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Impact of cooking method on phenolic composition and antioxidant potential of four varieties of *Phaseolus vulgaris* L. and *Glycine max* L.

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ABSTRACT

The present study aimed to investigate the effects of different cooking conditions - atmospheric (100 °C) and pressure cooking (115 °C) - on the phenolic composition and antioxidant activity of methanolic extracts of four *Phaseolus vulgaris* varieties and soy (*Glycine max*). Contrary to soy, in *P. vulgaris* varieties both cooking methods increased drastically the total phenolic, flavonoid, and *ortho*-diphenol content, as well as antioxidant capacity. These results were corroborated by HPLC analysis, where an overall increase of phenolic acids and flavonoids was detected in processed samples. However, draining the cooking water significantly decreased phenolic acids, flavonoids and antioxidant activity in all *P. vulgaris* varieties and as well as soy. The hypothesis that cooking increases the compound accessibility and nutritional value through increased release of phytochemicals was verified in the present study for *P. vulgaris* varieties. Keeping the cooking water is crucial to the increased nutritional value of all Phaseolus varieties. Overall, compared with the tested varieties of Phaseolus, soy, to which many health benefits are attributed, is not the best legume source of antioxidants.

1. Introduction

Legumes are the second most important food crop produced and consumed worldwide after cereal grains. They are a significant dietary protein source in several regions of the world (Kalogeropoulos et al., 2010) and constitute an important ingredient of the Mediterranean diet (Rosato et al., 2016).

Most dietary beans, also known as common beans, are varieties of *Phaseolus vulgaris* L., herbaceous annual plants of the Fabaceae (legume or bean) family that have been domesticated in two separate events, in Peru and Mexico, 8000 and 7000 years ago, respectively (Hayat, Ahmad, Masud, Ahmed, & Bashir, 2014). *Glycine max* L., known as soy bean, is a legume native to East Asia (including China, Japan and Korea) that has been domesticated more than 3000 years ago for its edible seeds and young pods. It is today the world's most important legume crop growing worldwide in diverse climates and the most widely used as oilseed (Waqas et al., 2015).

Nutritionally, legumes are excellent and affordable sources of protein, complex carbohydrates and dietary fiber, and are low in fat (Chávez-Mendoza & Sánchez, 2017; Ganesan & Xu, 2017). They exhibit a lower glycemic index compared to other starchy foods, such as cereals and potatoes. Legumes have also additional nutritional benefits due to their micronutrients, including minerals and vitamins, as well as, bioactive compounds including oligosaccharides, lectins, saponins, phytates and phenolic compounds (Kalogeropoulos et al., 2010; Siah, Konczak, Agboola, Wood, & Blanchard, 2012). These phytochemicals can have a health promoting effect and legumes have gaining interest in the food market for their antioxidant and other beneficial properties, suggesting that legumes can became added value functional foods or nutraceuticals (Campos-Vega, Loarca-Piña, & Oomah, 2010; Chávez-Mendoza & Sánchez, 2017).

Bean phytochemicals are secondary metabolites that are synthesized during normal development and as a response to stress conditions (Chon, 2013). The major bean polyphenolic classes of compounds are tannins, phenolic acids and flavonoids. Phenolic extracts from different types of beans have been shown to have antioxidant properties (Açar, Gokmen, Pellegrini, & Fogliano, 2009; Siah et al., 2012), protection against DNA damage (Madhujith, Amarowicz, & Shahidi, 2004), being antimutagenic and chemopreventive (Siah et al., 2012). Legumes consumption may help reduce the risks associated with the consumption of animal proteins in Western countries, leading to the prevention and management of several disease such as hypertension,

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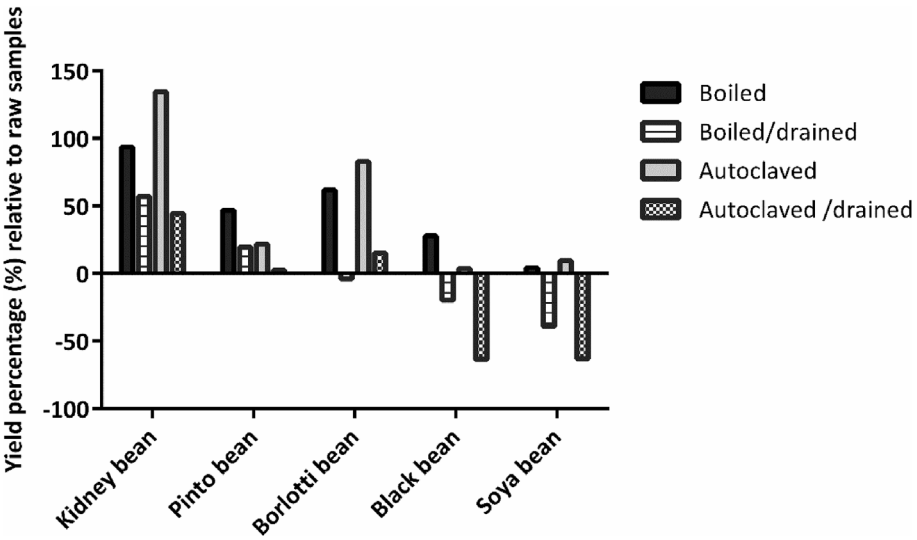

Fig. 1. Percentage (%) of gain or loss on extraction yield of different cooking conditions. Values were calculated based on the yield of raw bean. Extraction yield of raw kidney bean - 39,83; pinto bean - 57,76; borlotti bean - 57,79; black bean - 61,06; soy bean - 106,44; expressed in mg of extract per g of dry weight (mg Ex. g^{-1} DW).

Table 1
Effect of boiling and pressure cooking on the phenolic contents of crude methanolic extracts from the different beans.

	Raw	Boiled	Boiled/drained	Press. cooked	Press. cooked/drained
TPC (mg GAEq g^{-1} DW)					
Kidney bean	0.58 ± 0.05^{A,a}	1.60 ± 0.07^{A,B,c}	0.64 ± 0.05^{B,a}	1.60 ± 0.09^{B,c}	0.87 ± 0.03^{D,b}
Pinto bean	0.90 ± 0.07^{B,a}	1.62 ± 0.11^{D,c}	0.89 ± 0.07^{C,a}	1.32 ± 0.09^{A,b}	0.94 ± 0.07^{D,a}
Borlotti bean	0.61 ± 0.04^{A,b}	1.05 ± 0.02^{A,c}	0.48 ± 0.01^{A,a}	1.34 ± 0.07^{A,d}	0.58 ± 0.04^{B,a,b}
Black bean	0.90 ± 0.05^{B,c}	1.40 ± 0.06^{C,e}	0.72 ± 0.02^{B,b}	1.27 ± 0.05^{A,d}	0.38 ± 0.02^{A,a}
Soy bean	1.36 ± 0.04^{C,c}	1.34 ± 0.08^{B,C,c}	0.90 ± 0.03^{C,b}	1.63 ± 0.07^{B,d}	0.71 ± 0.04^{C,a}
TFC (mg CEq g^{-1} DW)					
Kidney bean	0.41 ± 0.03^{B,a}	0.81 ± 0.05^{C,c}	0.37 ± 0.03^{B,a}	1.04 ± 0.07^{D,d}	0.56 ± 0.01^{C,b}
Pinto bean	0.69 ± 0.05^{C,a}	1.19 ± 0.08^{D,b}	0.61 ± 0.06^{C,a}	1.07 ± 0.08^{D,b}	0.66 ± 0.06^{D,a}
Borlotti bean	0.41 ± 0.01^{B,b}	0.50 ± 0.03^{B,c}	0.22 ± 0.00^{A,a}	0.84 ± 0.05^{C,d}	0.34 ± 0.02^{B,b}
Black bean	0.36 ± 0.01^{B,b}	0.60 ± 0.05^{B,c}	0.32 ± 0.02^{B,b}	0.59 ± 0.03^{B,c}	0.17 ± 0.01^{A,a}
Soy bean	0.24 ± 0.01^{A,d}	0.26 ± 0.01^{A,d}	0.17 ± 0.00^{A,b}	0.21 ± 0.01^{A,c}	0.11 ± 0.00^{A,a}
***o*DC (mg GAEq g^{-1} DW)**					
Kidney bean	0.43 ± 0.06^{A,a}	0.75 ± 0.04^{B,b}	0.42 ± 0.02^{B,a}	1.04 ± 0.12^{C,c}	0.57 ± 0.02^{C,a}
Pinto bean	0.63 ± 0.05^{B,a,b}	0.94 ± 0.05^{C,c}	0.56 ± 0.05^{C,a}	0.90 ± 0.04^{B,C,c}	0.69 ± 0.02^{D,b}
Borlotti bean	0.37 ± 0.01^{A,b}	0.54 ± 0.04^{A,c}	0.31 ± 0.02^{A,a}	0.72 ± 0.03^{A,d}	0.35 ± 0.01^{A,a,b}
Black bean	1.13 ± 0.01^{D,c}	1.63 ± 0.08^{D,e}	0.89 ± 0.02^{D,b}	1.45 ± 0.01^{D,d}	0.53 ± 0.02^{B,a}
Soy bean	0.87 ± 0.00^{C,d}	0.82 ± 0.02^{B,C,c}	0.61 ± 0.02^{C,b}	0.87 ± 0.01^{A,B,d}	0.54 ± 0.01^{B,C,a}

Data presented as mean and SD from three replicates, n = 3. The data marked by the same letters were not significantly different ($p > 0.05$). Different uppercase letters for comparison within each column and lowercase letters for comparison within each row, indicating significant differences ($p < 0.05$) between varieties and processing, respectively. TPC – Total phenolic content; TFC - Total flavonoid content; oDC – *ortho*-diphenols content; mg GAEq g^{-1} DW - mg gallic acid equivalents per g of dry weight; mg CEq g^{-1} DW –mg of catechin equivalents per g of dry weight.

hypercholesterolemia, type II diabetes, cardiovascular diseases and cancer, contributing to overall health and wellness (Campos-Vega et al., 2010; Chávez-Mendoza & Sánchez, 2017; Hayat et al., 2014).

Traditionally, dietary dry seed legumes are prepared by soaking in water, followed by thermal processing such as boiling or pressure cooking, with exception of peas, chickpeas and fava beans that occasionally are roasted and eaten as snacks. It has been described that thermal cooking of legumes improves their nutritional value by reduction of antinutrients such as phytic acid and tannins and increases protein and starch digestibility (Deol & Bains, 2010; Ranilla, Genovese, & Lajolo, 2009; Rehman, Salariya, & Zafar, 2001). Furthermore, cooking procedures induce desirable sensory properties in beans such as sweet taste, flavor and soft texture (Mkanda, Minnaar, & de Kock, 2007; Ranilla et al., 2009).

There are abundant reports on the phenolic composition in raw beans (Açar et al., 2009; Itoh, Umekawa, & Furuichi, 2005; Marathe, Rajalakshmi, Jamdar, & Sharma, 2011; Siah et al., 2012), however, limited and contradictory information concerning the impact of heat processing of legumes on phytochemical composition and antioxidant activities are available (Eshraq, Mona, Sayed, & Emam, 2016; Kumar, Chauhan, Rani, Raghvanshi, & Jatav, 2012; Ranilla et al., 2009; Rocha-Guzmán, González-Laredo, Ibarra-Pérez, Nava-Berúmen, & Gallegos-Infante, 2007; Siah, Wood, Agboola, Konczak, & Blanchard, 2014).

The present study aims to investigate the *in vitro* antioxidant properties and phytochemical composition of raw, cooked and pressure cooked hydromethanolic extracts obtained from four varieties of common beans in a comparative study with soy, which is the most consumed legume worldwide.

2. Material and methods

2.1. Chemicals and equipment

The reagents of aluminium chloride, sodium nitrite, sodium hydroxide, acetic acid, formic acid and acetonitrile were purchased from Merck (Darmstadt, Germany). Methanol, sodium carbonate, gallic acid, Folin–Ciocalteau, sodium molybdate, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetremethychroman-2-carboxylic acid (Trolox), 2,2-azino-bis(3-ethylbenzothiazoline)-6 sulphonic acid (ABTS), potassium persulfate, 2,4,6-Tripyridyl-s-Triazine (TPTZ), Iron (III) chloride, hydrochloric acid, gallic acid and catechin were purchased from Sigma–Aldrich (St. Louis, USA). All the chemicals used

Table 2
Effect of boiling and pressure cooking on the antioxidant activity of crude methanolic extracts.

	Raw	Boiled	Boiled/drained	Press. cooked	Press. cooked/drained
DPPH (μmol TEq g^{-1} DW)					
Kidney bean	$3.14 \pm 0.25^{A,a}$	$6.57 \pm 0.15^{C,c}$	$3.07 \pm 0.19^{B,a}$	$8.45 \pm 0.62^{C,d}$	$4.57 \pm 0.43^{C,b}$
Pinto bean	$5.39 \pm 0.11^{B,a}$	$9.36 \pm 0.67^{D,c}$	$4.64 \pm 0.31^{C,a}$	$8.12 \pm 0.48^{C,b}$	$5.20 \pm 0.41^{C,a}$
Borlotti bean	$2.81 \pm 0.17^{A,b}$	$3.99 \pm 0.09^{B,c}$	$1.85 \pm 0.04^{A,a}$	$6.70 \pm 0.65^{B,d}$	$2.98 \pm 0.05^{B,b}$
Black bean	$5.42 \pm 0.38^{B,c}$	$8.88 \pm 0.29^{D,d}$	$4.59 \pm 0.30^{C,b}$	$8.21 \pm 0.17^{C,d}$	$2.29 \pm 0.12^{B,a}$
Soy bean	$2.66 \pm 0.25^{A,c}$	$2.17 \pm 0.17^{A,b}$	$1.31 \pm 0.06^{A,a}$	$1.83 \pm 0.21^{A,b}$	$0.91 \pm 0.06^{A,a}$
ABTS (μmol TEq g^{-1} DW)					
Kidney bean	$0.85 \pm 0.09^{C,a}$	$1.89 \pm 0.08^{C,c}$	$0.97 \pm 0.06^{B,a}$	$2.38 \pm 0.14^{D,d}$	$1.37 \pm 0.06^{D,b}$
Pinto bean	$1.46 \pm 0.06^{D,a}$	$2.34 \pm 0.07^{D,c}$	$1.43 \pm 0.10^{C,a}$	$1.98 \pm 0.02^{C,b}$	$1.56 \pm 0.09^{E,a}$
Borlotti bean	$0.47 \pm 0.02^{A,b}$	$0.79 \pm 0.04^{B,c}$	$0.33 \pm 0.02^{A,a}$	$1.05 \pm 0.04^{B,d}$	$0.41 \pm 0.02^{B,b}$
Black bean	$1.67 \pm 0.06^{E,c}$	$2.22 \pm 0.02^{D,e}$	$1.31 \pm 0.04^{C,b}$	$1.83 \pm 0.01^{C,d}$	$0.63 \pm 0.01^{C,a}$
Soy bean	$0.61 \pm 0.03^{B,c,d}$	$0.57 \pm 0.02^{A,c}$	$0.37 \pm 0.02^{A,b}$	$0.66 \pm 0.05^{A,d}$	$0.27 \pm 0.01^{A,a}$
FRAP (μmol TEq g^{-1} DW)					
Kidney bean	$4.83 \pm 0.39^{A,a}$	$10.72 \pm 0.44^{C,c}$	$5.19 \pm 0.28^{B,a}$	$14.19 \pm 1.29^{C,d}$	$7.37 \pm 0.31^{D,b}$
Pinto bean	$8.03 \pm 0.15^{B,a}$	$14.09 \pm 0.16^{D,b}$	$7.33 \pm 0.57^{C,a}$	$12.60 \pm 0.74^{B,C,b}$	$7.91 \pm 0.62^{D,a}$
Borlotti bean	$4.47 \pm 0.63^{A,ab}$	$8.43 \pm 0.17^{B,c}$	$3.82 \pm 0.10^{A,a}$	$11.65 \pm 0.80^{B,d}$	$5.13 \pm 0.10^{C,b}$
Black bean	$10.37 \pm 0.70^{C,c}$	$15.70 \pm 0.24^{E,d}$	$7.92 \pm 0.20^{C,b}$	$14.62 \pm 0.65^{C,d}$	$4.24 \pm 0.09^{B,a}$
Soy bean	$5.72 \pm 0.14^{A,c}$	$5.81 \pm 0.19^{A,c}$	$3.52 \pm 0.15^{A,b}$	$6.44 \pm 0.40^{A,d}$	$2.18 \pm 0.12^{A,a}$

Data presented as mean and SD from three replicates, n = 3. The data marked by the same letters were not significantly different ($p > 0.05$). Different upercase letters for comparison within each column and lowercase letters for comparison within each row, indicating significant differences ($p < 0.05$) between varieties and different processing, respectively. DPPH - 2,2-Diphenyl-1-picrylhydrazyl radical-scavenging activity; ABTS - 2,2-azino-bis(3-ethylbenzothiazoline)-6 sulphonic acid radical-scavenging activity; FRAP - Ferric reducing antioxidant power; μmol TEq g-1 DW – μmol Trolox equivalents per g of dry weight.

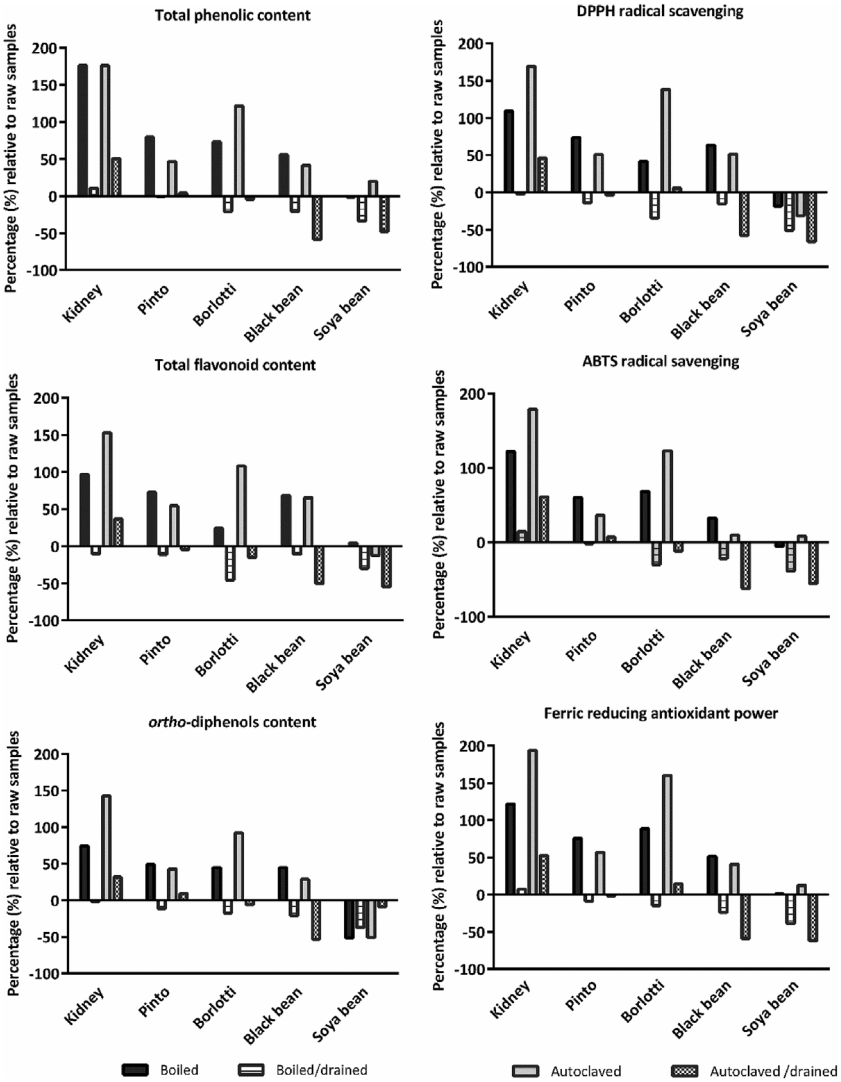

Fig. 2. Percentage gain or loss in the total phenolic content, total flavonoid content, *ortho*-diphenol content and antioxidant capacity of the different cooking conditions. Values were calculated based on the initial contents in raw beans.

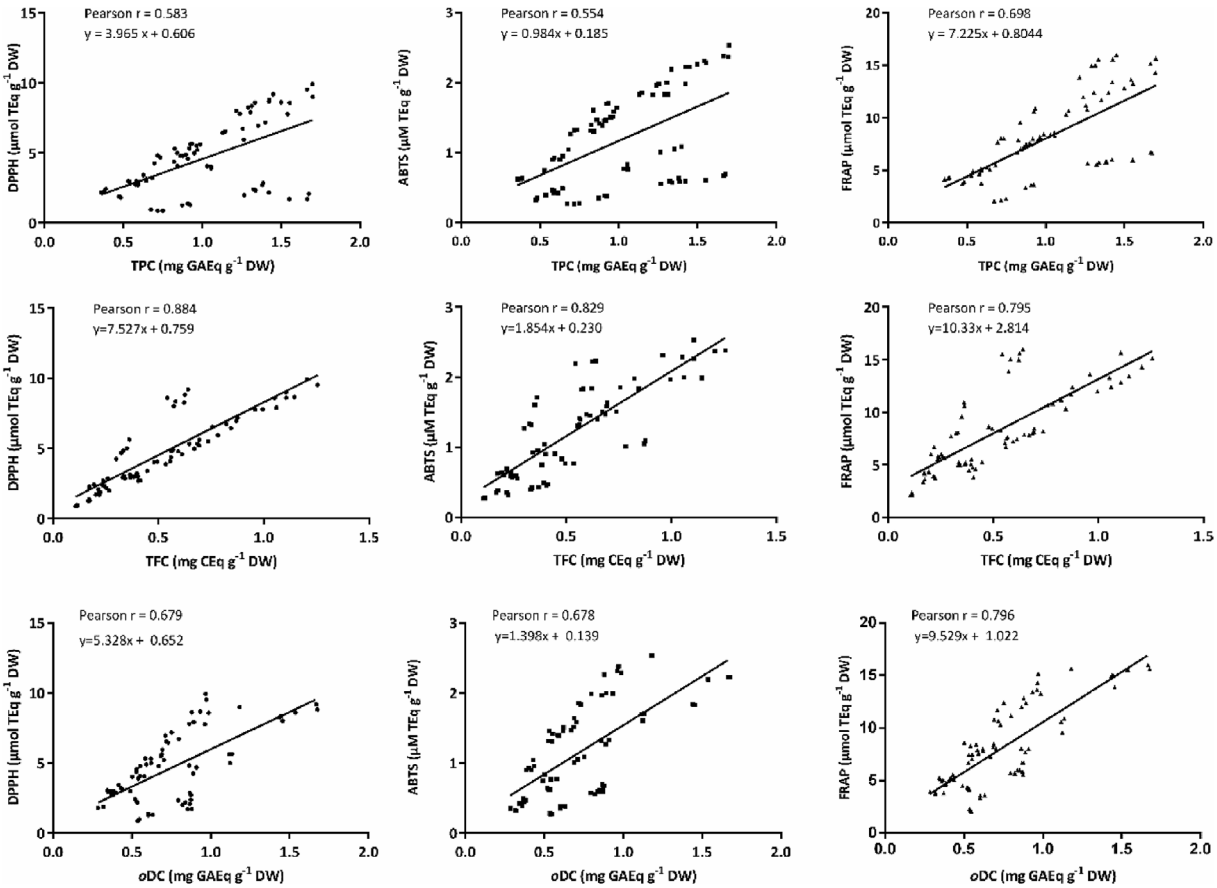

Fig. 3. Correlation of the total phenolic content (TPC), total flavonoid content (TFC), *ortho*-diphenol content (oDC) and antioxidant activity using DPPH - 2,2-Diphenyl-1-picrylhydrazyl radical-scavenging activity; ABTS - 2,2-azino-bis(3-ethylbenzothiazoline)-6 sulphonic acid radical-scavenging activity; FRAP - Ferric reducing antioxidant power. The results were based on three observations per legume by processing combination. Significant correlation at $p < 0.001$ were observed in all experiments.

were of analytical grade. Filters of 11 µm pore size from Whatman No. 1 paper (Maidstone, UK) and 96-well plates from Frilabo (Portugal) were used. The equipment used were an orbital shaker 501 (Bibby Stuart, UK), rotatory evaporator (BÜCHI 461 water bath REIII, Germany), freeze dryer Christ alpha 2–4 (Martin Christ, Germany), microplate reader (Infinite M200 microplate reader, Tecan, Austria), high performance liquid chromatography (HPLC) system with diode array detector (DAD) (Thermo Fisher Scientific, Inc., Waltham, USA) and ACE 5 C18 column (Advanced Chromatography Technologies Ltd, Scotland).

2.2. Plant material

Different varieties of *Phaseolus vulgaris* L. (kidney bean, pinto bean, black bean and borlotti bean) and *Glycine max* L. (soy bean) were used in this work. All legumes were purchased dried from the Portuguese market.

2.3. Processing

Beans (10 g) were soaked in 100 mL of distilled water for 12 h at room temperature. Samples of each legume were subjected to atmospheric boiling (100 °C, for 50 min) or pressure cooking (115 °C, for 20 min). In each case samples were prepared by keeping or draining the cooking water.

2.4. Preparation of crude phenolic extract

All samples, including raw beans, were freeze-dried and ground into a powder. Extraction was carried out in methanol:water (80:20, v/v) at a solid to solvent ratio of 1:10, ultrasound assisted during 30 min at 30 °C and maintained for 24 h in orbital shaker in the dark. The extracts were then vacuum filtrated through Whatman No. 1 paper and the extracts were concentrated using a rotatory evaporator under reduced pressure at 40 °C and then freeze-dried and stored in the dark vials sealed with cap and parafilm at 4 °C.

2.5. Total phenolic content

The total phenolic content (TPC) was determined by the Folin–Ciocalteau method described by Domínguez-Perles, Teixeira, Rosa, and Barros (2014) with some modification and optimized to 96-well plates. In brief, 20 µL of samples or standard were added, followed by 100 µL of Folin–Ciocalteau:H_20 (1:10) and 80 µL of sodium carbonate (7.5 %). The reaction was incubated during 30 min at 42 °C in the dark and the absorbance measured at 750 nm in a microplate reader. Results were calculated with the help of a standard curve and expressed as mg of gallic acid equivalents per g of dry weight (mg GAEq g^{-1} DW).

2.6. Total flavonoid content

The total flavonoid content (TFC) was determined by the aluminium chloride colorimetric method as described by Domínguez-Perles et al. (2014) with some modifications. To each of 96-wells, 24 µL of the sample or standard were added, followed by 28 µL of sodium nitrite (5 %), and 5 min later, 28 µL of aluminium chloride (10 %). The mixture was left to react for 6 min and then 120 µL sodium hydroxide (1 M)

Table 3A
Effect of boiling and pressure cooking on phenolic acid profile of methanolic extracts from the different beans.

	GA	CaftA	ChlA	CafA	*p*-CoumA	FerA
Kidney bean						
Raw	18.58 ± 6.07	7.62 ± 0.78	12.92 ± 2.22	13.61 ± 0.05	1.93 ± 0.03	9.57 ± 0.82
Boiled	64.52 ± 4.25	16.41 ± 1.64	19.72 ± 1.30	25.86 ± 1.54	2.79 ± 0.08	7.85 ± 0.3
Boil. drained	30.66 ± 1.71	10.15 ± 0.09	11.00 ± 0.25	14.89 ± 1.16	1.94 ± 0.04	4.04 ± 0.03
Press. cooked	68.95 ± 7.50	21.77 ± 0.27	25.37 ± 1.42	25.37 ± 1.42	2.81 ± 0.06	8.04 ± 0.36
Press. cooked drained	11.75 ± 1.68	12.91 ± 0.46	10.89 ± 0.90	19.13 ± 1.79	1.45 ± 0.06	3.34 ± 0.11
Pinto bean						
Raw	56.52 ± 5.46	29.41 ± 3.73	34.22 ± 2.17	45.54 ± 3.18	2.81 ± 0.14	13.20 ± 1.38
Boiled	79.41 ± 5.16	25.30 ± 2.36	32.48 ± 1.35	44.92 ± 3.09	5.33 ± 0.23	23.61 ± 0.9
Boil. drained	44.13 ± 2.31	17.39 ± 0.15	17.50 ± 0.78	27.99 ± 1.18	3.69 ± 0.19	16.33 ± 0.56
Press. cooked	56.53 ± 3.47	25.38 ± 0.10	27.34 ± 0.26	41.06 ± 0.54	4.20 ± 0.00	17.58 ± 0.45
Press. cooked drained	28.25 ± 1.49	11.35 ± 1.22	15.62 ± 0.10	21.75 ± 2.30	2.92 ± 0.38	14.20 ± 1.32
Borlotti bean						
Raw	32.88 ± 3.8	15.82 ± 0.84	17.61 ± 2.72	23.08 ± 3.79	1.82 ± 0.05	7.25 ± 0.67
Boiled	49.02 ± 3.96	20.90 ± 2.49	44.24 ± 2.37	32.53 ± 1.00	3.93 ± 0.14	14.19 ± 0.14
Boil. drained	17.77 ± 1.37	6.10 ± 0.64	12.90 ± 1.18	9.11 ± 0.75	1.70 ± 0.07	5.78 ± 0.21
Press. cooked	63.37 ± 4.95	54.57 ± 3.72	54.57 ± 3.72	41.99 ± 7.43	4.95 ± 0.25	16.30 ± 0.22
Press. cooked drained	22.38 ± 0.09	16.19 ± 0.35	16.19 ± 0.35	11.79 ± 0.27	2.03 ± 0.09	6.90 ± 0.23
Black bean						
Raw	67.88 ± 3.22	–	33.38 ± 2.78	54.96 ± 4.50	1.90 ± 0.26	9.35 ± 0.26
Boiled	44.78 ± 2.97	22.33 ± 0.18	32.57 ± 1.32	66.82 ± 5.14	3.48 ± 0.51	18.28 ± 1.51
Boil. drained	25.41 ± 0.25	11.35 ± 0.38	19.73 ± 0.07	34.66 ± 2.12	1.77 ± 0.08	10.18 ± 0.08
Press. cooked	36.02 ± 3.05	26.39 ± 1.75	31.13 ± 2.99	72.73 ± 9.40	2.56 ± 0.01	13.84 ± 1.89
Press. cooked drained	8.91 ± 0.32	5.09 ± 0.62	7.31 ± 1.17	9.55 ± 1.31	0.76 ± 0.02	3.06 ± 0.46
Soy bean						
Raw	54.96 ± 2.58	–	–	–	–	–
Boiled	84.48 ± 2.61	–	–	–	–	–
Boil. drained	39.49 ± 4.48	–	–	–	–	–
Press. cooked	79.81 ± 0.73	–	–	–	–	–
Press. cooked drained	27.32 ± 0.41	–	–	–	–	–

Data presented as mean and SD from four replicates. Results are expressed in μg g^{-1} of dry weight (DW). – not detected, GA: Gallic Acid, CaftA: Caftaric Acid, ChlA: Chlorogenic Acid, p-CoumA: p-Coumaric Acid, FerA: Ferulic Acid.

were added. Absorbance was read at 510 nm, results calculated with the help of a standard curve and expressed as mg catechin equivalents per g of dry weight (mg CEq g^{-1} DW).

2.7. *ortho*-diphenol content

The *ortho*-diphenol content (oDC) was assessed by the sodium molybdate complexation method according to Domínguez-Perles et al. (2014). The samples or standard (160 μL) were pipetted to each well followed by 40 μL of Na_2MO_4 (5 %). The reaction was incubated for 15 min in the dark and the absorbance measured at 370 nm. Results were calculated with the help of a gallic acid standard curve, therefore oDC content was expressed in mg GAE g^{-1} DW.

2.8. DPPH radical scavenging activity

DPPH radical scavenging activity assay was carried out as in Domínguez-Perles et al. (2014) with some modifications. To 10 μL of sample or Trolox standard, 190 μL of the DPPH solution were added. The mixture was placed in the dark at room temperature for 30 min, and absorbance was measured at 520 nm in a microplate reader. Inhibition of free radical DPPH in percent (%) was calculated using the formula:

$$\%\ \text{inhibition} = \frac{\text{Abs blank} - \text{Abs sample}}{\text{Abs blank}} \text{x } 100$$

DPPH radical scavenging activity of the samples was determined by interpolation of the calibration curve for Trolox. Results were expressed in μmol Trolox equivalent per g of dry weight (μmol TEq g^{-1} DW).

2.9. ABTS radical scavenging activity

ABTS radical scavenging activity was performed as previously reported by Domínguez-Perles et al. (2014) with some modifications. To assess ABTS radical inhibition, 12 μL of sample or standard were placed in the microplate followed by 188 μL of ABTS working solution. The plate was incubated for 30 min at room temperature in the dark and the absorbance was measured at 734 nm. Inhibition of ABTS radicals was calculated as previously described for DPPH.

2.10. Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay was performed according to Bolanos De La Torre, Henderson, Nigam, and Owusu-Apenten (2015) with minor alterations. To the 96-well microplate 20 μL of sample was added followed by 280 μL of FRAP working solution. The reaction was incubated at 37 °C in the dark for 30 min and read at 593 nm. Trolox was used as standard and results expressed in μmol TEq g^{-1} DW.

2.11. Phytochemical characterization by high performance liquid chromatography (HPLC)

The phenolic compounds profile was analyzed according to Lin, Harnly, Pastor-corrales, and Luthria (2008) in a HPLC system composed of a quaternary pump, auto sampler and diode array detector (DAD). Separation was performed on a ACE 5 C18 column (5 μm, 250 × 4.6 mm i. d.) at a flow rate of 1 mL min^{-1}. Column temperature was 25 °C and injection volume 20 μL. The mobile phase A consisted of water-formic acid (99.9:0.1; v/v) and B of acetonitrile-formic acid (99.9:0.1; v/v). The extract (10 mg mL^{-1}) was dissolved in water,

Table 3B
Effect of boiling and pressure cooking on flavonoid profile of methanolic extracts from the different beans.

	C	M-3-G	Q-3-G	Q-3-MG	K-3-G	M	K-3-MG	Q	K	G	D
Kidney bean											
Raw	140.54 ± 1.08	–	–	–	0.90 ± 0.07	–	–	–	–	–	–
Boiled	209.92 ± 15.19	–	–	29.11 ± 1.48	0.78 ± 0.03	10.69 ± 0.23	–	–	–	–	–
Boil. drained	93.40 ± 11.26	–	–	11.19 ± 0.08	0.40 ± 0.08	4.50 ± 0.01	–	–	–	–	–
Press. cooked	283.91 ± 23.28	–	–	36.33 ± 2.94	1.20 ± 0.03	13.53 ± 0.78	–	–	–	–	–
Press. cooked drained	104.17 ± 4.79	–	–	8.82 ± 0.2	0.55 ± 0.13	4.64 ± 0.17	–	–	–	–	–
Pinto bean											
Raw	188.70 ± 12.32	–	–	–	3.57 ± 0.13	–	–	–	–	–	–
Boiled	301.17 ± 23.69	–	–	–	3.52 ± 0.24	–	–	–	–	–	–
Boil. drained	284.82 ± 26.53	–	–	–	3.17 ± 0.32	–	–	–	–	–	–
Press. cooked	238.70 ± 14.92	–	–	–	2.35 ± 0.01	–	–	–	–	–	–
Press. cooked drained	162.92 ± 16.02	–	–	–	1.90 ± 0.10	–	–	–	–	–	–
Borlotti bean											
Raw	98.81 ± 1.57	–	–	–	3.50 ± 0.22	–	–	–	–	–	–
Boiled	118.01 ± 0.67	–	–	–	8.59 ± 0.51	–	–	–	–	–	–
Boil. drained	60.96 ± 5.47	–	–	–	0.57 ± 0.04	–	–	–	–	–	–
Press. cooked	143.30 ± 5.44	–	–	–	9.91 ± 0.06	–	–	–	–	–	–
Press. cooked drained	88.72 ± 5.44	–	–	–	0.82 ± 0.03	–	–	–	–	–	–
Black bean											
Raw	372.24 ± 37.83	44.87 ± 4.50	94.73 ± 8.93	–	8.35 ± 0.57	5.98 ± 0.18	4.53 ± 0.20	30.88 ± 2.11	2.51 ± 0.29	–	–
Boiled	485.21 ± 0.03	66.82 ± 5.14	128.01 ± 3.23	54.05 ± 1.54	11.60 ± 0.44	32.87 ± .85	4.46 ± 1.34	144.32 ± 15.17	7.73 ± 0.89	–	–
Boil. drained	162.95 ± 7.16	34.45 ± 2.12	73.88 ± 1.22	20.02 ± 0.01	6.50 ± 0.13	6.24 ± 0.74	2.48 ± 0.30	26.69 ± 1.75	2.13 ± 0.07	–	–
Press. cooked	287.87 ± 4.87	67.11 ± 1.83	104.49 ± 7.27	40.00 ± 3.23	9.94 ± 0.01	31.94 ± 3.36	3.08 ± 0.23	110.98 ± 9.87	8.32 ± 1.47	–	–
Press. cooked drained	61.23 ± 6.09	13.15 ± 0.94	29.18 ± 1.55	13.21 ± 1.15	2.94 ± 0.18	4.31 ± 0.99	4.31 ± 0.99	11.81 ± 1.55	0.90 ± 0.06	–	–
Soy bean											
Raw	1132.52 ± 108.1	–	–	–	–	–	–	–	–	260.41 ± 18.70	76.09 ± 1.65
Boiled	1498.1 ± 148.72	–	–	–	–	–	–	–	–	847.05 ± 90.68	238.82 ± 24.90
Boil. drained	518.51 ± 50.00	–	–	–	–	–	–	–	–	176.08 ± 14.52	56.65 ± 1.21
Press. cooked	1495.48 ± 50.37	–	–	–	–	–	–	–	–	1166.15 ± 3.83	1064.56 ± 68.11
Press. cooked drained	336.47 ± 15.88	–	–	–	–	–	–	–	–	164.60 ± 6.74	60.84 ± 3.12

Data presented as mean and SD from four replicates. Results are expressed in μg g^{-1} of dry weight (DW). – not detected, C: Catechin, M-3-G: Myricetin-3-*O*-glucoside, Q-3-G: Quercetin-3-*O*-glucoside, Q-3-MG: Quercetin-3-*O*-(6-O-manolyl)glucoside, K-3-G: Kaempferol-3-*O*-glucoside, M: Myricetin, K-3-MG: Kaempferol-3-*O*-(6-O-manolyl)glucoside), Q: Quercetin, K: Kaempferol, G: Genistein, D: Daidzein.

injected and eluted using the gradient reported by Lin et al. (2008). DAD was set at 280, 310 and 520 nm while a UV/VIS spectrum from 200 to 600 nm was continuously collected. The chromatograms were analyzed with Xcalibur (Thermo Fisher Scientific, Inc., Waltham, USA).

2.12. Statistical analysis

Statistical analyses were performed using IBM SPSS statistics 21.0 software (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) followed by Tukey's multiple comparisons tests was used to compare control and treated groups. Pearson's correlation coefficient r was used to quantify a relationship between 2 variables. A p value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Effect of processing on extraction yield

The efficiency of hydromethanolic extraction, expressed in mg of lyophilized extracts per g of dry weight of the different raw samples was found to decrease in the following order: soy > black > borlotti > pinto > kidney bean (data not shown). The effect of boiling and pressure cooking on the extraction yields, relative to raw samples, is presented in Fig. 1. In all *P. vulgaris*, boiling and pressure cooking significantly increased the extraction efficiency, while in soy only a slight increase was observed. The extraction yield in processed kidney and borlotti beans was found to be the highest, reaching an increase of 94 % for boiled and 130 % for pressure cooked kidney bean, and 62 % for boiled and 83 % for the pressure cooked borlotti bean. In pinto and black beans, boiling was more efficient than pressure cooking. In these samples, boiling generated an increase of 50 % and 30 %, while pressure cooking generated an increase of 20 % and 5 % for pinto and black bean, respectively.

A decrease in extraction yields in all processed samples was observed when the cooking water was discarded. This decrease was more evident in pressure cooked kidney bean and in both boiled and pressure cooked borlotti, black bean and soy bean.

These results are consistent with those of Siah et al. (2014) who found that boiling increased extraction yield by 2–34 % and pressure cooking by 265–275 %. The present study showed that heat treatment increased the extraction yield in broths after boiling and pressure cooking in *Phaseolus vulgaris*. This finding suggested that heat could cause destruction of seed structure enabling the release of phenolic compounds into the cooking broths, thereby increasing the extraction efficiency by the used solvent. Heat processing can also induce the release of other components, such as soluble sugars, proteins and soluble fibers, contributing to the increase of the generated material yields.

3.2. Total phenolic (TPC), total flavonoid (TFC) and ortho-diphenol content (oDC) and antioxidant activity

3.2.1. Raw samples

Results of colorimetric assays used for assessment of TPC, TFC and oDC are presented in Table 1. Comparing between raw samples, TPC was found to be the highest in soy bean and in decreasing order in pinto bean > black bean > borlotti bean > kidney bean. Raw soy bean TPC was significantly higher ($p < 0.05$) than any extracts of *Phaseolus vulgaris*.

Extraction of raw Phaseolus, all presented higher TFC content than soy bean extracts ($p < 0.05$), being pinto bean the richest source. In *P. vulgaris* varieties, 40–70 % of the phenolic compounds were flavonoids whereas in soy flavonoids corresponded only to 20 % of total phenols.

The antioxidant activity results are present in Table 2. In all assays, (DPPH, ABTS and FRAP) the antioxidant activity of raw black beans and pinto beans were higher that all the other varieties ($p < 0.05$). The antioxidant activity assessed by ABTS and FRAP showed the same pattern with black bean extracts having higher activity, followed by pinto, kidney, soy and finally borlotti bean. The DPPH radical scavenging activity was higher in black bean, followed by pinto, kidney, borlotti and soy bean.

There are many classes of compounds with antioxidant activity of which *ortho*-diphenols have been recognized as the class with highest antioxidant activity (Soufi, Romero, & Louaileche, 2014). Regarding *ortho*-diphenols, black bean was the richest source ($p < 0.05$) and kidney and borlotti beans showed the lowest levels ($p < 0.05$) in line with the higher antioxidant activity present in black bean extracts.

Overall, the antioxidant capacity of soy bean was approximately 2 times lower than that of black and pinto beans. Although a higher phenolic content in raw soy bean was observed, this is not reflected in a higher antioxidant activity. In black bean the antioxidant activity seems to be correlated with the oDC, while in the remaining varieties with the TFC.

3.2.2. Effect of processing

The effect of boiling and pressure cooking on TPC, TFC and oDC are presented in Table 1 and the percentage of gain or loss relative to the raw samples are shown in Fig. 2.

Overall, processing by both cooking methods increased all parameters in *P. vulgaris* varieties in contrast with the effects on soy, where cooking did not improve flavonoid content nor antioxidant activity of hydromethanolic extracts.

In kidney, pinto and black beans boiling and pressure cooking had a positive impact on total phenolic, flavonoid and *ortho*-diphenol content. Pressure cooking produced the highest increase in TPC, TFC and oDC in kidney and borlotti beans, while in pinto and black varieties boiling was more efficient. Importantly, in both processing methods, a significant part of TPC, TFC and oDC, as well as antioxidant activity was lost by draining the cooking water.

The antioxidant capacities of extracts are presented in Table 2. The relative changes found in extracts of processed legumes compared to raw samples are presented in Fig. 2. In agreement with phenolic content, the antioxidant activity of the extracts of all varieties of *P. vulgaris* significantly increased after the different cooking conditions, in contrast to soy bean.

Similarly to the phenolic content of extracts, boiling also increased the antioxidant activity, particularly in pinto and black beans but not in soy. Pressure cooking seemed to be more effective in the larger varieties, kidney and borlotti beans. Regardless of the processing method, discarding the cooking water significantly decreased the content in phenolics and in antioxidant activity ($p < 0.05$) in all beans.

It has been demonstrated in same vegetable foods, that phenolic compounds are generally bound covalently to amine functional groups and therefore heat treatment can hydrolyze them, increasing the extractability (Jiratanan & Liut, 2004). Ranilla et al. (2009) reported a significant increase of TPC and antioxidant activity assessed by DPPH assay in Brazilian bean cultivars cooked at 100 and 121 °C and without draining the cooking water. Rocha-Guzmán et al. (2007) also reported the increase of antioxidant activity in *Phaseolus vulgaris* cooked at 121 °C. In agreement with this, Sarmento, Barros, Fernandes, Carvalho, and Ferreira (2015) found in *Cicer arietinum* L. and *Lathyrus sativus* L., an increase in tocopherols, bioactive compounds and antioxidant activity in seeds previously soaked and pressure cooked for 15 min. In contrast, several researchers reported that thermal processing decrease the phenolic content and antioxidant activity in legumes. Granito, Yannellis, and Torres (2007) showed a decrease of TPC in boiled *Phaseolus lunatus*, suggesting that heat treatments could affect the aromatic rings of phenolic compounds making them more susceptible to polymerization and decomposition. Siah et al. (2014) also reported a decrease in TPC and TFC after boiling and pressure cooking *Vicia faba* L. In addition, in boiled black bean Eshraq et al. (2016) observed a decrease in TPC and TFC when compared with raw samples.

Our date shows a clear correlation between the levels of

polyphenols and the free radical scavenging activities of the extracts. The Pearson's correlations between the parameters TPC, TFC, oDC and DPPH, ABTS and FRAP of all beans are shown in Fig. 3. Correlation between polyphenol content and antioxidant activity was performed to evaluate which group of compounds are mainly responsible for the antioxidant activity. The strongest correlation was found between antioxidant activity (assessed by ABTS and TFC (Pearson $r = 0.829$), followed by FRAP activity) and oDC, as well as FRAP activity and TFC (Pearson $r = 0.796$ and 0.795, respectively).

3.3. Phytochemical composition

Quantitative and qualitative differences in phenolic composition of raw and processed samples were detected by HPLC and are presented in Tables 3A and 3B.

Gallic acid (GA) was found to be the predominant phenolic acid and was present in all varieties studied. In *P. vulgaris*, GA content was the highest and followed in decreasing order of abundance by caffeic acid (CafA), chlorogenic acid (ChlA), ferulic acid (FerA), caffeic acid (CafA) and *p*-coumaric acid (*p*-CoumA). In the different raw samples, GA concentration was found to decrease in the following order: black > pinto > soy > borlotti and kidney bean. In soy bean extracts, only gallic acid was detected. In all samples, an increase in GA content was found after processing, whereas in black beans the opposite was observed. Overall, processing whether by boiling or by pressure cooking, had a positive effect on the increase of the phenolic acid content in the extracts from the different varieties of beans compared to the raw samples, including soy bean.

With regard to flavonoids, as shown in Table 3B, catechin was detected in higher amount in soy followed by black bean but was present in all varieties both before and after cooking. Except for pressure cooked black bean, processing increased catechin in all other varieties. Kaempferol-3-*O*-glucoside (k-3-G) was found in all *P. vulgaris* and overall processing increased its content. Regarding the isoflavones genistein and daidzein, they were only detected in soy bean and were increased by processing, particularly by pressure cooking. In black and kidney bean extracts, myricetin, quercetin, and kaempferol with different glycosylation were also detected. Extracts of pinto and borlotti bean only presented derivatives of kaempferol. Higher total phenolic (TPC) and total flavonoid (TFC) content detected after cooking by the colorimetric assays are corroborated by the results obtained by HPLC analyses. Also visible on the HPLC data, content of phenolic acids and flavonoids dramatically decreased when the cooking water is drained. The highest oDC was observed in black bean, which reflects the high levels of the oDC species such as GA, ChlA and C, M, Q, K and derivatives. In soy bean, the high oDC was correlated only to the high amount of GA and genistein.

Our results are in agreement with those reported by Ranilla et al. (2009), using Brazilian *Phaseolus vulgaris*, where an increase in kaempferol and quercetin derivatives, *p*-coumaric and ferulic acids were found after cooking, independently of temperature. Also, draining had the effect of decreasing the levels of the different phenolics, independently of the heat treatment.

In the study performed by Price, Colquhoun, Barnes, and Rhodes (1998) no significant change in levels of quercetin and kaempferol derivates was found after thermal cooked green beans. In contrast, Díaz-Batalla, Widholm, Fahey, Castaño-Tostado, and Paredes-López (2006) reported a reduction of quercetin and kaempferol content in pressure cooked Mexican beans in comparison with the initial content. Also in contrast with our results, Kumar et al. (2012) observed a decrease in isoflavones (genistein, daidzein and glycitein) in boiled and pressure cooked soy.

4. Conclusion

In the present study, we evaluated the effect of two cooking methods (boiling and pressure cooking) on total phenolic content and individual composition as well as antioxidant activity of hydromethanolic extracts of several common bean varieties and soy. Total phenolic compounds of raw samples showed highest values in soy, although the antioxidant activity was higher in black bean and pinto bean methanolic extracts which were higher in flavonoids. Processing by the two cooking methods (boiling and pressure cooking) increased phenolic content and antioxidant activity in extracts of the four *Phaseolus vulgaris* varieties in contrast with the effects on soy. In spite of the modest increase in antioxidant activity, genistein and daidzein were substantially enriched in extract of boiled and particularly of pressure cooked soy compared to raw seeds. Discarding the cooking water resulted in loss of significant amounts of phytochemicals and decreased antioxidant potential.

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