

# Effect of Water Regimes on Dietary Fiber, Polyphenols and Antioxidant Capacity of Black and Pinto Beans

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## Abstract

Beans are rich in dietary fiber and polyphenols; however, growing conditions may affect the occurrence of these components. The effect of irrigation and rain fed conditions on dietary fiber, indigestible fraction, polyphenols and antioxidant capacity of Black 8025 and Pinto Durango bean cultivars grown in Mexico have been determined. Total dietary fiber decreased in beans grown under rain fed conditions compared to those grown under irrigation. The water regimes had an effect on the total indigestible fraction for Black 8025 bean. The extractable polyphenols were affected by the water regimes, while the antioxidant capacity of extractable and non-extractable polyphenols was dependent on the bean variety. Cooking the beans altered the extractable and non-extractable polyphenols and the antioxidant capacity. Also, the antioxidant properties and some extend, the digestibility of non-digestible carbohydrates of beans were affected by water regimes. This information could be taken into account for dry bean breeding programs to improve the nutritional quality of beans.

## Keywords

Water Regimes; Dietary Fiber; Indigestible Fraction; Polyphenols; Antioxidant Capacity; Cooking

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## 1. Introduction

Currently, interest in consumption of foods with high dietary fiber (DF) content has increased due to a focus on

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nutrition and healthy diet goals. The amount, concentration and composition of dietary fiber in beans are some factors that produce beneficial health effects [1]. The dietary fiber consists of two fractions: soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) [2]. SDF delays gastric emptying, decreases intestinal transit and cholesterol, in addition to helping reduce the risk of heart attack and colon cancer [1]. On the other hand, IDF is characterized by its water-holding capacity, increasing the fecal bolus, gastrointestinal motility and weight of the feces [2]. The most utilized method for determination of DF in food is an official method of the AOAC. Although this is the most common method, methodological errors associated with the analytical procedure have been found [3] [4], which can alter the starch digestibility and consequently the DF values [5]. One alternative method involves physiological aspects, simulating the digestion gastric and intestinal phase for the determination of SDF and IDF contents [3]. This methodology allows the *in vitro* determination of the indigestible fraction (IF), which aside from DF includes resistant starch (RS), resistant protein (RP), polyphenols like tannins, and others [4] [5]. Alternatively, it has been stated that during determination of DF and IF, a significant amount of phenolic compounds or polyphenols, which have shown interesting effects on human health, are overlooked in this method [5]. The common bean is considered as a good source of phenolic compounds with antioxidant properties, which mainly come from the seed coat [6] [7]. Chemical composition of Black and Pinto beans has gained attention due to the presence of flavonols, anthocyanins and proanthocyanidins or condensed tannins. These compounds are considered antioxidant because they have the ability to interact with free radicals [6].

The common bean has been cultivated year round under different environmental conditions for many years, which could affect the composition of the bean seed. Currently, drought is a big problem for crops. We think that water deficit can affect the biosynthesis of components such as DF and polyphenols. The aim of this research was to evaluate the effect of the irrigation and rain fed conditions on dietary fiber, indigestible fraction and polyphenols and their antioxidant capacity of two bean varieties.

## 2. Materials and Methods

### 2.1. Materials

Two dry bean cultivars (Black 8025 and Pinto Durango) were used for this experiment. Both cultivars were grown in irrigated and rain fed (“temporal”) conditions in two localities of Guanajuato, Mexico (**Figure 1**). Black 8025 and Pinto Durango grown under rain fed conditions in Ocampo (2200 masl; 21°1'51"N, 101°28'47"O), were planted in July and harvested in October 2008 (300 mm). While the same varieties grown under irrigation conditions in Celaya (1875 masl; 20°31'20"N, 100°48'44"O) were planted in February and harvested in June of the same year (500 mm, in 3 times). Samples were donated by National Institute of Research Forest, Agricultural and Livestock (INIFAP) of Celaya, Guanajuato, Mexico. Samples were cleaned to remove foreign material and damaged seeds prior to analysis.

### 2.2. Preparation of Raw and Cooked Bean Samples

Raw and cooked bean samples were analyzed in the form of flour. The raw bean flour of each variety was prepared using the method described by Chung *et al.* [8].

Cooked bean samples were obtained by cooking the beans according to their optimum cooking time and prepared like was reported previously [9].

### 2.3. Total Dietary Fiber and Indigestible Fraction of Cooked Beans

The determination of dietary fiber was done in cooked beans because this is the form which beans are consumed. The dietary fiber content was determined using AACC approved method 32.05 [10]. As an alternative to dietary fiber, the indigestible fraction of the bean samples was quantified. This procedure follows near-physiological analytical conditions. Soluble indigestible fiber (SIF) and insoluble indigestible fiber (IIF) fractions were assessed using the method described by Saura-Calixto *et al.* [3].

### 2.4. Determination of Neutral and Uronic Acids in SIF

Neutral sugars (NS) were quantified using the anthrone method with glucose as a standard according to Laurentin and Edwards (2003) [11]; while the uronic acids (UA) were measured colorimetrically by m-hydroxydiphenyl method [12] using glucuronic acid as a standard.



**Figure 1.** Map of Mexico and Guanajuato State. Ocampo is the area 022 and Celaya is the area 007.

## 2.5. Determination of Polyphenols in Raw and Cooked Beans

**Extractable polyphenols.** Samples were extracted by shaking at room temperature with methanol-water acidified with HCl (50:50 v/v, pH 2, 50 mL/g sample, 60 min, room temperature; constant shaking) and acetone-water (70:30 v/v, 50 mL/g sample, 60 min, room temperature; constant shaking). After centrifugation (15 min, 25°C, 3000 g) supernatants were combined and used to determine extractable polyphenols and antioxidant capacity. Extractable polyphenols were determined by the Folin-Ciocalteu procedure [13], while condensed tannins were determined with the vanillin method [14]. The results were expressed as gallic acid and catequin equivalents, respectively.

**Phenolic acids.** The phenolic acid profile was determined using the method of Ramamurthy *et al.* [15] (1992). The solution from the extractable polyphenol determination was injected onto a high-performance liquid chromatography (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a diode array detector (HPLC-DAD), dual wavelength UV/VIS detector, acquisition system (Agilent ChemStation Software Plus A.09.xx, Santa Clara, CA, USA), and Zorbax octadecylsilane (ODS-C18) reverse-phase column. The UV detector was set at 280 nm and the compounds identified and quantified by comparison of retention times and absorption spectra of sample chromatographic peaks with those of authentic standards (gallic, protocatechuic, *p*-hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, benzoic and salicylic acids, as well as vanillin aldehyde, catechin, epicatechin, and epigallocatechin gallate) using the same HPLC operating conditions.

**Non extractable polyphenols.** Hydrolysable polyphenols comprise hydrolysable tannins, phenolic acids, and hydroxycinnamic acids that are released from the food matrix by strong acidic hydrolysis. They were determined by a methanol/H<sub>2</sub>SO<sub>4</sub> 90:10 (v/v) hydrolysis at 85°C for 20 h from the residues of methanol/acetone/water extraction [16]. After centrifugation (15 min, 25°C, 3000 g), supernatants were combined and used to determine the hydrolysable polyphenols by the Folin Ciocalteu method [13]. The results were expressed as gallic acid equivalents. Condensed tannins or proanthocyanidins: residues from the methanol/acetone/water extraction were treated with 5 mL/L HCl-Butanol (3 h, 100°C) for condensed tannin determination [17]. Proanthocyanidins were calculated from the absorbance at 550 nm of the anthocyanidin solutions. Condensed tannins were determined using a standard curve from Mediterranean carob pod (*Ceratonia siliqua* L.) supplied by Nestlé S.A.

**Free radical scavenging assay (ABTS).** The antioxidant capacity was estimated in terms of radical scavenging activity following the procedure described elsewhere [18] with some modification [19]. Briefly, ABTS radical cation (ABTS<sup>•+</sup>) was produced by reacting 7 mmol/L ABTS stock solution with 2.45 mmol/L potassium persulphate in the dark at room temperature for 12 - 16 h before use. The ABTS<sup>•+</sup> solution was diluted with methanol to an absorbance of  $0.70 \pm 0.02$  at 658 nm. After addition of 0.1 mL of sample to 3.9 mL of diluted ABTS<sup>•+</sup> solution, absorbance readings were taken every 20 s using a UV/VIS spectrophotometer (UNICO 2800 UV/VIS). The reaction was monitored for 6 min. Inhibition of absorbance vs time was plotted and the area below the curve (0 - 6 min) was calculated. It was used an internal standard. The results were expressed as  $\mu\text{mol}$  of Trolox equivalents per g of dry matter.

## 2.6. Statistical Analysis

Results are presented as mean  $\pm$  SEM (standard error of mean) of three determinations. Analysis of variance was done using a commercial program (Sigma Stat version 2.03, Jandel Corporation, San Rafael, CA). Tukey's multiple comparison test was used to determine significant differences among means.

## 3. Results and Discussion

### 3.1. Total Dietary Fiber in Cooked Beans

The TDF contents ranged from 29.51% to 44.05%. Significant differences were found in the TDF content among bean varieties grown under irrigation and rain fed conditions (Table 1). Also, it was observed that water deficit caused a decrease in the TDF content with respect to the samples grown under irrigation. This pattern is related to the metabolism of carbohydrates which are utilized for maintenance of the plant metabolism during water deficit [20] [21]. Stoughton-Ens *et al.* [22] found that locality affected the TDF, IDF and SDF of pea, while the pea genotype affected the TDF and IDF content; this is due to the nutrients present in the soil. The IDF contents ranged from 21.35% and 38.06%, which was similar to that found in cooked beans by Rosin *et al.* (19.27%) [23], de Almeida-Costa *et al.* (22.6%) [24] and Silva-Cristobal *et al.* (25.59%) [7]. Black 8025 bean cultivated under irrigation conditions presented the highest IDF content while, Pinto Durango bean grown under rain fed conditions had the lowest IDF. It was found that the effect of the variety and water regimes resulted in significant differences in the IDF content ( $p < 0.05$ ) for both bean varieties. Regarding the SDF content, Black 8025 bean grown under irrigation conditions displayed the lowest value (5.99%) and Pinto Durango bean cultivated in the same conditions showed the highest value (12.75%). Under rain fed conditions, there were no significant differences in the SDF content of the two bean varieties ( $p < 0.05$ ). Between the same bean varieties, it was observed that SDF content decreased in Pinto Durango bean under rain fed conditions with respect to irrigation, while Black 8025 bean showed the opposed trend. Possibly, the change in dietary fiber content of the samples

**Table 1.** Total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) content of two common bean varieties grown under irrigation and rain fed conditions (%)\*.

Sample*	TDF	IDF	SDF
<b>Irrigation</b>			
Black 8025	44.05 ± 1.01 <sup>c</sup>	38.06 ± 1.31 <sup>d</sup>	5.99 ± 0.85 <sup>a</sup>
Pinto Durango	36.79 ± 1.15 <sup>b</sup>	24.04 ± 0.55 <sup>b</sup>	12.75 ± 1.29 <sup>c</sup>
<b>Rain Fed</b>			
Black 8025	37.70 ± 1.08 <sup>b</sup>	29.50 ± 0.81 <sup>c</sup>	8.20 ± 1.14 <sup>b</sup>
Pinto Durango	29.51 ± 1.15 <sup>a</sup>	21.35 ± 0.39 <sup>a</sup>	8.16 ± 1.21 <sup>b</sup>

Values are mean ± SEM, n = 3. Data with different superscripts in the same column are significantly different ( $p < 0.05$ ) by Tukey test. The samples were cooked before the analysis. SDF = TDF-IDF.

could be principally due to the adaptation of each bean variety to the conditions where they were cultivated (climatic change, type of soil, amount of water, etc.). However, it is known that water is necessary to carry out photosynthesis and obtain triose phosphate, which is used in the biosynthesis of starch, amino acids and sucrose. It has been reported that sucrose is a precursor in the synthesis of the cell wall polysaccharides [21]. Thus, the water deficit may cause the reduction of the sucrose transport to the seed [20]. More specifically, the water deficit might have caused a decrease in sucrose synthesis, which could affect the content of non-starch polysaccharides and might result in a low TDF content in the samples cultivated under the rain fed condition.

### 3.2. Indigestible Fraction of Cooked Bean

Soluble indigestible fraction (SIF) and insoluble indigestible fraction (IIF) values showed a similar trend, as the dietary fiber, and were more highly affected by the bean variety than the water regimes, except for Black 8025 bean, which was affected by the water deficit (Table 2). Total indigestible fraction (TIF) content was higher than that reported by Saura-Calixto *et al.* [3], Menezes *et al.* [25] and Silva-Cristobal *et al.* [7]. It is important to point out that the TIF values show a different behavior to the TDF, because the indigestible fraction of the samples has higher values than dietary fiber.

This is due to the inclusion of resistant starch, indigestible protein, polyphenolic compounds and non-starch polysaccharides like cellulose, hemicelluloses and lignin in the IF [7] [26]. The IIF of Black 8025 and Pinto Durango beans ranged from 37.18% to 40.69%, where Black 8025 bean grown under irrigation and rain fed conditions presented higher IIF values. The samples did not present significant differences between bean varieties or water regimes (irrigation and rain fed) ( $p < 0.05$ ). The IIF values reported in this work were lower than those reported by Silva-Cristobal *et al.* [7] in Black bean (41.66%) and higher than that found by Saura-Calixto and Goñi [26] in kidney bean (31.14%). The SIF values ranged between 19.60% and 23.82%. Only Black 8025 bean was affected by the water deficit, because under rain fed conditions the SIF content decreased compared to its counterpart grown under irrigation conditions. On the other hand, significant difference in the SIF content was not observed between bean varieties. It is worth mentioning that the SIF contents reported here were higher than reported by others authors. Silva-Cristobal *et al.* [7] reported 10.78% SIF for Black bean, whereas Saura-Calixto *et al.* [3] reported 9.74% SIF for Pinto bean. These differences are related to the content of neutral sugars and uronic acids in the bean varieties, as well as the climatic conditions and soil where the bean varieties were cultivated. For this reason, it was important to determine the uronic acids and neutral sugars in the samples to verify whether or not the SIF values obtained with the DNS method are correct or underestimated. Table 3 shows the values of neutral sugars (NS) and uronic acids (UA) determined in the SIF of the each sample. The NS content did not show significant differences between bean varieties or water regimes. In the case of UA, there were significant differences between bean varieties, but no significant differences were found between water regimes. The sum of NS and UA was similar to the SIF content determined by the DNS method.

Based on the data obtained, beans are an excellent source of fermentable substrate, producing beneficial health effects, due to formation of short chain fatty acids (SCFA) such as acetic, propionic and butyric acids, the

latter being of the most interest [27].

### 3.3. Polyphenols Content in Raw and Cooked Beans

**Table 4** presents the extractable polyphenols (EP), condensed tannins (CT<sup>1</sup>) and non-extractable polyphenols (NEP) (hydrolysable polyphenols (HP) and condensed tannins (CT<sup>2</sup>)) in raw beans grown under different water regimes. In general, the bean variety had an effect on the EP, CT<sup>1</sup> and NEP and the growing conditions (irrigation and rain fed) affected the EP and CT<sup>1</sup> contents. Black and Pinto beans grown under irrigation conditions

**Table 2.** Content of total indigestible fraction (TIF), insoluble indigestible fraction (IIF) and soluble indigestible fraction (SIF) of two common bean varieties grown under irrigation and rain fed conditions (%)\*.

Sample*	TIF	IIF	SIF
<b>Irrigation</b>			
Black 8025	64.50 ± 0.68 <sup>b</sup>	40.69 ± 0.46 <sup>c</sup>	23.82 ± 0.90 <sup>b</sup>
Pinto Durango	58.34 ± 0.61 <sup>a</sup>	38.59 ± 0.34 <sup>a,b</sup>	19.75 ± 0.51 <sup>a</sup>
<b>Rain Fed</b>			
Black 8025	59.43 ± 1.44 <sup>a</sup>	39.82 ± 0.51 <sup>b,c</sup>	19.60 ± 1.20 <sup>a</sup>
Pinto Durango	58.48 ± 0.39 <sup>a</sup>	37.18 ± 0.38 <sup>a</sup>	21.30 ± 0.42 <sup>a,b</sup>

Values are mean ± SEM, n = 3. Data with different superscripts in the same column are significantly different (p < 0.05) by Tukey test. The samples were cooked before the analysis.

**Table 3.** Neutral sugars (NS) and uronic acids (UA) present in the soluble indigestible fraction of cooked bean (%)\*.

Sample*	NS	UA	TSF
<b>Irrigation</b>			
Black 8025	20.64 ± 3.56 <sup>a</sup>	2.62 ± 0.25 <sup>a</sup>	23.26 ± 3.35 <sup>a</sup>
Pinto Durango	16.54 ± 2.28 <sup>a</sup>	3.30 ± 0.20 <sup>b</sup>	19.84 ± 2.15 <sup>a</sup>
<b>Rain Fed</b>			
Black 8025	15.52 ± 3.36 <sup>a</sup>	2.40 ± 0.21 <sup>a</sup>	17.92 ± 3.25 <sup>a</sup>
Pinto Durango	17.24 ± 2.27 <sup>a</sup>	3.41 ± 0.12 <sup>b</sup>	20.65 ± 2.34 <sup>a</sup>

Values are mean ± SEM, n = 3. Data with different superscripts in the same column are significantly different (p < 0.05) by Tukey test. TSF = NS + UA, where TSF is total soluble fraction.

**Table 4.** Polyphenolics and phenolic acid content of raw common bean varieties grown under irrigation and rain fed conditions.

Sample	Polyphenols (mg/g)				Phenolic Acids (mg/100g)		
	Total Phenolics		NEP		Caffeic	Hydroxibenzoic	<i>p-Coumaric</i>
	EP	CT <sup>1</sup>	HP	CT <sup>2</sup>			
<b>Irrigation</b>							
Black 8025	4.86 ± 0.18 <sup>b</sup>	3.89 ± 0.27 <sup>b</sup>	10.01 ± 0.40 <sup>b</sup>	25.56 ± 0.14 <sup>c</sup>	20.6 ± 0.98 <sup>ab</sup>	10.1 ± 0.5 <sup>a</sup>	4.63 ± 0.20 <sup>b</sup>
Pinto Durango	6.20 ± 0.29 <sup>c</sup>	7.47 ± 0.15 <sup>d</sup>	8.26 ± 0.10 <sup>a</sup>	16.31 ± 0.71 <sup>b</sup>	19.3.3 ± 1.2 <sup>a</sup>	13.3 ± 1.2 <sup>b</sup>	6.3 ± 0.92 <sup>c</sup>
<b>Rain Fed</b>							
Black 8025	3.49 ± 0.17 <sup>a</sup>	2.54 ± 0.28 <sup>a</sup>	8.61 ± 0.22 <sup>a</sup>	11.97 ± 0.19 <sup>a</sup>	22.4 ± 1.4 <sup>b</sup>	9.9 ± 1.1 <sup>a</sup>	5.34 ± 0.71 <sup>b</sup>
Pinto Durango	4.66 ± 0.21 <sup>b</sup>	4.67 ± 0.13 <sup>c</sup>	8.94 ± 0.26 <sup>a</sup>	15.60 ± 1.10 <sup>b</sup>	26.2 ± 0.91 <sup>c</sup>	17.7 ± 0.38 <sup>c</sup>	3.6 ± 1.15 <sup>a</sup>

Values are mean ± SEM, n = 3. Data with different superscripts in the same column are significantly different (p < 0.05) by Tukey test. EP: extractable polyphenols. HP: hydrolysable polyphenols. CT1: soluble condensed tannins present in the EP extract, determined with the vanillin method (Reynoso-Camacho *et al.* 2007). CT2: insoluble condensed tannins determined with the proanthocyanidins method (Reed *et al.* 1982).



presented higher EP values compared to their counterparts grown under rain fed conditions. This could be due to the fact that water deficit resulted in changes to the distribution and content of the phenolic compounds in the seed coat and cotyledon of bean. André *et al.* [28] reported that changes in the polyphenol content could cause the alteration of the sucrose flux in the plant due to water deficit. Tannins are also found in the non-extractable polyphenols (NEP), which have a high degree of polymerization and are associated with dietary fiber or protein. Due to the high molecular weight, NEP may remain in the extraction residues and not be fully quantified [29]. Due to this fact, the NEP of the bean samples was determined. Black 8025 bean grown under rain fed conditions showed lower condensed tannins (CT<sup>2</sup>), whereas the same bean variety cultivated under irrigation conditions had higher values. Diaz *et al.* [30] demonstrated that CT present in the seed coat of different bean varieties are composed principally of catechin and gallic acid, and the amount of these monomers is different among varieties.

Water regimes affected the EP in cooked samples. Both Pinto Durango and Black 8025 bean grown under irrigation conditions showed higher values of EP than their counterparts grown under rain fed conditions (Table 5). This pattern was similar to that found in raw bean samples. On the other hand, cooking reduced the EP content. Xu and Chang [6] reported that cooking of Black and Pinto beans resulted in a decrease of the total phenolic compounds due to its degradation and release of their monomers into the cooking water. However, the differences observed in the phenolic compound profile caused by the heat treatment of Black and Pinto beans could be attributed to differences in the distribution and composition of the phenolic compounds in the seed coat and cotyledon of bean. The HP content of both bean varieties did not show significant differences ( $p < 0.05$ ) due to differences in varieties or water regimes, but the cooking caused an increase in the HP compared to raw bean. In the case of CT<sup>1</sup>, Pinto Durango (grown in both conditions) and Black 8025 bean (under rain fed conditions) showed a more marked decrease in the CT<sup>1</sup> content due to cooking. These results indicate that the cooking of bean may have caused the CT<sup>1</sup> to bind with other components, such as fiber and/or protein in a way that decreases their solubility in organic solvents. Black 8025 bean grown under irrigation conditions showed a decrease in the CT<sup>2</sup> content, but the rest of the samples had an increase in CT<sup>2</sup>. The increase of CT<sup>2</sup> may be due to the decrease of CT<sup>1</sup>, which could be triggered by the cooking causing the CT<sup>1</sup> to become bound by the fiber matrix or linked to proteins. Also, the tannins present in the seed coat and cotyledon of each bean variety may have differences in the chemical structure as well as in the amount and type of monomer present in the structure, and so they may be affected differently by cooking or water regimes.

### 3.4. Phenolic Acids

Derivates of benzoic acid: *p*-hydroxybenzoic acid, as well as cinnamic acid derivates: caffeic acid and *p*-coumaric acids were detected in raw and cooked beans (Table 4). Caffeic acid was the main simple phenolic com-

**Table 5.** Polyphenolics and phenolic acid content of cooked common bean varieties grown under irrigation and rain fed conditions.

Sample	Polyphenols (mg/g)				Phenolic Acids (mg/100g)		
	Total Phenolics		NEP		Caffeic	Hydroxybenzoic	<i>p</i> -Coumaric
	EP	CT <sup>1</sup>	HP	CT <sup>2</sup>			
<b>Irrigation</b>							
Black 8025	4.56 ± 0.04 <sup>d</sup>	3.64 ± 0.15 <sup>d</sup>	9.95 ± 0.48 <sup>a</sup>	22.39 ± 0.17 <sup>d</sup>	21.54 ± 0.8 <sup>a</sup>	7.9 ± 0.9 <sup>a</sup>	3.7 ± 0.2 <sup>b</sup>
Pinto Durango	3.80 ± 0.04 <sup>c</sup>	2.66 ± 0.15 <sup>c</sup>	9.70 ± 0.31 <sup>a</sup>	17.09 ± 0.30 <sup>c</sup>	25.3 ± 1.1 <sup>b</sup>	12.9 ± 1.2 <sup>b</sup>	1.7 ± 0.3 <sup>a</sup>
<b>Rain Fed</b>							
Black 8025	2.67 ± 0.13 <sup>b</sup>	1.65 ± 0.15 <sup>b</sup>	10.68 ± 0.16 <sup>a</sup>	13.27 ± 0.33 <sup>a</sup>	25.7 ± 1.7 <sup>b</sup>	6.4 ± 0.91 <sup>a</sup>	4.63 ± 0.5 <sup>c</sup>
Pinto Durango	2.35 ± 0.06 <sup>a</sup>	1.08 ± 0.12 <sup>a</sup>	9.50 ± 0.30 <sup>a</sup>	16.70 ± 0.16 <sup>b</sup>	28.1 ± 0.4 <sup>c</sup>	17.7 ± 0.38 <sup>c</sup>	3.8 ± 0.29 <sup>b</sup>

Values are mean ± SEM, n = 3. Data with different superscripts in the same column are significantly different ( $p < 0.05$ ) by Tukey test. EP: extractable polyphenols. HP: hydrolysable polyphenols. CT1: soluble condensed tannins present in the EP extract, determined with the vanillin method (Reynoso-Camacho *et al.* 2007). CT2: insoluble condensed tannins determined with the proantocyanidins method (Reed *et al.* 1982).

pound found in dry-bean samples, followed by *p*-hydroxybenzoic and *p*-coumaric acids. Phenolic acids detected and levels reported in this work are in agreement with those reported by Díaz-Batalla *et al.* [31] and Espinoza-Alonso *et al.* [32] for Pinto and Black beans. However, we did not detect vanillic, ferulic or sirynigic acids. In raw Pinto Durango beans, rain fed condition significantly increased the caffeic acid and hydroxybenzoic acid ( $p < 0.05$ ). There was an increase in caffeic and *p*-coumaric acids in Black 8025, but it was not significant ( $p < 0.05$ ). On the other hand, in cooked beans the rain fed conditions resulted in significantly higher contents of all phenolic acids, except for hydroxybenzoic acid in Black 8025, than the irrigated condition for both bean varieties (Table 4). The presence of these compounds may contribute in different degrees to the antioxidant capacity and health related effects attributed to simple phenolics, which are extensively reported elsewhere [33].

### 3.5. Antioxidant Capacity of Raw and Cooked Beans

The antioxidant capacity (AOC) of EP in the beans grown under irrigation conditions did not show significant differences ( $p < 0.05$ ) due to variety (Table 6). The beans grown under rain fed conditions showed a significant ( $p < 0.05$ ) decrease in the AOC of EP compared to the beans grown under irrigation. There is a lack of information about the effect of the environmental factor on the AOC, with respect to legumes. In the case of cereals, Mpofo *et al.* [34] demonstrated that environmental factors had a high effect on the AOC compared to the effect caused by the wheat variety, while the variety-environmental interaction did not result in great variation of this parameter. Although raw bean varieties showed a high CT<sup>2</sup>, their AOC was lower compared to the AOC of EP. Xu and Chang [6] reported that the main biological activity of the phenolic acids is their antioxidant capacity. For this reason, it can be seen that EP has higher AOC than CT<sup>2</sup>. There were no observed significant differences ( $p < 0.05$ ) in the AOC of CT<sup>2</sup> between bean varieties, except for Black 8025 bean grown under irrigation conditions. Black 8025 (irrigation) presented the highest value of CT<sup>2</sup>. In regard to AOC of HP, significant differences in the AOC were observed due to the bean variety ( $p < 0.05$ ), but not due to the water regimes. The AOC values were lower than EP values, although the HP content was higher in raw beans.

The AOC of the EP of cooked beans was affected by the bean variety and water regimes (Table 7). Also, cooking resulted in a decrease in the AOC, except for Black 8025 bean cultivated under irrigation conditions which showed a similar AOC to that of the raw bean. The decrease in the AOC can be attributed to the cooking which caused the reduction of the EP in the cooked samples, except for Black 8025 (irrigation). The AOC of Black 8025 may not have been affected by the cooking because this bean variety had a high indigestible fraction and dietary fiber content compared to all samples. This indicated that the dietary fiber present in Black 8025 bean grown under irrigation conditions has a more pronounced ability to act as antioxidant dietary fiber. The AOC of CT<sup>2</sup> of both Pinto Durango and Black 8025 beans increased after cooking. As was observed in raw beans, there were not significant differences due to the effect of the variety under rain fed conditions, however under irrigation Black 8025 had significantly higher AOC of CT<sup>2</sup> ( $p < 0.05$ ). In the case of HP, significant differences were observed between bean varieties. These compounds presented higher AOC values in cooked

**Table 6.** Antioxidant capacity of two raw common bean varieties grown under irrigation and rain fed conditions.

Sample*	Antioxidant Capacity (μmol TE/g)		
	EP	NEP	
		HP	CT <sup>2</sup>
<b>Irrigation</b>			
Black 8025	59.00 ± 1.00 <sup>c</sup>	19.50 ± 2.49 <sup>c</sup>	27.15 ± 1.13 <sup>b</sup>
Pinto Durango	63.33 ± 1.54 <sup>c</sup>	5.62 ± 0.61 <sup>a</sup>	6.42 ± 0.31 <sup>a</sup>
<b>Rain Fed</b>			
Black 8025	41.19 ± 0.99 <sup>a</sup>	10.10 ± 1.18 <sup>b</sup>	6.66 ± 0.44 <sup>a</sup>
Pinto Durango	46.02 ± 1.30 <sup>b</sup>	4.39 ± 0.56 <sup>a</sup>	5.49 ± 0.33 <sup>a</sup>

Values are mean ± SEM, n = 3. Data with different superscripts in the same column are significantly different ( $p < 0.05$ ) by Tukey test. EP: extractable polyphenols. HP: hydrolysable polyphenols. CT<sup>2</sup>: insoluble condensed tannins.



**Table 7.** Antioxidant capacity of two cooked common bean varieties grown under irrigation and rain fed conditions.

Sample*	Antioxidant Capacity ( $\mu\text{mol TE/g}$ )		
	EP	NEP	
		HP	CT <sup>2</sup>
<b>Irrigation</b>			
Black 8025	58.39 $\pm$ 0.28 <sup>d</sup>	19.47 $\pm$ 2.34 <sup>c</sup>	26.15 $\pm$ 0.79 <sup>b</sup>
Pinto Durango	42.34 $\pm$ 0.24 <sup>c</sup>	10.21 $\pm$ 0.85 <sup>b</sup>	11.18 $\pm$ 0.74 <sup>a</sup>
<b>Rain Fed</b>			
Black 8025	33.45 $\pm$ 0.18 <sup>b</sup>	16.71 $\pm$ 1.02 <sup>c</sup>	11.74 $\pm$ 0.73 <sup>a</sup>
Pinto Durango	24.99 $\pm$ 0.12 <sup>a</sup>	7.70 $\pm$ 0.116 <sup>a</sup>	12.42 $\pm$ 1.10 <sup>a</sup>

Values are mean  $\pm$  SEM, n = 3. Data with different superscripts in the same column are significantly different ( $p < 0.05$ ) by Tukey test. EP: extractable polyphenols. HP: hydrolysable polyphenols. CT<sup>2</sup>: insoluble condensed tannins.

beans than in raw beans, except for Black 8025 bean grown under irrigation. The changes in the AOC of the processed beans can be attributed to synergic combinations or different chemical reactions, release of soluble antioxidants in the cooking water and the formation or breaking of the antioxidant structure [6].

#### 4. Conclusion

Total dietary fiber (TDF) and insoluble dietary fiber (IDF) contents were affected by the bean variety and water regimes. The extractable polyphenols (EP), condensed tannins (CT) and antioxidant capacity (AOC) of raw beans were affected by the bean variety and water regimes. The content and AOC of EP decreased after cooking, while the AOC of CT<sup>2</sup> and HP increased due to cooking. Some parameters evaluated were affected both by the bean variety and water regimes. However, it is necessary to measure these factors over a longer period of time to determine if these parameters change among generations. We suggest that the phenolic acid profile of the Black 8025 and Pinto Durango should be studied over several growing seasons to determine how the water regimes affect the amount and structure of the polyphenols and phenolic acids present in the samples and how these changes can affect the bioavailability of the components in the human body.

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## Abbreviations

CT	Condensed tannins
EP	Extractable polyphenols
HP	Hydrolysable polyphenols
IDF	Insoluble dietary fiber
IIF	Insoluble indigestible fraction masl, metres above sea level
NS	Neutral sugars
SDF	Soluble dietary fiber
SIF	Soluble indigestible fraction
TDF	Total dietary fiber
TIF	Total indigestible fraction
UA	Uronic acids