.05$ ) than TF of PB ( $1.27 \pm 0.14$  mg CE/g sample). Oomah et al. [18] reported that total phenols of six bean cultivars were varied from 3.3 to 16.6 mg CE/g sample while total flavonoids were changed between 0.41 mg rutin/g sample and 1.02 mg rutin/g sample. Lin et al. [19] 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.

Soaking has been used as pretreatment step in cooking of common beans to soften texture and to reduce cooking time. In Turkish cuisine dry beans can be soaked either in cold water during a night or in hot water for several hours. In the present study two soaking conditions were performed and the effects of soaking on TP and TF of cooked common beans were shown in Table 1. Increase in the weight of the beans following

soaking treatment were 97.92% and 103.22% for DB and 93.43% and 87.29% for PB soaked in cold and hot water, respectively.

Table 1. Effect of soaking on TP and TF contents of cooked common beans

Treatment	TP (mg GAE/g sample)		TF (mg CE/g sample)	
	DB	PB	DB	PB
Soaked in hot water	31.37 ± 4.82 <sup>a</sup> (10.08 ± 1.56)**	35.47 ± 5.25 <sup>a</sup> (13.44 ± 1.99)	2.52 ± 0.50 <sup>a</sup> (0.81 ± 0.16)	6.18 ± 0.74 <sup>a</sup> (2.34 ± 0.28)
Soaked in cold water	32.33 ± 4.98 <sup>a</sup> (10.85 ± 1.67)	39.74 ± 6.00 <sup>a</sup> (14.31 ± 2.18)	0.63 ± 0.21 <sup>b</sup> (0.21 ± 0.07)	6.05 ± 0.56 <sup>a</sup> (2.18 ± 0.20)
Not soaked	10.41 ± 1.44 <sup>b</sup> (8.24 ± 1.14)	14.70 ± 2.04 <sup>b</sup> (11.51 ± 1.6)	0.24 ± 0.20 <sup>b</sup> (0.19 ± 0.24)	2.92 ± 0.42 <sup>b</sup> (2.29 ± 0.33)

\*Results were given as mean ± standard deviation.

\*\*Values in the parentheses are based on wet basis.

Different letters within the same column indicate statistical significances at  $p < 0.05$  level.

Soaking treatment caused significant increase in TP contents of both DB and PB. TF contents of PB also increased upon soaking treatments; however soaking in cold water did not affect TF content of DB significantly. In the study of Xu and Chang [6] total phenols of green pea, yellow pea, chick pea and lentil soaked to different hydration rates changing between 50% and 100% were found to be significantly different. In addition, the degree of the loss in total phenols following soaking was about 2-12% for peas and chickpeas and about 9-38% for lentil. However, the results of the two studies are not comparable because of different samples and methodology used in two studies. In our study effect of soaking was evaluated after cooking procedure. Samples cooked after soaking were compared with the samples cooked without soaking treatment. Differences between TP content of beans cooked without soaking and cooked after soaking in water can be due to the increase in the effectiveness of heat process to extract phenolic compounds from food matrix. Besides this, slightly lower TP content obtained for beans cooked after soaking in hot water can be explained by the shorter soaking time than those soaked in cold water. According to similar explanation reported by Xu and Chang [6], differences in distribution and content of phenolic compounds in legumes can determine the effect of soaking. For instance, longer soaking time can cause the cotyledon to absorb phenolics in water like in the case of peas and chickpeas or more phenolics can remain in the water than those can diffuse into the cotyledon like in the case of lentil. TF content of DB soaked in hot water was found to be significantly higher

than those of DB soaked in cold water and cooked without soaking ( $p < 0.05$ ). However, soaking type did not cause any significant differences in the TF contents of PB (Table 1).

The percentage of TP diffusing into cold and hot soaking water of DB and PB were shown in Figure 1. The percentage of TP leaking from PB into soaking water was higher than that from DB ( $p < 0.05$ ). Results obtained for PB were similar with the findings obtained for *B. purpurea* by Vijayakumari et al. [3]. Soaking of *B. purpurea* in distilled water resulted in significant reduction in the levels of phenolics (58%-65%) and tannins (64%-71). This reduction was observed during the first 2-4 hours of soaking and prolonging the soaking time did not cause any significant reduction [3].

Contrary to the percentage of TP diffusing into soaking water, the percentage of TF retained in both cold and hot soaking water of DB were significantly higher ( $p < 0.05$ ) than that of PB (Figure 1). Another difference between the results of the percentage of TP and TF diffusing into soaking water of DB was that the significantly higher percentage of TF ( $p < 0.05$ ) retained in cold soaking water than that of TF retained in hot soaking water. TP contents of cold and hot soaking water of DB were similar. However, the results obtained for PB were totally opposite. The ratio of TP diffusing into soaking water was higher than the percentage of TF diffusing into soaking water. This difference can be due to the differences between distribution of phenolics in both beans such as in seedcoat and cotyledon.

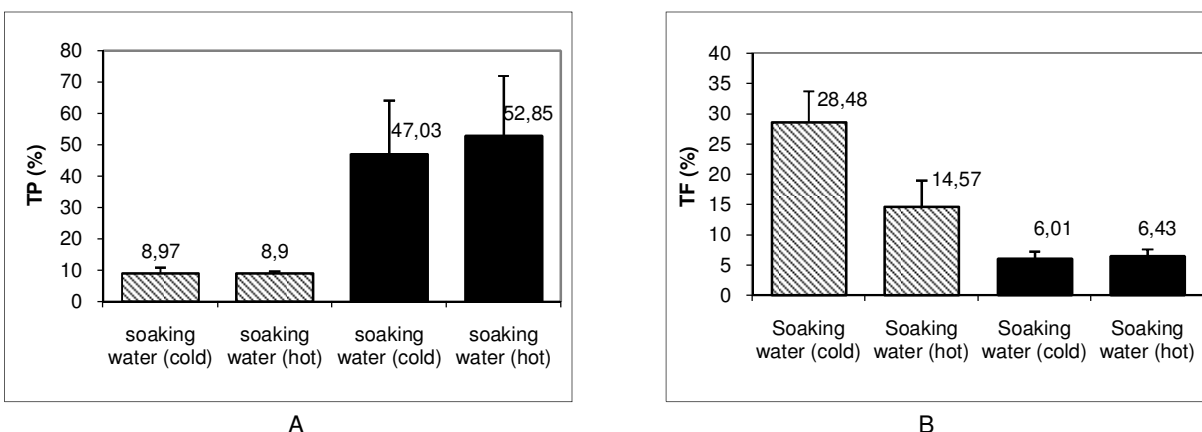


Figure 1. The percentage of TP diffusing into both cold and hot soaking water of DB (striped) and PB (solid) (A) and the percentage of TF diffusing into both cold and hot soaking water of DB (striped) and PB (solid) (B)

Cooking common beans with the addition of  $\text{NaHCO}_3$  did not cause significant increase ( $p > 0.05$ ) in TP contents of DB and PB (Table 2). TF content of PB increased when cooked with the addition of  $\text{NaHCO}_3$  whereas the increase for DB was not significant ( $p > 0.05$ ). This difference might have been resulted because of the difference of the varieties of beans. Increase in TF content can be explained by solubilisation of flavonoids which are favored during cooking, and which are increased with the help of alkaline medium.

In the study of Vijayakumari et al. [3] soaking *B. purpurea* seeds in  $\text{NaHCO}_3$  solution for 6 h resulted in 72% reduction in the level of phenols. Greater reduction in the level of phenols in  $\text{NaHCO}_3$  solution than soaking

in distilled water was explained by the possible diffusing of phenols into soaking medium or increase in the solubilisation of phenols in alkaline conditions. In the present study, possible increase in the solubilisation of phenols from food matrix can be the reason of an increase in the TP and TF contents. Although the results of the two study seem to be conflicting, in fact they support each other. In the study of Vijayakumari et al. [3], the addition of  $\text{NaHCO}_3$  was applied in soaking step. They observed a decrease due to the solubilisation of phenolics. However in the present study,  $\text{NaHCO}_3$  was added during cooking. After cooking, the extraction was carried out with cooking water. So, the phenolics diffused into cooking medium were not discarded.

Table 2. Effect of  $\text{NaHCO}_3$  on TP and TF contents of cooked common beans

Type of Cooking	TP (mg GAE/g sample)		TF (mg CE/g sample)	
	DB	PB	DB	PB
with $\text{NaHCO}_3$	$13.28 \pm 2.41^a$	$18.18 \pm 2.94^a$	$0.69 \pm 0.32^a$	$3.40 \pm 0.28^a$
	$(10.51 \pm 1.91)^{**}$	$(14.24 \pm 2.30)$	$(0.55 \pm 0.25)$	$(2.66 \pm 0.22)$
without $\text{NaHCO}_3$	$11.30 \pm 1.67^a$	$15.23 \pm 1.80^a$	$0.38 \pm 0.30^a$	$2.39 \pm 0.40^b$
	$(8.94 \pm 1.32)$	$(11.93 \pm 1.41)$	$(0.30 \pm 0.24)$	$(1.87 \pm 0.31)$

\*Results were given as mean  $\pm$  standard deviation.

\*\*Values in the parentheses are based on wet basis.

Different letters within the same column indicate statistical significances at  $p < 0.05$  level.

### Antioxidant Activities of Common Beans

Cooking caused an increase in the Trolox equivalent antioxidant capacity of common beans (Table 3). This result is important because common beans can not be consumed unless cooking. The samples cooked after soaking treatment had higher antioxidant activity than the samples cooked without soaking. Although DPPH radical scavenging activity (TEAC) of cooked DB after soaking treatment increased by 6 to 9 times according to the antioxidant activity of raw DB, this increase was

not found to be statistically significant for the samples cooked without soaking. However, ABTS radical scavenging activity of raw DB was 5 to 15 folds lower than those of cooked DB ( $p < 0.05$ ). DPPH radical scavenging activities of cooked PB were statistically higher (5 to 12 folds) than that of raw PB ( $p < 0.05$ ). Same finding was observed for ABTS radical scavenging activity of PB (6 to 16 folds). Addition of sodium bicarbonate did not have significant effect on the TEAC values.

Table 3. Antioxidant activities of common beans ( $\mu\text{mol TE/g}$  sample)

Treatment	Cooking Type	DPPH radical scavenging activity <sup>*</sup>		ABTS <sup>+</sup> radical scavenging activity <sup>*</sup>	
		DB	PB	DB	PB
Soaked in hot water	with NaHCO <sub>3</sub>	1.12 ± 0.5 <sup>a,b</sup> (0.36 ± 0.4) <sup>**</sup>	3.03 ± 0.78 <sup>c</sup> (1.15 ± 0.3)	6.29 ± 0.80 <sup>c</sup> (2.02 ± 0.25)	5.34 ± 0.65 <sup>c</sup> (1.60 ± 0.56)
	without NaHCO <sub>3</sub>	1.37 ± 0.14 <sup>b</sup> (0.44 ± 0.4)	2.71 ± 0.91 <sup>c</sup> (1.03 ± 0.3)	6.07 ± 0.84 <sup>c</sup> (1.95 ± 0.28)	5.40 ± 0.78 <sup>c</sup> (1.63 ± 0.53)
Soaked in cold water	with NaHCO <sub>3</sub>	1.67 ± 0.58 <sup>b</sup> (0.56 ± 0.2)	3.01 ± 0.95 <sup>c</sup> (1.08 ± 0.3)	6.05 ± 0.73 <sup>c</sup> (2.03 ± 0.23)	5.62 ± 0.63 <sup>c</sup> (1.54 ± 0.61)
	without NaHCO <sub>3</sub>	1.25 ± 0.35 <sup>b</sup> (0.42 ± 0.4)	2.54 ± 1.07 <sup>b,c</sup> (0.92 ± 0.4)	5.75 ± 0.69 <sup>c</sup> (1.93 ± 0.22)	5.35 ± 0.62 <sup>c</sup> (1.53 ± 0.61)
Not soaked	with NaHCO <sub>3</sub>	0.42 ± 0.41 <sup>a</sup> (0.33 ± 0.3)	1.29 ± 0.33 <sup>b</sup> (1.01 ± 0.3)	2.32 ± 0.05 <sup>b</sup> (1.84 ± 0.09)	2.35 ± 0.11 <sup>b</sup> (1.57 ± 0.25)
	without NaHCO <sub>3</sub>	0.39 ± 0.31 <sup>a</sup> (0.31 ± 0.2)	1.23 ± 0.33 <sup>b</sup> (0.96 ± 0.3)	2.29 ± 0.06 <sup>b</sup> (1.81 ± 0.04)	2.31 ± 0.06 <sup>b</sup> (1.58 ± 0.09)
Raw		0.19 ± 0.07 <sup>a</sup> (0.17 ± 0.06)	0.25 ± 0.04 <sup>a</sup> (0.23 ± 0.03)	0.42 ± 0.04 <sup>a</sup> (0.37 ± 0.03)	0.35 ± 0.04 <sup>a</sup> (0.32 ± 0.04)

<sup>\*</sup>Results were given as mean ± standard deviation.

<sup>\*\*</sup>Values in the parentheses are based on wet basis.

Different letters within the same column indicate statistical significances at  $p < 0.05$  level.

Similarly, in the study of Rocha-Guzmán et al. [20], DPPH radical scavenging activity of cooked beans was found to be higher than that for crude beans. On the other hand Xu and Chang [6] reported that soaking, boiling and steaming caused a decrease in the DPPH radical scavenging capacities of cool season food legumes including green pea, yellow pea, chickpea and lentil. One of the differences between their study and our study was the cooking method. The other difference was the different sample preparation methods used in these studies. In our study all analysis were performed by using legumes and cooking water together whereas cooking water was discarded in the other one.

Examination of soaking waters for antioxidant activity showed that hot and cold soaking waters of DB inhibited ABTS radical activity by 35.66% and 32.88%, respectively and also inhibited DPPH radical activity by 18.55% and 7.31%, respectively. Hot and cold soaking waters of PB inhibited ABTS radical activity by 99.23% and 99.19% and also inhibited DPPH radical activity by 79.02% and 82.41%, respectively.

Comparison of the phenolic content of DB with antioxidant activities on DPPH and ABTS revealed strong correlation. Antioxidant activity of DB against DPPH ( $r = 0.740$ ,  $p < 0.01$ ) and ABTS radicals ( $r = 0.741$ ,  $p < 0.01$ ) showed significant positive correlation with phenolic content. A significant positive correlation was observed between TP of PB and antioxidant activity against both DPPH ( $r = 0.797$ ,  $p < 0.01$ ) and ABTS radicals ( $r = 0.912$ ,  $p < 0.01$ ). Similar results were obtained by Oomah et al. [18]. They reported that flavonoid and flavonol contents of bean cultivars were the best indicators of antioxidant activity while anthocyanin content was strongly associated with antiradical activity ( $r = 0.826$ ). Our results were also in agreement with the study of Malencic et al. [21] who reported that there was a linear relationship between DPPH free radical scavenging activity and total phenols,

tannin and proanthocyanidin contents of soybean extracts. Similarly in the study of Mktan et al. [22], total phenol contents of kinema and CNF soybean were found to be positively correlated ( $p < 0.01$ ) with the respective values of DPPH free radical scavenging activity ( $r = 0.96$  and  $0.82$ ), reducing power against Fe<sup>3+</sup> ( $r = 0.91$  and  $0.82$ ), Fe<sup>2+</sup>-chelating activity ( $r = 0.95$  and  $0.76$ ) and lipid peroxidation inhibitory activity ( $r = 0.91$  and  $0.82$ ).

A significant positive correlation ( $p < 0.01$ ) was observed between DPPH radical scavenging activities and ABTS radical scavenging activities of DB ( $r = 0.957$ ) and PB ( $r = 0.912$ ). In fact this result was expectable, because antioxidant reaction mechanisms of ABTS and DPPH, which involve single electron transfer mechanism, are similar.

Positive significant correlation ( $p < 0.01$ ) was observed between TF content of PB and antioxidant activities of PB ( $R = 0.673$  for antiradical activity on DPPH and  $R = 0.666$  for antiradical activity on ABTS). However there was no significant correlation between TF content of DB and antioxidant activities of DB.

## CONCLUSION

Although cooking methods differ by means of traditions of the communities, some of them are very common all over the world. The present study was conducted to evaluate the effects of the most common techniques applied to beans in Turkey. Soaking beans in water prior to cooking favored the subsequent release of phenolics and flavonoids during cooking. Addition of NaHCO<sub>3</sub> also favored TF content of PB. This result seems to be occurred due to the solubilisation of phenolics with the help of alkaline medium. However, it is well known that alkaline medium lead to loss in B group vitamins, especially in thiamine. The amount of sodium bicarbonate used in cooking process is important; on

that account further studies may be beneficial to determine vitamin stability during cooking in alkaline medium. Cooked beans had stronger antioxidant activity when compared with the raw ones. As a consequence, this study revealed that cooked DB and PB which are common dishes in Turkish cuisine had substantial benefits because of their polyphenol contents and antioxidant activities.

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