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ARTICLE

Effect of Different Cooking Conditions on Phenolic Compounds and Antioxidant Capacity of Some Selected Brazilian Bean (*Phaseolus vulgaris* L.) Cultivars

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The effects of different cooking conditions such as soaking, atmospheric (100 °C) or pressure boiling (121 °C), and draining of cooking water following thermal treatment on phenolic compounds and the DPPH radical scavenging capacity from two selected Brazilian bean cultivars (black and yellow-brown seed coat color) were investigated using a factorial design (2^3). Factors that significantly reduced the total phenolic contents and antioxidant capacity in both cultivars were the soaking and draining stage. Independent of cooking temperature, total phenolics and antioxidant capacities were enhanced in treatments without soaking and where cooking water was not discarded, and this was likely linked to an increase of specific phenolic compounds detected by high performance liquid chromatography such as flavonols and free phenolic acids in both cultivars. Cooking of beans either at 100 or 121 °C, without a soaking stage and keeping the cooking water, would be recommendable for retaining antioxidant phenolic compounds.

KEYWORDS: Brazilian bean cultivars; *Phaseolus vulgaris* L.; polyphenols; antioxidant capacity; cooking

INTRODUCTION

Common beans (*Phaseolus vulgaris* L.), widely grown and consumed throughout the world, are a rich and relatively inexpensive source of proteins (20−25%) and carbohydrates (50−60%) for a large part of the world's population, mainly in developing countries (*1*). Brazil is one of the main producers of these pulses, and the consumption per year is around 15.9 kg/capita (*2*). Besides their nutritional importance, beans also serve as a rich source of bioactive compounds such as enzyme inhibitors, lectins, phytates, oligosaccharides, and phenolic compounds with potential health implications (*3*).

Different types of polyphenolic compounds have been widely reported in common beans (*4*), and their presence in these pulses has been associated with several biological effects such as antioxidant and antiproliferative effects on cancer cells lines (*5*, *6*). This would explain the numerous experimental and epidemiological studies that have shown a correlation between the consumption of dry beans and decreasing incidences of coronary heart diseases, diabetes, and obesity risk (*7*).

Thermal treatment applied to legumes improves their nutritive value by reduction of antinutrients such as phytic acid and tannins, along with an improvement in protein and starch digestibility (*8*). Further, cooking procedures induce the production of desirable sensory properties in beans such as sweet taste, cooked-bean flavor, and soft and mushy textures (*9*). Rocha-Guzman et al. (*10*) found that the antioxidant-linked radical scavenging activity of beans from Mexico increased after an autoclaving process. However, information regarding the effect of thermal treatments on phenolic compounds and antioxidant capacity from beans is still limited, especially for cultivars commonly consumed in countries such as Brazil, where beans are the staple food, and their consumption is declining due to changes in traditional dietary habits (*11*).

In a previous study (*4*), methanolic extracts from commercially important Brazilian bean cultivars were characterized in relation to their phenolic profiles and in vitro antioxidant capacity. The black bean FT Nobre cultivar showed the highest total anthocyanin content along with a high antioxidant activity assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Meanwhile, the yellow-brown seed coat Jalo Precoce cultivar showed the highest content in flavonols, especially kaempferol derivatives. However, the potential changes on phenolic compounds and antioxidant capacity of these cultivars following thermal treatment have not been investigated yet. The aim of this work was to evaluate the effect of different domestic cooking conditions such as atmospheric or pressure boiling, the presence/absence of the application of a soaking step prior to thermal treatment, and the effect of draining or not draining the cooking liquid on total phenolic contents, condensed tannins, phenolic profiles, and antioxidant capacity of two selected Brazilian bean cultivars (FT Nobre and Jalo Precoce) by using a 2^3 factorial design.

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MATERIALS AND METHODS

Materials. Selected bean (*Phaseolus vulgaris* L.) cultivars FT Nobre and Jalo Precoce (*4*) were obtained from the Brazilian Company of Agricultural Research (EMBRAPA) (Rice and Bean department). Mature dry seeds (harvested in 2007, in the state of Goias) were stored at 4 °C and analyzed in a period of not more than 1 year. For analyses of raw beans, seeds were milled under refrigeration into flour (60 mesh), and the final flours were stored at −18 °C until analysis.

Chemicals. The HPLC standards quercetin, kaempferol, chlorogenic, *p*-coumaric, and ferulic acids, and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin−Ciocalteu reagents were purchased from Sigma Co. (St. Louis, MO). The cyanidin chloride standard was from Extrasynthese (Genay Cedex, France), and the hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was from Aldrich (Milwaukee, WI). All chemicals and solvents used for extraction and quantification of phenolics were of HPLC grade.

Moisture Determination of Raw Beans. Moisture in beans was determined in triplicate ($n = 3$) according to the Association of Official Analytical Chemists (AOAC) (*12*), by drying 2 g samples in an oven (Precision Scientific, Winchester, VA) set at 100 ± 0.5 °C until constant weight.

Use of a Factorial Design for the Evaluation of Different Thermal Treatments on Bean Phenolic Compounds and Antioxidant Capacity. Thermal treatments were conducted trying to mimic the different types of cooking procedures commonly used for beans. The experimental design corresponded to a factorial design at two levels with three factors or variables at each level (2^3). Thermal treatment at 121 °C was performed using a vertical autoclave model 103 (FABBE-PRIMAR, Sao Paulo, Brazil). Experiments were carried out in duplicate, and analyses of total phenolics, antioxidant capacity, condensed tannins, and phenolic profiles by HPLC were determined in thermally treated and further freeze-dried bean samples, and the results were expressed in dried weight (DW).

Determination of Cooking Times. The cooking times for treatments with and without a soaking stage prior to thermal treatment at 100 °C were determined by the Mattson cooker method (*13*). The Mattson cooker consists of 25 plungers and a cooking rack with 25 reservoir-like perforated saddles, each of which holds one bean and a plunger calibrated to a specific weight. Soaked or nonsoaked beans (*25*) were randomly selected and then positioned in each of the 25 saddles of the rack so that the tip of each plunger rested on top of each bean. All plungers were calibrated to 89.9 g. The rack was then placed in a 2 L glass beaker containing 1.5 L of boiling water. When beans became sufficiently tender, the plunger penetrated them and dropped through the hole in the saddle. The time required for 50% + 1 of plungers to penetrate beans was defined as the cooking time in this study. The analysis was carried out in triplicate.

For treatments at 121 °C, the cooking time was defined as the time required for getting the same final texture obtained at 100 °C. Therefore, several tests were performed using a digital texture analyzer to get more objective results than the tactile or the sensory panel methods. Cooked beans (*25*) were placed in covered plastic containers and cooled at room temperature for 1 h prior to texture analysis. The firmness values of each cooked bean were measured by using a TAXT2 texture analyzer (Stable Microsystems, Ltd., Surrey, UK) equipped with a load cell of 25 k and a Kramer Compression-Shear cell. The cross-head speed was set at 1 mm/s, and the maximum force needed for the shear process (maximum peak) was taken to indicate the degree of firmness of the autoclaved bean. The analysis was performed in duplicate, and the results were expressed as *g* force.

Flavonoid and Phenolic Acids Extraction. The extraction was performed according to the method of Arabbi et al. (*14*) with some modifications, as follows. Raw bean flours or freeze-dried samples (1 g) were placed in a 50 mL flask, mixed with 20 mL of methanol/water (70:30) or methanol/water/acetic acid (70:30:5) (samples with anthocyanins) and shaken for 2 h at 4 °C. The extracts were filtered under reduced pressure through filter paper (Whatman No. 1), evaporated under vacuum at 40 °C to ∼6 mL in a rotatory evaporator, and made up to 10 mL with water. An aliquot of 1 to 6 mL (depending on the flavonoid and phenolic acid concentrations) was added to a polyamide (1 g) SC6 column (Macherey-Nagel Gmbh and Co., Düren, Germany) preconditioned with methanol (20 mL) followed by water (60 mL). The column was washed with water (20 mL) and further eluted with methanol (50 mL), to elute the neutral flavonols, and with methanol/ammonia (99.5:0.5) (50 mL) to elute the acidic flavonols. These fractions were evaporated to dryness under reduced pressure at 40 °C, redissolved in HPLC grade methanol (1 mL), filtered through 0.22 μm PTFE (polytetrafluoroethylene) filters (Millipore Ltd., Bedford, MA), and analyzed by high performance liquid chromatography (HPLC). All extractions were performed in duplicate.

HPLC Quantification. Identification and quantification of flavonoids and phenolics acids was achieved according to Arabbi et al. (*14*) by using analytical reversed-phase HPLC in a Hewlett-Packard 1100 system with an autosampler and quaternary pump coupled to a diode array detector controlled by Chemstation software. The column used was 250×4.6 mm, i.d., 5 μm, Prodigy ODS3 reversed-phase silica (Phenomenex Ltd., Torrance, CA), and the elution solvents were A, water/tetrahydrofuran/trifluoroacetic acid (98:2:0.1), and B, acetonitrile. Eluates were monitored at 270, 328, 370, and 525 nm, and samples were injected in duplicate. Calibration was performed by injecting the standards three times at five different concentrations ($R^2 \geq 0.999$). Peak identification was performed by comparison of retention times and diode array spectral characteristics with the standards and the library spectra. Cochromatography was used when necessary. In the case of quercetin and kaempferol derivatives, results were expressed as mg of aglycone, anthocyanins were expressed as mg of cyanidin, and phenolic acids (chlorogenic, *p*-coumaric and ferulic acids) were expressed as mg of the respective standard. Quantification of hydroxycinnamic acid derivatives was effected on the basis of chlorogenic acid as a reference standard for peaks detected at 328 nm with spectroscopic characteristics similar to those of chlorogenic acid, but with different retention times (the areas under the curves were combined to obtain a single value). All analyses were carried out in duplicate, and the results were expressed by 100 g of sample in dried weight (DW).

Sample Extraction for Total Phenolics, Condensed Tannins, and Antioxidant Capacity Assays. Raw bean flours or freeze-dried samples (1 g) were placed in a 50 mL flask and mixed with 20 mL of methanol/water (70:30) or methanol/water/acetic acid (70:30:5) (samples with anthocyanins) for 2 h at 4 °C and centrifuged at 10 000*g* for 10 min. The supernatant recovered was stored in the dark at −18 °C until analysis. All extractions were performed in duplicate, and the subsequent assays were run in triplicate.

Total Phenolics. The analysis was performed according to Zielinski and Kozlowska (*15*), with some modifications. A 0.25 mL aliquot was mixed with 0.25 mL of the Folin−Ciocalteu reagent and 2 mL of distillated water. After 3 min at room temperature, 0.25 mL of a saturated sodium carbonate (Na_2CO_3) solution was added and the mixture placed at 37 °C in a water bath for 30 min. The absorbance was measured at 750 nm using a model Ultrospec 2000 UV/visible spectrophotometer (Amersham Biosciences, Cambridge, UK). (+)-Catechin was used as the reference standard, and the results were expressed as mg of catechin equivalents/g sample DW.

Condensed Tanins. Tannins were assayed with the vanillin-HCl method of Price et al. (*16*). (+)-Catechin was used as the reference standard, and tannins were expressed in mg of catechin equivalents/g sample DW. In order to correct for interference from natural pigments in seed coats, a blank sample was prepared by subjecting the original extract to the same reaction conditions but without the vanillin reagent.

Antioxidant Capacity. The antioxidant capacity was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging method according to Brand-Williams et al. (*17*). A 50 μL aliquot of the extract previously diluted and 250 μL of DPPH (0.5 mM) were shaken, and after 25 min, the absorbance was measured at 517 nm using a Benchmark Plus microplate spectrophotometer (BioRad, Hercules, CA). The control consisted of a Trolox solution at different concentrations. The antioxidant capacity was expressed as μmol Trolox equivalents/g sample DW.

Statistical Analysis. Results from HPLC ($n = 4$) and spectrophotometric ($n = 6$) analyses were expressed as means ± standard deviation (SD). Data were subjected to 3-way analysis of variance (ANOVA), the Tukey test ($p < 0.05$) was used to determine significant differences between means, and the calculated effects for the 2^3 factorial design were obtained by using the Statistica software package version 5.0 (StatSoft, Tulsa, OK).

RESULTS AND DISCUSSION

Determination of Cooking Times. Table 1 shows that cooking times varied according to the different treatments. Times were shorter in treatments with a soaking stage prior to the thermal process than in treatments without a soaking step, and shorter times were also obtained for treatments at 121 °C (<10 min). In addition, cooking times at 121 °C for previously soaked beans were the shortest among all evaluated treatments in both cultivars (just the time necessary to reach 121 °C). Longer times (> 1 min) under those cooking conditions led to lower values of texture than those obtained at 100 °C. Traditionally, beans are soaked in water before the thermal treatment to facilitate the cooking process; a previous soaking allows better thermal transference resulting in shorter cooking times (*18*). However, treatments without a previous soaking stage were included to determine if losses of phenolic antioxidants are significant during this step.

In general, the Jalo Precoce cultivar required higher cooking times than the FT Nobre cultivar, and values of texture measured in *g* force were higher for the Jalo Precoce cultivar than for black beans. This might be related to differences in carbohydrate and protein chemical characteristics for these bean cultivars (*19*). Similarly, Rocha-Guzman et al. (*10*) observed that different bean cultivars varied significantly not only in their phenolic distribution within the cooked seed but also in their cooking times when processed under the same conditions.

Effect of Cooking Conditions on Flavonoid and Phenolic Acid Profiles. In general factorial designs, the researchers selects a fixed number of levels for each of a number of factors (independent variables) and then run experiments with all possible combinations. The calculated values of main effects quantitatively indicate the variation in the response caused by the change of all levels of a specific factor. Through these designs, it is possible to calculate either the main effects due to independent factors or to estimate and detect interactions between factors. Further, the determination of the statistical significance of the effect of one or more factors or their interactions on the response variable is also possible (*20*).

In this work, three independent factors (cooking temperature, soaking prior to cooking, and draining following the cooking process) were evaluated at two levels (100 and 121 °C, with or without soaking and with or without draining, respectively). Therefore, a 2^3 factorial design with 8 treatments or total combinations was planned. The response variables corresponded to the total phenolic contents, condensed tannin contents, antioxidant capacity, and the HPLC phenolic profiles following the thermal treatment.

The flavonoid and phenolic acid contents detected by HPLC in raw and cooked FT Nobre black bean cultivar under different cooking conditions are shown in **Table 2**. The calculated effects of main factors and their interactions on bean phenolic compounds along with the statistical significance of all effects following the different thermal treatments are provided in a table included in Supporting Information.

According to **Table 2**, treatments without a previous soaking and without a draining step after the cooking process had higher flavonoid and phenolic acid contents than treatments that included soaking and draining stages. Among treatments without soaking and without draining, no significant differences were found in quercetin and kaempferol derivatives and phenolic acid contents in black beans cooked either at 100 or 121 °C. However, anthocyanin levels were significantly reduced following the thermal treatment at 121 °C. It is well known that anthocyanins are easily degraded when exposed to heat. In this regard, Sadilova et al. (*21*) reported that successive deglycosylation reactions represent the initial steps of anthocyanin degradation following thermal treatment at pH 1 yielding the corresponding aglycones. Further, Jing and Giusti (*22*) observed a consistent decrease of protein at 100 °C in a purple corn water extract indicating a possible protein denaturation at high temperatures, which could result in anthocyanin complexation and precipitation leading to a decline in total anthocyanin content.

The calculated effect values indicate that main factors (soaking, temperature, and draining) had a significant ($p < 0.05$) and an inverse effect (negative sign) on flavonoid and phenolic acid contents. The effect of factors defined as draining and soaking showed the highest influence (highest quantitative values) on flavonoid and phenolic acid contents. In the case of anthocyanins, the effect of a draining step following the thermal treatment was to reduce the mean by about 6.53 mg/100 g DW, and this was approximately irrespective of the tested levels of the other factors such as temperature and soaking. Similarly, when black beans were cooked with a previous soaking step, the mean value for anthocyanins decreased in 4.45 mg/100 g DW, independent of the other evaluated factors. The same could be interpreted for the

Table 1. Determination of Cooking Times for the Different Treatments

cultivar	soaking [a]	temperature (°C)	time (min)	texture (*g* force)
FT Nobre	without	100	93	578 ± 51
	without	121	10	554 ± 65
	with	100	50	514 ± 59
	with	121	0[b]	560 ± 10
Jalo Precoce	without	100	119	800 ± 50
	without	121	8	750 ± 60
	with	100	58	870 ± 70
	with	121	0[b]	770 ± 80

[a] Soaking prior to thermal treatment. [b] The autoclave was turned off when the temperature reached 121 °C.

Table 2. Phenolic Compound Content Detected by HPLC in Raw and Cooked FT Nobre Bean Cultivar under Different Cooking Conditions[a]

		without soaking (mg/100 g sample in dried weight)				with soaking (mg/100 g sample in dried weight)			
		100 °C		121 °C		100 °C		121 °C	
phenolic compound	raw bean[b]	draining	without draining	draining	without draining	draining	without draining	draining	without draining
total anthocyanins	37 ± 2	7 ± 1 c	16.9 ± 0.2 a	3.9 ± 0.3 d	10.1 ± 0.1 b	3.0 ± 0.4 de	9.1 ± 0.9 b	2.0 ± 0.1 e	5.9 ± 0.2 c
quercetin derivatives	3.0 ± 0.3	2.5 ± 0.3 b	3.6 ± 0.2 a	1.7 ± 0.1 cd	3.8 ± 0.1 a	0.70 ± 0.01 e	1.6 ± 0.1 d	0.80 ± 0.03 e	2.1 ± 0.2 bc
kaempferol derivatives	0.63 ± 0.04	0.6 ± 0.1 b	0.8 ± 0.1 a	0.47 ± 0.04 c	0.83 ± 0.05 a	0.16 ± 0.02 e	0.32 ± 0.02 d	0.19 ± 0.01 e	0.40 ± 0.05 cd
ferulic acid	0.94 ± 0.05	0.8 ± 0.1 c	1.22 ± 0.04 b	0.80 ± 0.03 c	1.7 ± 0.1 a	0.20 ± 0.01 e	0.52 ± 0.03 d	0.24 ± 0.01 e	0.80 ± 0.03 c
hydroxycinnamic acid derivatives	36 ± 4	4 ± 1 c	18 ± 1 a	4.4 ± 0.3 c	16.1 ± 0.5 a	1.6 ± 0.2 d	12 ± 1 b	1.59 ± 0.03 d	13 ± 1 b
total flavonoids	41 ± 3	10 ± 1 cd	21.3 ± 0.4 a	6.2 ± 0.3 e	14.7 ± 0.2 b	3.9 ± 0.5 f	11 ± 1 c	3.0 ± 0.1 f	8.4 ± 0.5 d
total phenolic acids	37 ± 4	5 ± 1 c	19 ± 1 a	5.2 ± 0.3 c	17.7 ± 0.5 a	1.8 ± 0.2 d	13 ± 1 b	1.83 ± 0.02 d	14 ± 1 b

[a] Values are means ± SD. [b] Moisture of raw bean: 9.65 ± 0.14%. Means in the same line with different letters are significantly different ($p < 0.05$).

Table 3. Phenolic Compound Content Detected by HPLC in Raw and Cooked Jalo Precoce Bean Cultivar under Different Cooking Conditions[a]

		without soaking (mg/100 g sample in dried weight)				with soaking (mg/100 g sample in dried weight)			
		100 °C		121 °C		100 °C		121 °C	
phenolic compound	raw bean[b]	draining	without draining	draining	without draining	draining	without draining	draining	without draining
quercetin derivatives	0.78 ± 0.02	0.84 ± 0.04 b	1.3 ± 0.1 a	0.50 ± 0.02 c	1.2 ± 0.1 a	0.30 ± 0.03 d	0.51 ± 0.01 c	0.21 ± 0.01 d	0.55 ± 0.02 c
kaempferol derivatives	30.7 ± 0.2	33.3 ± 0.1 c	48.8 ± 0.1 a	16.3 ± 0.1 f	47.1 ± 0.4 b	13.5 ± 0.3 g	23.4 ± 0.1 d	13.6 ± 0.2 g	22.3 ± 0.1 e
p-coumaric acid	0.36 ± 0.04	0.33 ± 0.05 b	0.51 ± 0.06 a	0.30 ± 0.01 b	0.4 ± 0.1 a	0.05 ± 0.00 d	0.18 ± 0.02 c	0.05 ± 0.00 d	0.27 ± 0.01 b
ferulic acid	0.95 ± 0.04	0.90 ± 0.03 bc	1.6 ± 0.1 a	0.84 ± 0.01 c	1.57 ± 0.04 a	n.d	0.82 ± 0.01 c	n.d	0.94 ± 0.01 b
hydroxycinnamic acid derivatives	23.9 ± 0.1	6.4 ± 0.1 e	19 ± 1 a	8.00 ± 0.05 d	17.8 ± 0.3 b	2.06 ± 0.04 g	15.6 ± 0.4 c	3.1 ± 0.1 f	15.9 ± 0.5 c
total flavonoids	31.5 ± 0.1	34.1 ± 0.1 c	50.1 ± 0.1 a	16.8 ± 0.1 f	48.3 ± 0.4 b	13.8 ± 0.3 g	23.9 ± 0.1 d	13.8 ± 0.2 g	22.8 ± 0.1 e
total phenolic acids	25.2 ± 0.1	7.7 ± 0.2 e	21 ± 1 a	9.15 ± 0.03 d	19.8 ± 0.4 b	2.10 ± 0.05 g	16.6 ± 0.4 c	3.1 ± 0.1 f	17.2 ± 0.5 c

[a] Values are means ± SD. [b] Moisture of raw bean: 11.13 ± 0.08%. n.d: not detected. Means in the same line with different letters are significantly different ($p < 0.05$).

other response variables such as quercetin and kaempferol derivatives and phenolic acids. The increase of the cooking temperature from 100 to 121 °C did not show a significant effect either on flavonol or hydroxycinnamic acid derivatives, independent of the evaluated levels of the other factors. However, in the case of anthocyanins, the effect of increasing the cooking temperature was to reduce the mean by about 3.47 mg/100 g DW.

In general, the calculated effects of factor interactions were positive; this likely indicates that treatments with adequate combinations of temperature, soaking, and draining could lead to a better retention of phenolic compounds from black beans. However, these calculated effects were quite low and in some cases not significant ($p > 0.05$) when compared to those showed by main factors as previously mentioned.

The flavonoid and phenolic acid contents detected by HPLC in raw and cooked Jalo Precoce bean cultivar under different cooking conditions are shown in **Table 3**. Although flavonoid and phenolic acid profiles were quite different between the black bean FT Nobre and Jalo Precoce cultivars, retention of phenolic compounds from the Jalo Precoce cultivar was better in treatments without soaking and draining steps, as was also observed in the FT Nobre cultivar.

According to statistical analysis (Supporting Information), overall all factors and their interactions had an inverse (negative sign) and a significant effect ($p < 0.05$) on phenolic compounds. Factors such as soaking and draining showed the highest effect on flavonoid and phenolic acid contents as was also observed for the black bean cultivar. In the case of kaempferol derivatives (the major flavonols in the Jalo Precoce cultivar), the effect of thermal treatment with a previous soaking step was to decrease the mean by 18.19 mg/100 g DW, independent of the evaluated levels of the other factors. Similarly, the effect of a draining stage following thermal treatment was to decrease the mean of kaempferol derivatives by 16.25 mg/100 g DW, independent of the other factors and their tested levels. The draining factor had a negative and a higher effect on hydroxycinnamic acid contents (−12.10) than the soaking factor (−3.51), indicating that losses of these compounds might occur mainly during the draining step than during the bean soaking step prior to thermal treatment.

Independent of factors such as soaking and draining and their evaluated levels, the increase of cooking temperature from 100 to 121 °C did not influence the *p*-coumaric and ferulic acid contents. However, the effect of increasing the cooking temperature was to reduce the mean of kaempferol derivatives by about 4.94 mg/100 g DW.

On the basis of flavonoid and phenolic acid contents in raw beans for both FT Nobre and Jalo Precoce cultivars, it was possible to calculate the percentage of losses or increases of each phenolic compound due to the different cooking conditions. The variations in flavonoid and phenolic acid contents from the FT Nobre bean cultivar in relation to its initial levels prior to thermal treatment are shown in **Figure 1**. Significant losses in total anthocyanin contents (> 70%) were observed in all treatments; however, this reduction was lower (54%) in the treatment without a soaking and draining stage, and cooked at 100 °C. Similarly, kaempferol and quercetin derivatives were reduced significantly (> 70%) in treatments with a soaking and draining step following thermal treatment, independent of cooking temperature. Conversely, in treatments without soaking and without draining of boiling water, flavonol contents increased around 25% compared to that in raw black beans.

Hydroxycinnamic acid derivatives decreased in all treatments as was also observed for anthocyanin contents, but losses were lower in treatments without a soaking and draining step. Thermal treatment of the FT Nobre black bean cultivar previously soaked in water and drained after the cooking process led to a significant loss (~80%) of ferulic acid. On the contrary, treatments without soaking and without draining significantly enhanced the contents of such phenolic acids. This increase was higher in beans cooked at 121 °C than those at 100 °C (increase of 80 and 30%, respectively).

The variability in flavonoid and phenolic acid contents from the cooked Jalo Precoce cultivar in relation to its raw equivalent exhibited trends similar to those observed for the black bean cultivar after thermal treatment (**Figure 2**). A previous soaking step and draining of cooking water produced significant losses of flavonoids (~60%); however, flavonol contents increased in treatments without soaking and without draining. Independent of cooking temperatures, kaempferol derivatives increased significantly following thermal treatment (~60%). This indicates the potential of the cooked Jalo Precoce cultivar as an interesting source of kaempferol derivatives (48.8 ± 0.1 and 47.1 ± 0.4 mg/100 g DW for treatments without soaking and without draining, and cooking at 100 and 121 °C, respectively).

Higher losses in phenolic acid contents were observed in treatments with soaking and draining. Free phenolic acids such as *p*-coumaric and ferulic acids were reduced in all treatments except for those where beans were not soaked, and the boiling water was not discarded. In this regard, contents of ferulic acid were significantly enhanced (60%) in such treatments, independent of cooking temperature. However, this phenolic acid was not detected by HPLC in treatments with a previous soaking and draining of cooking water, which indicates the high solubility of ferulic acid into the soaking and cooking water.

According to the results shown above for both bean cultivars, decreases of hydroxycinnamic acid derivatives in treatments with a soaking and draining stage following thermal treatment might indicate losses of those compounds by leaching or diffusion into the soaking or cooking water. In addition, the increase of certain phenolic acids such as ferulic and *p*-coumaric acids (Jalo Precoce

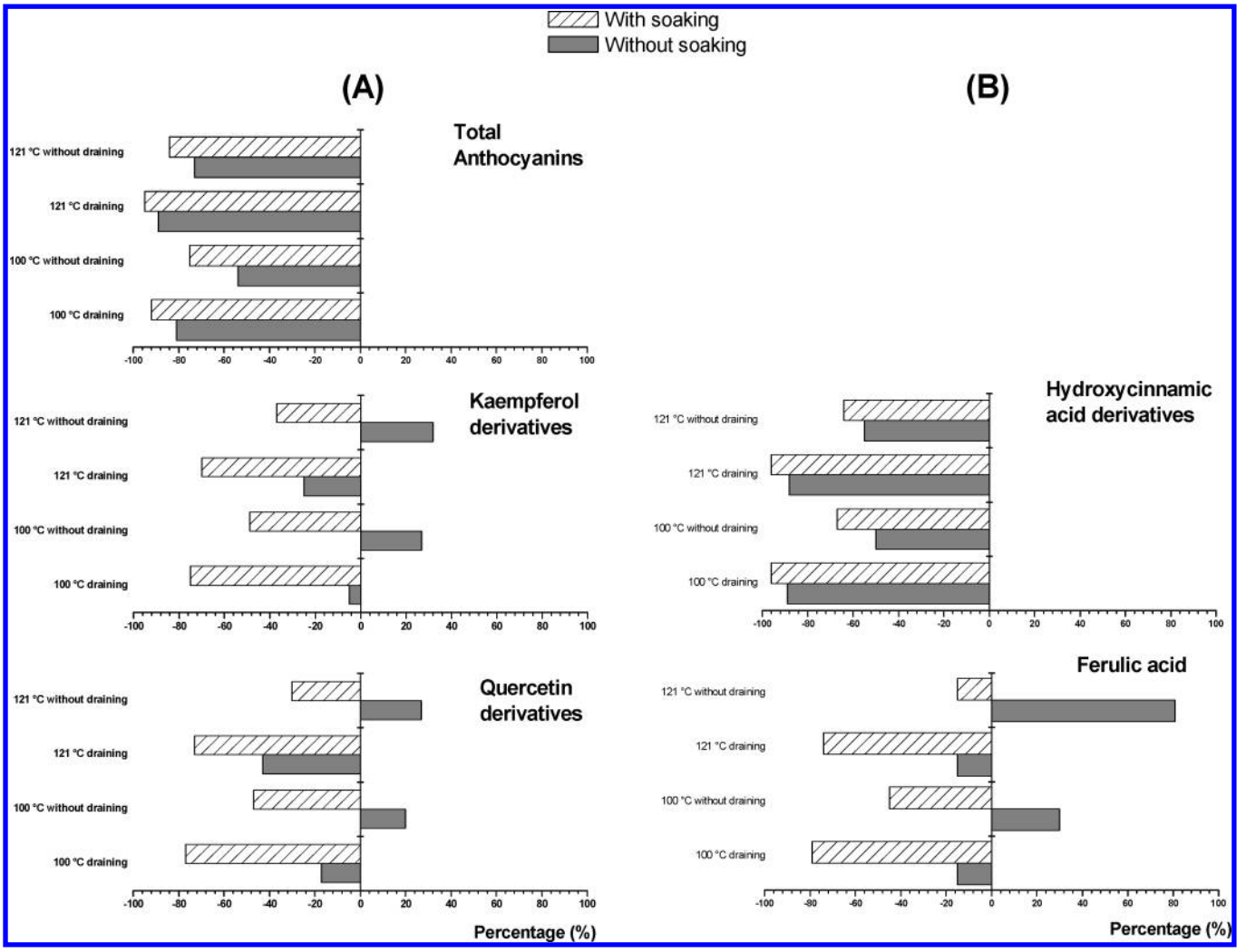

Figure 1. Losses or increases (%) of flavonoid (**A**) and phenolic acid (**B**) contents in black bean FT Nobre cultivar following different cooking conditions (values calculated on the basis of the initial contents in raw bean in dried weight).

cultivar) in treatments without soaking and without draining was associated with a moderate reduction of hydroxycinnamic acid derivatives (or conjugated hydroxycinnamic acids) under the same cooking conditions. This may indicate that thermal treatment likely induced the hydrolysis of conjugated hydroxycinnamic acids resulting in the release of free phenolic acids. Rakic et al. (*23*) observed that following thermal treatment of oak acorns from Serbia, nontannin phenolics, including gallic acid, increased significantly, whereas tannin contents decreased, indicating that during thermal treatment hydrolyzable tannins were degraded resulting in an increase of simple phenolics such as gallic acid. Additionally, the significant increase of ferulic acid contents in treatments without soaking and without draining and cooking at 121 °C might be also related to the thermal stability of such phenolic acids. Morello et al. (*24*) reported that phenolic acids in general exhibit better thermal stability than other phenolic compounds.

Flavonol contents from both evaluated bean cultivars were significantly lost in the soaking and cooking water as was also observed for hydroxycinnamic acid derivatives. However, when beans were not soaked, and the cooking water was kept, a moderate increase of kaempferol derivatives was observed in black beans, whereas contents of such compounds were enhanced significantly in the Jalo Precoce cultivar in relation to the initial concentrations found in its uncooked equivalent. Diaz-Batalla et al. (*3*) observed a high variability in the reduction of initial quercetin and kaempferol contents (12−65% and 5−71%, respectively) when evaluated different autoclaved Mexican beans previously soaked and lyophilized together with the cooking water. In contrast, Price et al. (*25*) did not report significant losses in levels of quercetin and kaempferol glycosides from green beans (*Phaseolus vulgaris* L.) due to thermal treatment. Further, the same authors highlighted that glycosylated flavonols were not hydrolyzed under heat treatment. According to Bunea et al. (*26*), the increase in concentrations of certain phenolic compounds after thermal treatment may be explained either by their better release from the food matrix as a result of the breakdown of supramolecular structures containing phenolic groups or because of their thermal stability.

Effect on Total Phenolic Contents and Antioxidant Capacity. **Table 4** shows the total phenolic contents and antioxidant capacity determined in raw and cooked FT Nobre and Jalo Precoce bean cultivars.

Total phenolic contents and antioxidant capacity of both cultivars varied significantly following the different cooking conditions. The highest total phenolic contents and antioxidant capacities were obtained in treatments where beans were not soaked prior to thermal treatment, and the cooking water was not drained. Among treatments without soaking and draining, no significant differences in the total phenolic contents were observed between beans cooked at 100 and 121 °C. Independent of whether or not the cooking water was discarded, the results

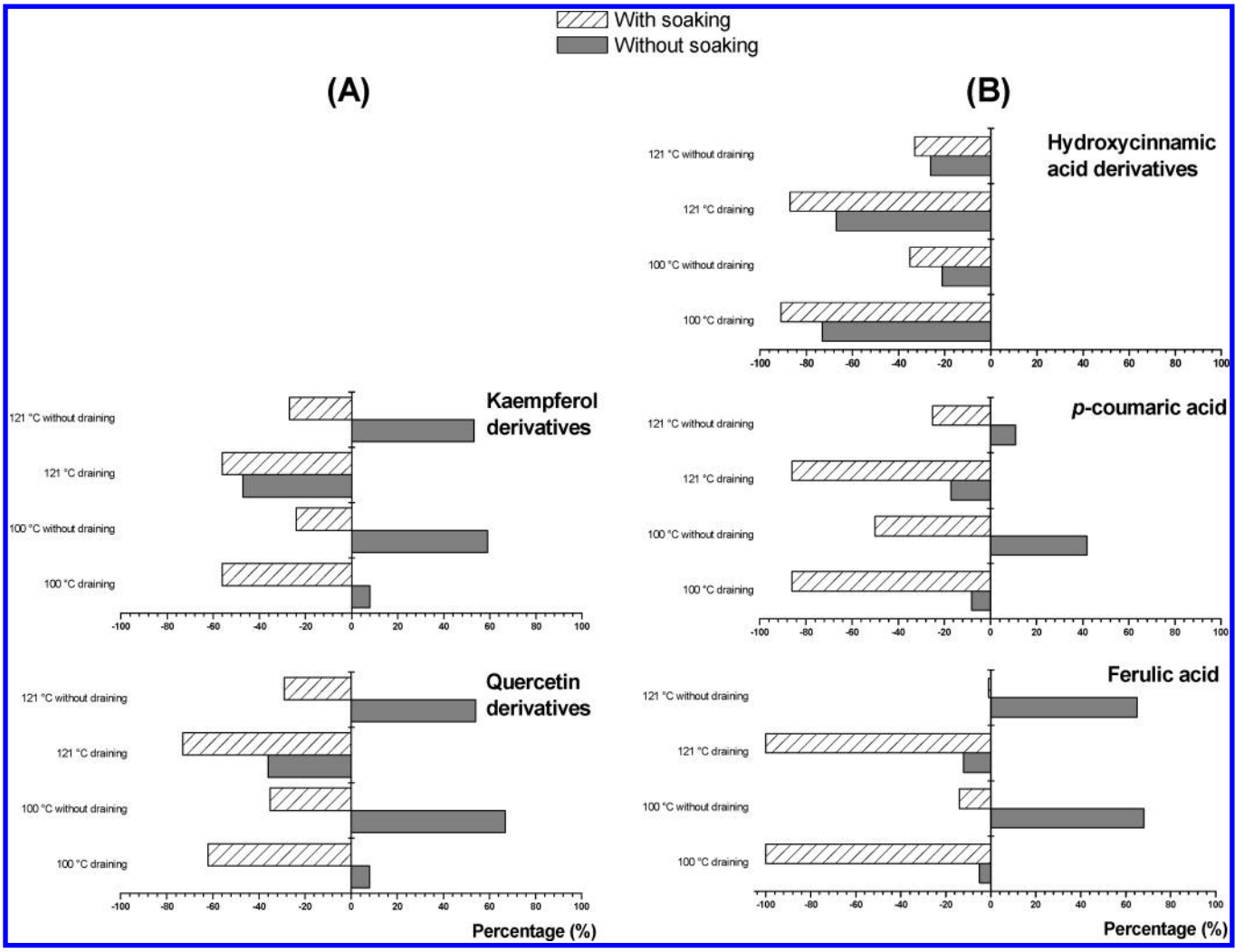

Figure 2. Losses or increases (%) of flavonoid (**A**) and phenolic acid (**B**) contents in Jalo Precoce bean cultivar following different cooking conditions (values calculated on the basis of the initial contents in raw bean in dried weight).

Table 4. Total Phenolic Contents and Antioxidant Capacity in Raw and Cooked FT Nobre and Jalo Precoce Bean Cultivars under Different Conditions[a]

		without soaking				with soaking			
		100 °C		121 °C		100 °C		121 °C	
	raw bean	draining	without draining	draining	without draining	draining	without draining	draining	without draining
					FT Nobre				
TP[b]	2.2 ± 0.1	1.7 ± 0.1 e	3.1 ± 0.1 b	1.5 ± 0.1 f	3.7 ± 0.1 a	0.51 ± 0.03 g	2.02 ± 0.15 d	0.65 ± 0.02 g	2.46 ± 0.12 c
AC[c]	5.8 ± 0.4	3.6 ± 0.6 c	7.2 ± 0.3 a	3.7 ± 0.2 c	7.3 ± 0.4 a	1.9 ± 0.1 d	4.2 ± 0.4 c	2.3 ± 0.1 d	4.9 ± 0.4 b
					Jalo Precoce				
TP	2.4 ± 0.1	1.93 ± 0.05 d	3.59 ± 0.04 a	1.48 ± 0.02 e	3.67 ± 0.04 a	0.77 ± 0.04 f	2.3 ± 0.1 c	0.71 ± 0.05 f	2.67 ± 0.05 b
AC	6.4 ± 0.6	5.7 ± 0.2 d	11.5 ± 0.3 a	4.1 ± 0.3 e	11.9 ± 0.5 a	2.6 ± 0.1 f	8.1 ± 0.6 c	2.5 ± 0.2 f	9.4 ± 0.6 b

[a] Values are means ± SD. [b] mg catechin equivalents/g sample in dried weight. [c] μmol Trolox equivalents/g sample in dried weight. TP, total phenolics; AC, antioxidant capacity. Means in the same line with different letters are significantly different ($p < 0.05$).

indicate that antioxidant capacity was quite stable in thermally treated beans (without soaking) either at 100 or 121 °C.

In general, all factors and their interactions showed a significant ($p < 0.05$) and an inverse effect on total phenolic contents and antioxidant capacity of the FT Nobre bean cultivar. (Statistical results are provided in Supporting Information.) Factors defined as soaking and draining exhibited the highest influence on those response variables, similar to that observed in the case of flavonoid and phenolic acid concentrations. The effect of draining the cooking water following thermal treatment was to reduce the mean of total phenolic contents in 1.74 mg catechin equivalents/g DW, independent of the other factors; meanwhile, the effect of soaking prior to the cooking process was to decrease the mean value in 1.09 mg catechin equivalents/g DW, and this was irrespective of the levels of the other evaluated factors.

In the case of the Jalo Precoce cultivar, the effects of the main factors and their interactions on total phenolic contents and antioxidant capacity exhibited trends similar to those observed in

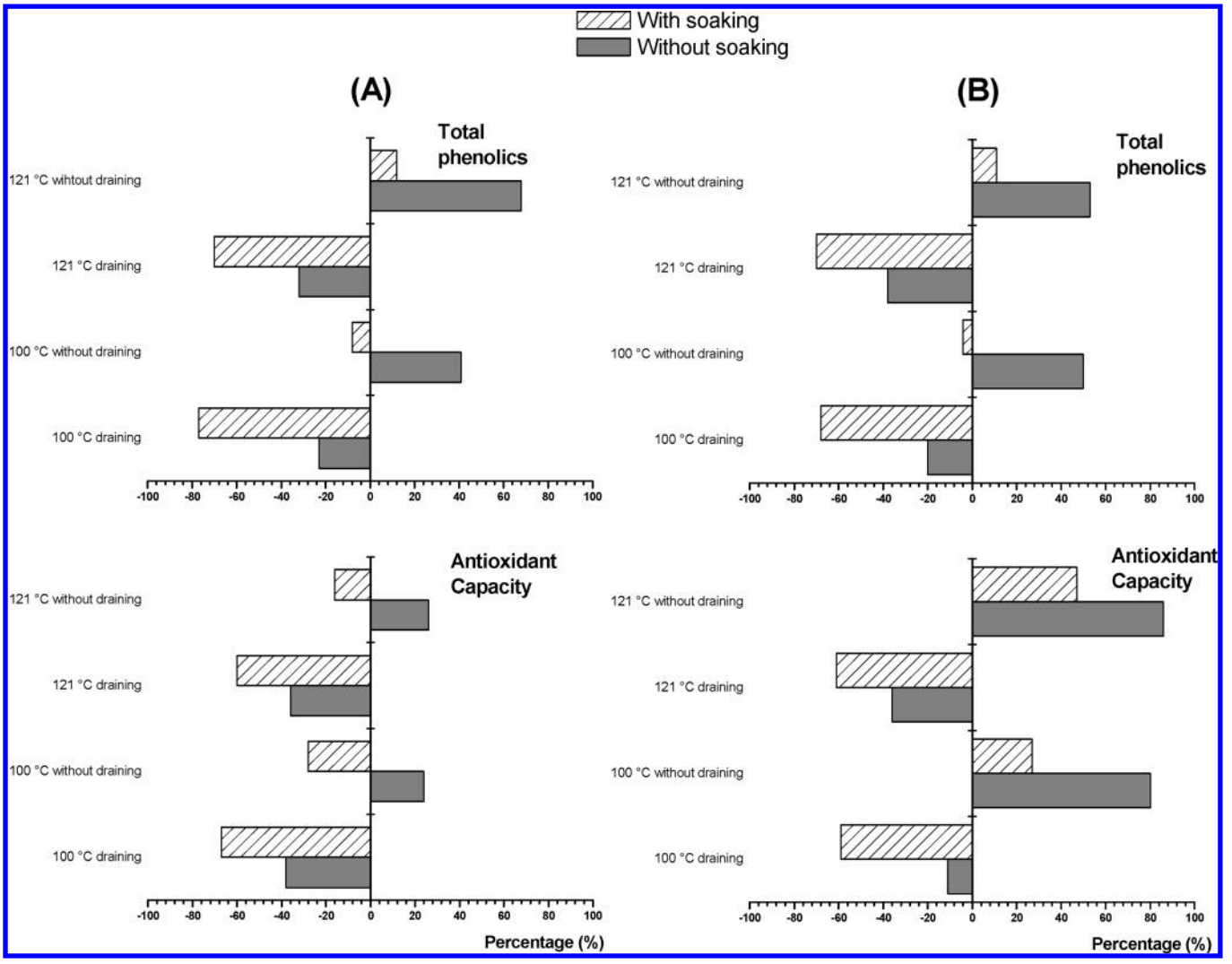

Figure 3. Losses or increases (%) of total phenolics and antioxidant capacity in FT Nobre (**A**) and Jalo Precoce (**B**) bean cultivars following different cooking conditions (values calculated on the basis of the initial total phenolic contents and antioxidant capacity in raw bean in dried weight).

the black bean cultivar. However, the influence of draining on the antioxidant capacity was higher than the effect shown by the soaking factor. The effect of discarding the cooking water following thermal treatment was to decrease the mean value of the antioxidant capacity in 6.53 μmol Trolox equivalents/g DW, whereas the effect of soaking was to reduce the antioxidant capacity in 2.61 μmol Trolox equivalents/g DW. This suggest that the antioxidant capacity of the Jalo Precoce cultivar could be significantly lost during the draining step, rather than during the previous soaking, independent of the cooking temperature.

Statistically, the temperature factor did not affect the total phenolic contents and antioxidant capacity of the Jalo Precoce cultivar; thus, when the cooking temperature was increased from 100 to 121 °C, the variation of total phenolic contents and the DPPH radical scavenging-linked antioxidant capacity from those beans were not significant, independent of beans were soaked previously or drained after thermal treatment.

A previous study (*4*) has shown that several Brazilian bean cultivars are rich in condensed tannins, and those compounds are more concentrated in the seed coat fractions. In contrast, no condensed tannins were detected in evaluated whole bean cultivars under current experimental conditions in this study. Granito et al. (*27*) observed significant losses in condensed tannin concentrations (~84%) in *Phaseolus lunatus* following the cooking process at 100 °C, and losses increased with more severe thermal treatments. The authors concluded that losses were more associated with cooking temperatures than with losses by lixiviation of such compounds into the soaking or cooking water. Similarly, Aparicio-Fernandez et al. (*28*) reported a high reduction (70%) in condensed tannin contents when beans (*Phaseolus vulgaris* L.) were cooked at 100 °C for 2.5 h.

Depending on the temperature and time of cooking, condensed tannins are significantly reduced during thermal treatment either due to leaching of these compounds into the soaking and cooking water or due to the breakdown of phenolics during processing (*27*). However, tannins are also susceptible to the formation of insoluble complexes with proteins under thermal and pressure conditions. Siddharaju and Becker (*29*) indicated that thermal treatment can be inducing the formation of insoluble tannin−protein and tannin−carbohydrate, including cell wall polysaccharide complexes. As a consequence, tannins are not extracted by the solvent, and therefore, they are not detected by routine tannin analyses such as the vanillin method. Guzman-Maldonado et al. (*30*) pointed out that in order to obtain the real content of tannins of beans, these compounds should be determined in the seed coat flour rather than in whole bean flours. In this work, the tannin analysis was performed in whole bean flours in order to obtain results comparable with the other response variables; therefore, an interference of cotyledon proteins could explain the results obtained here.

On the basis of the total phenolic contents and antioxidant capacities in raw FT Nobre and Jalo Precoce cultivars, losses or increases of these variables following the different cooking conditions were calculated and are shown in **Figure 3**.

Significant losses were observed in both total phenolic contents and antioxidant capacity from the FT Nobre cultivar (70−77% and 60−67%, respectively) in treatments that included a soaking and draining step, independent of the cooking temperature. Similarly, in the Jalo Precoce cultivar, losses due to soaking and draining of cooking water were 68−70% and 59−61% for total phenolic contents and antioxidant capacity, respectively. Lower losses were observed in treatments without soaking but with the draining of cooking water following the thermal treatment for both bean cultivars.

In the case of the FT Nobre cultivar, either total phenolic contents or antioxidant capacity increased in 41−68% and 24−26%, respectively, in treatments without a soaking and draining stage. A similar trend was observed in the Jalo Precoce cultivar under the same cooking conditions; however, in this case, the increase in antioxidant capacity was higher (80−86%) than that in total phenolic content (50−53%). These observed increases were likely related to increases of quercetin and kaempferol derivatives and some phenolic acids, as was mentioned above for both bean cultivars.

According to the literature, thermal processing generally decreases the total polyphenol content in legumes. Granito et al. (*27*) reported that more severe treatments such as drying, could affect the aromatic rings of the polyphenolic compounds, making them more susceptible to polymerization reactions and/or to the decomposition of the aromatic structure. However, phenolic compounds are generally linked covalently to amine functional groups in some vegetal foods, and thus, it has been shown that heat treatment can hydrolyze them, significantly increasing or not changing the phenol content of legumes (*31*). Rocha-Guzman et al. (*10*) reported a significant increase in antioxidant capacity assessed by the DPPH method in beans (*Phaseolus vulgaris* L.) cooked at 121 °C without soaking and not draining the cooking water, independent of the evaluated cultivar. Similarly, Khatun et al. (*32*) observed that total phenolic content and antioxidant capacity increased following the heat treatment of several spices. Further, Seung-Cheol et al. (*33*) observed that thermal treatment seemed to release some low molecular phenolic compounds with the concomitant increase of the antioxidant capacity in extracts from citrus peels.

In this research, the increase of the antioxidant capacity in treatments without a soaking and draining stage was likely linked to better release of some antioxidant phenolic compounds such as flavonols and phenolic acids because of thermal treatment. However, other phenomena such as better solubility of antioxidant nonphenolic compounds following thermal treatment (*34*) and the formation of Maillard products with increased free radical scavenging properties may be involved (*35*).

The results obtained in this study suggest that phenolic compounds, such as flavonols and phenolic acids, total phenolic contents, and antioxidant capacity from analyzed bean cultivars depend on the cooking process in analyzed bean cultivars. Factors with the highest effect on the reduction of phenolic compounds and antioxidant capacity were the soaking step prior to thermal treatment and the draining of cooking water in both evaluated Brazilian bean cultivars. Independent of cooking temperature, thermal treatment increased the total phenolic concentrations and antioxidant capacities of both bean cultivars in treatments without soaking and where the cooking water was not discarded, and this was likely linked to a significant increase of certain phenolic compounds such as flavonols and free phenolic acids. Therefore, cooking of beans either at 100 or 121 °C, without a soaking stage and keeping the cooking water would be recommended for retaining antioxidant phenolic compounds. Insights from this study may provide the basis for further research on potential changes in sensory characteristics linked to different cooking procedures.

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Supporting Information Available: Values of calculated effects on flavonoids, phenolic acids, total phenolic contents, and antioxidant capacity for both FT Nobre and Jalo Precoce bean cultivars following thermal treatments. This material is available free of charge via the Internet at http://pubs.acs.org.

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