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Starch characteristics of bean (*Phaseolus vulgaris* L.) grown in different localities

Maribel Ovando-Martínez[a], Luis A. Bello-Pérez[a], Kristin Whitney[b], Perla Osorio-Díaz[a], Senay Simsek[b,*]

[a] Centro de Desarrollo de Productos Bióticos del IPN, Apartado Postal 24 C.P. 62731 Yautepec, Morelos, Mexico
[b] Department of Plant Sciences, North Dakota State University, P.O. Box 6050, Department 7670, Fargo, ND 58108-6050, USA

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ABSTRACT

Bean variety and environmental conditions (locality) might have an effect on functional properties of the starch. The digestibility and physicochemical characteristics of starch isolated from Black 8025 and Pinto Durango beans grown in two different localities with different water regimes were evaluated. Amylose content showed significant differences between localities. Pasting properties of bean starches obtained under rain fed conditions were higher than starches from beans grown in irrigation conditions. Bean starches from both localities presented a high degree of polymerization (DP) of starch chains. Starch from bean varieties grown in Celaya (irrigated) exhibited high resistant starch content and low glycemic index value compared to starch from bean varieties grown in Ocampo (rain fed). This study demonstrated that the variety of bean, locality and rain fed or irrigated conditions, affected the internal structure of bean starch and therefore some of their physicochemical and digestibility properties.

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1. Introduction

The common bean (*Phaseolus vulgaris* L.) is a legume, which is an excellent source of protein (20–25%) and complex carbohydrates (50–60%) (Chung, Liu, Pauls, Fan, & Yada, 2008). Starch is the most abundant carbohydrate in the legume seed (22–45%) (Hughes et al., 2009) and is deposited in partially crystalline granules varying in morphology and molecular structure between and within varieties of plant species (Kaur, Sandhu, & Lim, 2010). Two polysaccharide components contribute to the molecular structure of starch: amylose, a linear chain molecule, and amylopectin, a highly branched molecule (Munzing, 1991). The ratio between these components varies depending on the starch source. The molecular weight of amylose has been shown to range from 1×10^5 to 1×10^6 Da while amylopectin has an average molecular weight of the order 10^7–10^9 Da (Dona, Pages, Gilbert, & Kuchel, 2010; Hoover, Hughes, Chung, & Liu, 2010).

Legume starches, including bean starch, are digested slowly, have low glycemic index values and are fermented in the large intestine to produce short chain fatty acids, which are beneficial for colon health (Hughes et al., 2009). Differences in digestibility of starches have been attributed to many factors such as starch source, amylose/amylopectin ratio, granule size, degree of crystallinity, amylose–lipid complex, molecular structure of amylopectin and amylose chain length. In legume starches, the reduced digestibility has been attributed to the absence of pores on the granule surface, the amylose content, B-type crystallites and strong interactions between amylose chains (Hoover et al., 2010).

The effect of the environmental conditions on the properties of starches is well documented in cereal starches, such as wheat and rice. In the case of rice, genotype, environmental conditions, and crop year had major impacts on the molecular structure and consequently the physicochemical properties of medium-grain rice starch (Cameron, Wang, & Moldenhauer, 2007). Water stress conditions on wheat showed a pronounced effect on characteristics of starch and proteins (Singh, Singh, Singh, & Singh, 2008). For legume starches, there are studies about the effect of the legume varieties cultivated in the same location, but there is lack of information about how growing season, location and field conditions could be affecting the molecular structure of starch. Consequently, it is difficult to ascertain whether the variations in starch properties among cultivars were controlled by genetic and/or environmental factors (Hughes et al., 2009). This suggests that the properties of common bean starch could be affected by different environmental factors, such as growing location and different water regimes. Therefore, the objective of this study was to evaluate the effect of variety and growing conditions on chemical composition, morphology, physicochemical, structural and digestibility properties of starch from common bean.

2. Materials and methods

2.1. Materials

Two common bean cultivars (Black 8025 and Pinto Durango) were used for this experiment. Both cultivars were grown in

* Corresponding author. Tel.: +1 701 231 7737; fax: +1 701 231 8474.
E-mail address: senay.simsek@ndsu.edu (S. Simsek).

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irrigated and temporal conditions (rain fed) in two localities of Guanajuato, Mexico. Black 8025 and Pinto Durango grown under rain fed conditions in Ocampo were planted in July 2008 and harvested in October 2008. While the same varieties grown under irrigation conditions in Celaya were planted in February and were harvested in June of the same year. Samples were donated by National Institute of Research Forest, Agricultural and Livestock (INIFAP) of Celaya, Guanajuato, Mexico.

2.2. Starch isolation

Bean starch was isolated from milled bean flour according to the method described by Otto, Baik, and Czuchajowska (1997). Samples (200 g) were blended with 500 ml of water for 3 min using a blender. The slurry was then centrifuged at 1500 g for 15 min. The supernatant was decanted, and remaining solid layers were blended in 500 ml of water and centrifuged again. The same procedure was repeated once more. The supernatant was decanted, and tailing starch was carefully separated from the bottom prime starch using a spatula. The prime starch was further purified using 500 ml of water and centrifuged again. The bean starch was air dried at 40 °C. Finally, the bean starch was milled and passed through a sieve with 125 μm openings.

2.3. Chemical composition of bean starch

The moisture and ash were determined gravimetrically using approved methods 44-15A and 08-01, respectively (AACC, 2000). Nitrogen was determined using a Leco combustion nitrogen analyzer (approved method 46-30, AACC, 2000). Crude protein content was calculated as N × 6.25. A total starch assay kit (Megazyme International Ireland) was used to determine the starch content on a dry weight basis for each of the samples following the approved method 76-13 (AACC, 2000). All analyses were done in duplicate.

2.4. Granular morphology

Bean starch was mounted on aluminum mounts using colloidal silver or carbon adhesive tabs and coated with gold using a Balzers SCD 030 sputter coater (BAL-TEC RMC, Tucson, AZ). Images were obtained using a JEOL JSM-6300 Scanning Electron Microscope (JEOL USA, Peabody, MA) while using an accelerating voltage of 10 kV.

2.5. Swelling volume

The swelling volume of the bean starch was determined according to the method described by Huang et al. (2007). Bean starch (0.2 g) was weighed into aluminum cans and 10 ml of deionized water was added. Samples were equilibrated at 25 °C for 30 min and then heated at 50, 60, 70, 80 or 90 °C for 30 min using a Rapid Visco Analyzer. The samples were cooled at room temperature and centrifuged at 1000 rpm for 15 min. The supernatant was measured. Finally, the swelling volume was calculated from the gel volume of each sample and was reported in ml/g of sample. The analysis was done in duplicate.

2.6. Thermal properties

Thermal characteristics of bean starch were studied with a Perkin-Elmer Differential Scanning Calorimeter, DSC-7 using the method described by Kim, Wiesenborn, and Grant (1997) with minor modifications. Samples (3.5 mg) were weighed into aluminum pans and deionized water (8 μl) was added. The pans were sealed hermetically and kept at room temperature overnight before analysis. The samples were heated at 10 °C/min from 20 to 120 °C. An empty aluminum pan was used as a reference. From the curve, enthalpy of gelatinization (ΔH), the onset (T_o), peak (T_p) and end (T_c) temperatures were obtained using the data processing software supplied with the DSC instrument.

A second DSC scan of these gelatinized starch samples was performed after storage for 7 days at 4 °C to characterize the extent of retrogradation. Samples taken from storage were held at room temperature for 2 h prior to analysis. During the second DSC scan the samples were heated at the same interval of temperature used for gelatinization properties. All analyses were done in triplicate.

2.7. Pasting properties

Pasting properties of bean starch samples were determined using a Newport Scientific Rapid Visco-Analyzer (RVA) according to approved method 76-21. Bean starch (3 g, 14% moisture basis) was added to 25 ml deionized water in a RVA canister. The starch slurries were held at 50 °C for 1 min before heating from 50 to 95 °C at a rate of 12 °C/min and held at 95 °C for 2 min. The slurry was then cooled at rate of 12 °C/min to 50 °C and held for 2 min (AACC, 2000).

2.8. Freeze–thaw stability

The freeze–thaw stability of bean starch was tested following the method of Huang et al. (2007). Aqueous suspensions of starch (5%, w/w) were shaken for 30 min at room temperature, then were heated in a boiling water bath (30 min) with constant stirring. Immediately 1 g of starch slurry was dispensed into eppendorf tubes. The gel obtained was placed in the freezer (24 h) and thawed at 30 °C for 1.5 h. The tubes, being measured were drained on tissue paper for 5 min, and the remaining tubes were placed back in the freezer. A total of five freeze thaw cycles were completed. The freeze–thaw stability was calculated as the ratio of exuded water weight to original paste weight. The syneresis of the starch slurries was measured by storage of samples in the refrigerator for 5 days. Every 24 h the exuded water was measured.

2.9. X-ray diffraction in bean starch

The relative degree of crystallinity of the bean starch samples was determined by X-ray powder diffraction operating at 50 kV and 40 mA (Cu Kα radiation of 0.154 nm). The diffracted intensity was measured from 5° to 45° as a function of 2θ. The degree of crystallinity of the starch samples was defined by the intensity ratio of the diffraction peaks and of the sum of all measured intensity using Xpert High Score Plus, version 2.2b software. Background intensity was subtracted from the total intensity. The standard reference material was Respirable Alpha Quartz which determined the constant background.

2.10. Analysis of starches with high performance size exclusion chromatography

The molecular weight distribution of the bean starch was determined by high performance size exclusion chromatography (HPSEC) analysis according to the method of Grant, Ostenson, and Rayas-Duarte (2002). The starch (20 mg) was solubilized by adding 4.5 ml of 1.0 M KOH and 0.5 ml of 6.0 M urea. After purging the tubes with nitrogen the samples were heated at 100 °C for 90 min. After heating, 1 ml of the sample was neutralized with 1.0 M HCl and filtered through a hydrophilic nylon syringe filter before analysis. A Waters Ultrahydrogel linear column (Waters Co. Milford, MA) was used for separation. The samples were run using a Agilent 1200 series high-performance liquid chromatography (Agilent Technologies, Wilmington, DE), equipped with an

auto sampler. An Agilent refractive index detector and PC with ChemStation (HP ChesmStation for LC Rev. A.04.01) were used for control and integration. The samples were analyzed at 40 °C with filtered HPLC grade water as the mobile phase. The flow rate was 0.45 ml/min and injection volume was 20 μl. Weight-averaged molecular weights of starch samples were calculated using a series of gel permeation chromatography grade dextrans as standards. The dextran standard molecular weights were as follows: 48,600, 147,600, 273,000, 409,800, 667,800, 1.4 million, and 5–40 million Da.

2.11. Gel permeation chromatography (GPC)

Starch was fractionated into amylose and amylopectin using gel permeation chromatography (GPC). Native starch (20 mg) was dissolved in 2 M NaOH (2 ml) and heated at 70 °C with constant stirring for 2 h. The mixture was filtered using 1.5 μm nylon syringe filters. The sample was loaded into the GPC column (1.6 cm × 100 cm, Pharmacia Inc., Piscataway, NJ) packed with Sephadex CL-2B gel (Sigma–Aldrich, St. Louis, MO). The mobile phase was sodium hydroxide (10 mM) with a flow rate of approximately 0.4 ml/min. Fractions were collected (2 ml, measured for 9 min) and every third fraction was selected for analysis of total carbohydrates content by phenol–sulfuric acid assay and the identification of amylose and amylopectin by the blue value assay.

For the phenol–sulfuric acid assay, 100 μl of 5% phenol solution and 500 μl of concentrated sulfuric acid was added to 100 μl of sample. The sample was placed into a boiling water bath for 10 min. After, the sample was cooled; the absorbance was read at 490 nm. The blue value was determined by adding 100 μl of I_2/KI solution (0.2 g I_2, 2 g KI in 100 ml of 0.1 M acetate buffer pH 5, diluted 10×) to 100 μl of sample. The absorbance was read at 630 nm. After completion of the assays each fraction of the sample containing amylopectin was combined and placed into dialysis membrane. The samples were placed in distilled water and dialyzed for 3 days at 4 °C. The dialyzed sample was freeze-dried for the analysis of chain length distribution of amylopectin.

2.12. Amylopectin branch chain length distribution

The chain length distribution of purified amylopectin was characterized by high performance anion exchange chromatography equipped with a pulsed amperometric detector (HPAEC-PAD). The amylopectin samples were prepared following the methodology described by Patindol, Gonzalez, Wang, and McClung (2007) with modifications. Purified amylopectin (2–4 mg) obtained after freeze-drying was dissolved in 3.2 ml of deionized water by heating in a boiling water bath with stirring for 1 h. The amylopectin was cooled to room temperature and 0.4 ml of 0.1 M acetate buffer (pH 3.5) and 20 μl of isoamylase was added. The mixture was incubated during 48 h in a water bath shaker at 40 °C. Finally, the enzyme activity was arrested by heating the sample in a boiling water bath for 20 min. The sample (25 μl) was injected into the HPAEC-PAD system (DX 500) onto a Carbopac PA-100 column. The eluent gradient was as follows: (A) 100 mM NaOH, (B) 400 mM sodium acetate in 100 mM NaOH, with a linear gradient of 0–62.5% of eluent B for 30 min, then a linear gradient from 62.5 to 100% eluent B for 15 min. The samples were monitored with an ED-40 detector in pulsed amperometric mode with the following potentials and times: E_1, +0.05 V (t_1 = 400 ms); E_2, +0.75 V (t_2 = 200 ms); E_3, −0.15 V (t_3 = 400 ms) (Swennen, Courtin, Van der Bruggen, Vandecasteele, & Delcour, 2005). The amylopectin chain length distribution was calculated based on total area percent.

2.13. In vitro starch digestibility

In vitro starch digestibility of bean starch was analyzed using the method described by Englyst, Kingman, and Cummings (1992). Amyloglucosidase (140 AGU/ml, Megazyme International Ireland) (1.07 ml) was brought to 25 ml with deionized water. Invertase (Sigma I-4504) 60 mg was added to deionized water (8 ml). Pancreatin (Sigma P-7545) (3 g) was dispersed in deionized water (20 ml), stirred for 10 min at 4 °C and centrifuged. One hundred eight milliliters of supernatant was collected and mixed with 8 ml invertase and 12 ml of amyloglucosidase. The solution was freshly prepared for the digestion analysis.

The samples (0.3 g) with 0.1 M sodium acetate buffer (20 ml, pH 5.2) were gelatinized in boiling water for 30 min and were placed in a water bath (37 °C) with agitation (100 strokes/min). Guar gum (50 mg) and 5 glass beads were added to each tube. Blank and glucose standard tubes were prepared. Five milliliters of enzyme solution was added to each tube at 1 min intervals. At 20 and 120 min aliquots (0.5 ml) were taken, mixed with 5 ml of absolute ethanol and centrifuged. The glucose content was measured using glucose oxidase and peroxidase assay kit (Megazyme International Ireland). Glucose content in the samples was calculated against a standard curve and the rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) were determined. The same procedure was used for the analysis of bean starch without gelatinization.

The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of the sample by the area obtained for white bread (hydrolysis curve 0–180 min). The glycemic index of the samples was estimated using the equation described by Granfeldt, Bjorck, Drews, and Tovar (1992): pGI = 8.198 + 0.862HI.

2.14. Statistical analysis

DSC and *in vitro* starch digestion analysis was done in triplicate. All other analyses were done in duplicate. The mean and SEM (standard error of the mean) were reported for each of the samples. 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. Chemical composition and amylose and amylopectin content determined by HPSEC

The chemical composition of bean starch from Black 8025 and Pinto Durango is shown in Table 1. The total starch (TS) content ranged from 88.56 to 95.28% (Table 1). Significant differences were observed between Pinto Durango bean starch grown in both irrigated and rain fed conditions, while Black 8025 did not show significant differences ($P > 0.05$). Irrigation conditions affected total starch content for Pinto Durango. The total starch content from these bean starches was similar to those reported by Chung, Liu, Pauls, et al. (2008) (94–100%). Black 8025 bean starch showed no significant differences between localities. Pinto Durango bean starch from irrigation conditions had significantly ($P > 0.05$) higher TS content compared to the same variety from rain fed conditions. The ash and protein content did not differ significantly and were less than one percent (Table 1).

The HPSEC chromatograms of bean starches showed three peaks (Fig. 1). The first two peaks correspond to amylopectin and the

Table 1
Yield and chemical composition of starches from Black 8025 and Pinto Durango beans grown under irrigation and rain fed conditions[a].

Characteristics	Variety			
	Celaya: irrigation		Ocampo: rain fed	
	Black 8025	Pinto Durango	Black 8025	Pinto Durango
Yield (%)[b]	29.52 ± 1.42a	38.67 ± 0.15b	30.50 ± 1.28a	29.27 ± 4.60a
Moisture (%)	11.90 ± 0.16b	12.79 ± 1.09b	11.28 ± 0.11a	11.42 ± 0.17a
Ash (%)[b]	0.05 ± 0.05a	0.03 ± 0.01a	0.10 ± 0.06a	0.02 ± 0.02a
Protein (%)[b]	0.58 ± 0.02b	0.56 ± 0.01b	0.54 ± 0.01a	0.59 ± 0.01b
TS[c] (%)[b]	91.91 ± 0.77b	95.28 ± 1.38c	91.69 ± 0.69b	88.56 ± 0.56a
Amylose (%)	28.06 ± 0.21c	23.20 ± 0.77a	25.17 ± 1.05b	24.92 ± 0.88b
HMW-AP (%)	29.53 ± 0.79a	26.31 ± 2.44a	27.39 ± 1.69a	29.14 ± 0.69a
LMW-AP (%)	42.42 ± 0.86a	50.49 ± 2.93c,b	47.44 ± 1.73b	45.94 ± 1.39b

Data with the same letters in the same row are not significantly different ($P > 0.05$) by Tukey test. HMW-AP: high molecular weight amylopectin, LMW-AP: low molecular weight amylopectin.

[a] Values are mean ± SEM, $n = 2$.
[b] Values expressed in dry weigh basis.
[c] Total starch.

third represents amylose (Grant et al., 2002). The amylose content in bean starches varied from 23.20 to 28.06% (Table 1) and was similar to values reported for common bean (Yoshimoto, Matsuda, Hanashiro, Takenouchi, & Takeda, 2001). Significant differences ($P > 0.05$) between varieties were observed in amylose content for varieties grown under irrigation, but not for varieties grown under rain fed conditions. Lower temperatures during growing caused an increase in the amylose content of rice starch (Cameron et al., 2007). In our study, Ocampo had lower minimum growing temperature (5.0 °C) than Celaya (9.3 °C). However, only starch isolated from Pinto Durango had an increase in amylose content when grown in Ocampo, while starch from Black 8025 bean showed an opposed trend. This indicates that bean variety has an influence on the amylose content. The first two peaks corresponding to amylopectin (Fig. 1) were assigned to high molecular weight amylopectin (HMW-AP) and low molecular weight amylopectin (LMW-AP). The HMW-AP content ranged from 26.31% to 29.53% (Table 1). There were no significant differences among varieties, water regimes and localities. Starch from Black 8025 bean grown in Celaya had the lowest LMW-AP content (42.42%), while the other bean starches did not show significant differences ($P > 0.05$).

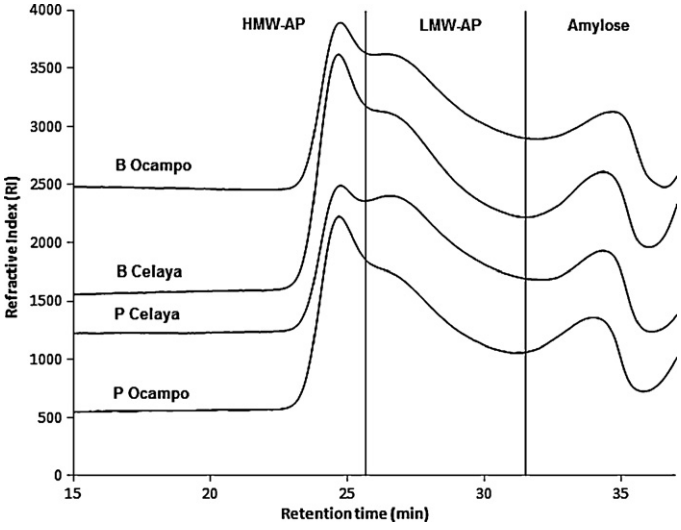

Fig. 1. Size exclusion chromatography (HPSEC) analysis of isolated bean starches. **BOcampo**: Black 8025 bean grown in Ocampo, **BCelaya**: Black 8025 bean grown in Celaya, **POcampo**: Pinto Durango bean grown in Ocampo and **PCelaya**: Pinto Durango bean grown in Celaya. HMW-AP: high molecular weight amylopectin; LMW-AP: low molecular weight amylopectin.

3.2. Granular morphology of bean starch

Scanning electron microscopy (SEM) images of isolated bean starches from Black 8025 and Pinto Durango bean grown in Celaya and Ocampo are shown in Fig. 2. All of the bean starches showed round and oval shapes and different granule size (left panel, ×550). The surface of bean starch granules is smooth and did not show evidence of fissures (right panel, ×2500). Pinto Durango starches grown in both conditions (Celaya and Ocampo) showed the presence of grooves, such as it was reported for pigeon pea starch (Hoover, Swamidas, & Vasanthan, 1993). The granule size distribution of bean starches is shown in Table 2. The mean granule length and width of bean starches ranged from 22.61 to 24.88 μm and 17.87 to 18.74 μm, respectively. The bean starches from beans grown in Celaya presented the highest granule size variation (length and width) compared to the same bean varieties grown in Ocampo. However, the granules of bean starches from Celaya were larger than those from Ocampo. Zhong-Min et al. (2008) reported in wheat starch that soil water deficit caused the increase in amount of B type granules (small granules) and the decrease in A type granules (large granules). The decrease in the granule size in bean starches from rain fed conditions in Ocampo can be attributed to changes in the activity of enzymes related to starch synthesis due to water deficit. The differences in the granule size and granule size distribution may have influence on the physicochemical and digestibility properties of bean starch (Kaur et al., 2010).

3.3. Swelling volume (SV)

The weakening of starch granules when heating in excess water causes the absorption of water (due to replacement of hydrogen bonds with water), swelling of starch granules and increase in volume (Huang et al., 2007). The SV of the starches from Black 8025 and Pinto Durango beans grown in Celaya (irrigation) and Ocampo (rain fed) tested at different temperatures are presented in Fig. 3. The SV value for all bean starches increased as the temperature increased. The SV values to 50 and 60 °C for all starches were low because these temperatures are below the gelatinization temperature of the starch samples. At 70 °C, an increase in the SV values was observed, due to the temperature being greater than the gelatinization temperature. Black 8025 did not show much difference between growing locations at 70 °C, however Pinto Durango from Celaya had a higher SV than from Ocampo at 70 °C. Between bean starch varieties, Pinto Durango showed higher SV value than Black 8025 bean at 70 °C. This pattern can be related to the difference in the gelatinization temperature of bean starches. At the highest temperatures of the test (80 and 90 °C) Black 8025 bean starch from

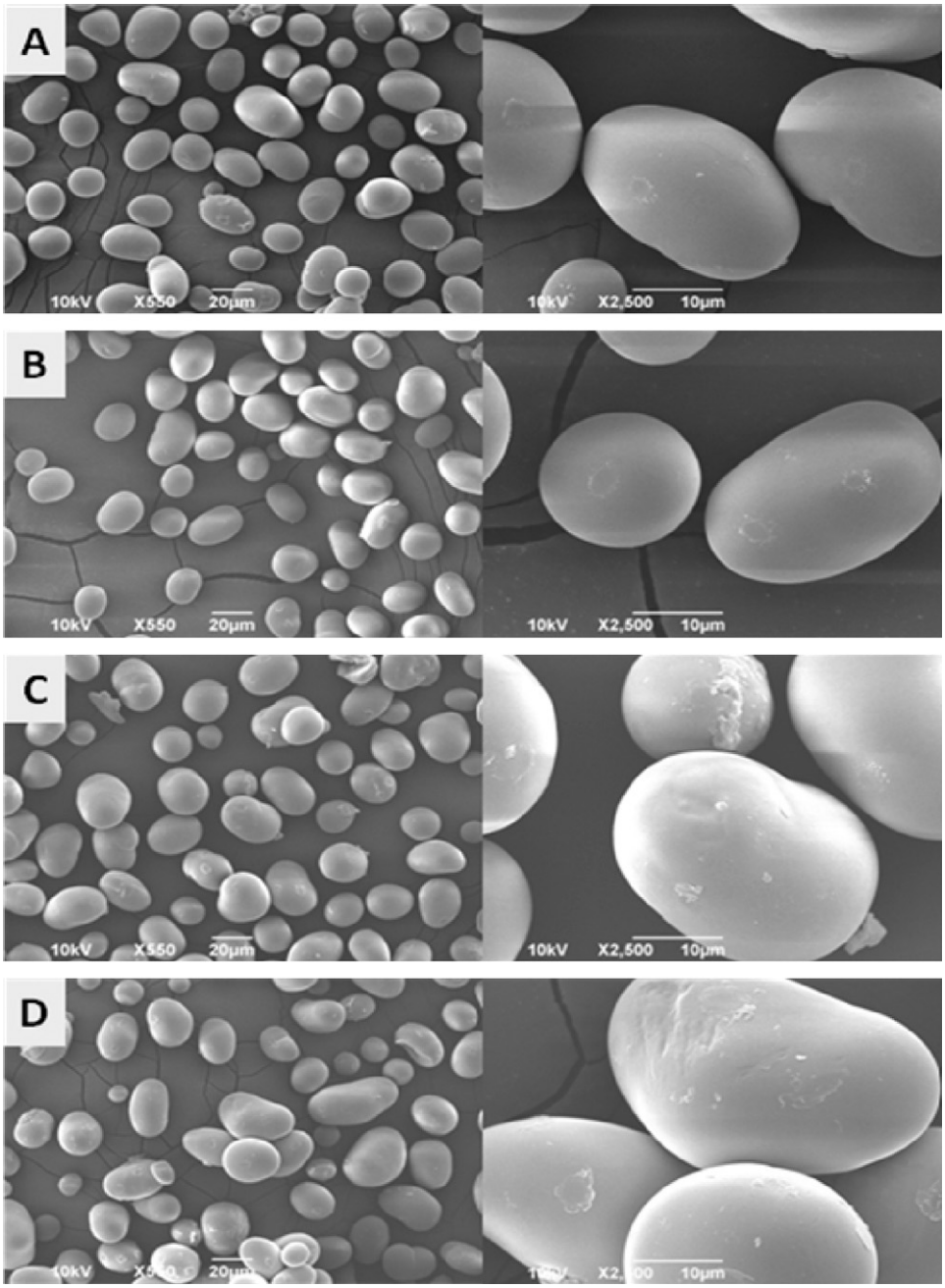

Fig. 2. Scanning electron microscopy (SEM) images of bean starches. **Bean grown in Celaya**: (A) Black 8025 and (D) Pinto Durango. **Bean grown in Ocampo**: (B) Black 8025 and (C) Pinto Durango. Left and right panel are SEM images at different magnification. Left panel (×550) and right panel (×2500).

Ocampo had the highest SV values and the other starches did not show differences. At these temperatures the starches have been fully gelatinized and the rupturing of the starch granules may have begun. It was reported that swelling of starch is influenced by the amylose–lipid complexes, amylose content, interaction between starch chains within the amorphous and crystalline region of the granule, and the molecular structure of amylopectin (Zhou, Hoover, & Liu, 2004).

Table 2
Size of bean starch granules of Black 8025 and Pinto Durango grown under irrigation and rain fed conditions.

Sample	Length (µm)		Width (µm)	
	Mean	Range	Mean	Range
Celaya: irrigation				
Black 8025	24.30	15.65–36.52	18.74	13.91–25.22
Pinto Durango	24.88	9.57–43.48	18.55	9.57–26.96
Ocampo: rain fed				
Black 8025	22.71	13.91–32.17	17.87	12.17–23.48
Pinto Durango	22.61	12.17–40	18.02	11.30–25.22

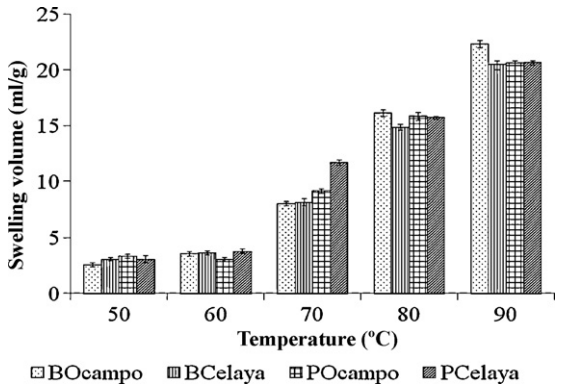

Fig. 3. Swelling volume for bean starches influenced by temperature. Swelling volume was calculated as gel volume per unit dry weight of starch. **BOcampo**: Black 8025 bean grown in Ocampo, **BCelaya**: Black 8025 bean grown in Celaya, **POcampo**: Pinto Durango bean grown in Ocampo and **PCelaya**: Pinto Durango bean grown in Celaya.

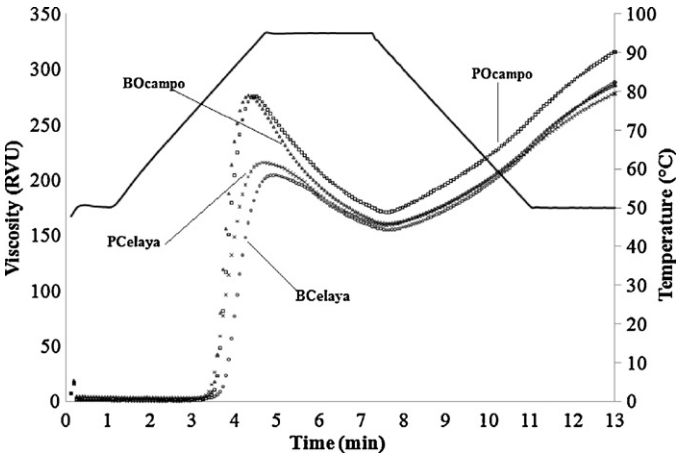

Fig. 4. RVA viscograms of common bean starches. **BOcampo**: Black 8025 bean grown in Ocampo, **BCelaya**: Black 8025 bean grown in Celaya, **POcampo**: Pinto Durango bean grown in Ocampo and **PCelaya**: Pinto Durango bean grown in Celaya.

3.4. Thermal properties

Gelatinization properties of bean starches from Black 8025 and Pinto Durango bean grown in two locations and water regimes are summarized in Table 3. The gelatinization temperatures, T_o, T_p and T_c, ranged from 62.67 to 69.14, 70.14 to 75.42 and 78.22 to 82.33 °C, respectively. In general, Black 8025 bean starches had higher transition temperatures (T_o, T_p and T_c) than Pinto Durango bean starches. The isolated starches from bean varieties grown under rain fed conditions showed higher T_o and T_p compared with bean starches from irrigation conditions. Tester and Karkalas (2001) reported that the planting/harvesting dates and growing conditions affected gelatinization properties of cassava, sweet potato and maize starches. The gelatinization temperatures are influenced by the molecular architecture of the crystalline region corresponding to the distribution of amylopectin short chains (degree of polymerization, DP 6–11) (Noda et al., 1998). The low T_o, T_p and T_c in Pinto Durango bean starches from both growing conditions is related to the high content of short amylopectin chains in these samples (Table 8). The gelatinization temperature range ($T_c - T_o$) varied from 12.49 to 15.55 °C and was higher in Pinto bean starches than Black 8025 bean starches. Therefore, Pinto Durango bean starches have a higher degree of heterogeneity of crystallites within the starch granule. The gelatinization enthalpy (Table 3) did not showed significant differences ($P > 0.05$) among variety or growing conditions, indicating similar arrangement of the double helices of amylopectin chains. The gelatinization enthalpy found in these bean starches is associated with a higher proportion of amylopectin short branch chains (Table 8), which may reduce the packing order within the crystalline lamellae.

Upon refrigerated storage, the amylose and amylopectin components begin to re-organize causing recrystallization of the chains. The retrogradation properties of bean starches are summarized in Table 3. The T_o, T_p and T_c values of retrograded starches were lower than those obtained for the initial gelatinization. Pinto Durango starch from Ocampo presented the lowest T_o and the bean starch isolated from the same variety grown in Celaya showed the lowest T_p. The bean starches did not present significant differences ($P > 0.05$) in the T_c between variety and locality. The bean starches have high percentage of B1 (DP 13–24) short chains in their amylopectin structure. These chains have higher mobility and take more time to reassociate, resulting in a low retrogradation rate. Perhaps the slow rate of retrogradation caused the temperature range of the phase transition to increase, causing the formation of heterogeneous and/or imperfect crystallite requiring less energy to melt (lower enthalpy value than gelatinization).

3.5. Pasting properties

The pasting properties are determined to give a picture of the functional behavior of starch during heating and cooling periods during processing (Bello-Pérez & Paredes-López, 2009). The RVA profiles of bean starches are represented in Fig. 4 and summarized in Table 4. Bean starches from Celaya (irrigation) presented lower peak viscosity compared to bean starches from Ocampo (rain fed). These variations can be attributed to the granule swelling, starch crystallinity, amylose content and chain length distribution (Chung, Liu, Pauls, et al., 2008). Only Black 8025 bean starch from Celaya (irrigation) (Table 4) showed a significant difference ($P > 0.05$) in pasting temperature from the other starches. Black 8025, had the highest pasting temperature (84.68 °C), which might be due to the high amylose content resulting in restricted starch granule swelling. With respect to the peak viscosity, bean starches from Ocampo (rain fed) had higher peak viscosity than bean starches from Celaya (irrigation). It has been reported that low swelling factor, stronger crystalline structure (high proportion of B1 chains) and a large amount of relative crystallinity, cause a low peak viscosity in starch (Chung, Liu, Donner, et al., 2008). The higher proportion of chains with DP 25–36 and 37–40 (Table 8) present in bean starches from Ocampo, hold the integrity of the swollen granules during the heating cycle and contributed to the formation of high peak viscosity as was reported by Jayakody et al. (2007). However, during the holding cycle bean starches from Ocampo (rain fed) showed the highest breakdown viscosity. The breakdown viscosity presented significant differences ($P > 0.05$) among bean starches. Black 8025 bean starch from Celaya had the lowest breakdown viscosity (49.67 RVU), while Black 8025 from Ocampo had the highest breakdown (115.38 RVU). Starches grown under irrigation conditions had lower breakdown viscosity than bean starches grown under rain fed conditions. High breakdown viscosity reflects granular swelling that makes the starch granules more susceptible to shear (Hughes et al., 2009). This indicates that starches from Ocampo (rain fed) have less resistance to the shear and the starch granules have more susceptibility to disintegration than bean starches from Celaya (irrigation). Setback viscosity ranged between 119.25 and 145.13 RVU. Among bean starches grown in the same locality significant differences ($P > 0.05$) were observed. Black 8025 bean starch from Celaya (irrigation) had higher setback than Pinto Durango grown in the same location, but showed the opposite trend when grown in Ocampo (rain fed). The final viscosity was not different between bean starches grown under irrigation (Celaya) but was different between varieties grown under rain fed (Ocampo). Pinto

Table 3
Gelatinization and retrogradation properties of bean starches from Black 8025 and Pinto Durango beans grown under rain fed and irrigation conditions using Differential Scanning Calorimetry (DSC)[a].

Starch	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH (J/g)
Gelatinization					
Celaya: irrigation					
Black 8025	68.13 ± 0.65c	75.22 ± 0.16c	82.33 ± 0.57c	14.20 ± 0.99b	9.45 ± 1.57a
Pinto Durango	62.67 ± 0.21a	70.14 ± 0.11a	78.22 ± 0.45a	15.55 ± 0.66c,b	9.11 ± 0.46a
Ocampo: rain fed					
Black 8025	69.14 ± 0.19d	75.42 ± 0.08c	81.63 ± 0.15b	12.49 ± 0.30a	9.51 ± 0.70a
Pinto Durango	67.27 ± 0.07b	74.69 ± 0.06b	81.62 ± 0.10b	14.36 ± 0.15b	9.57 ± 0.33a
Retrogradation					
Celaya: irrigation					
Black 8025	44.18 ± 0.33b	56.52 ± 0.23b	74.08 ± 0.96a	29.89 ± 1.29b	7.48 ± 0.53b
Pinto Durango	43.73 ± 0.26b	55.37 ± 0.46a	73.72 ± 0.75a	29.99 ± 0.75b	5.09 ± 0.31a
Ocampo: rain fed					
Black 8025	44.59 ± 0.51c,b	56.84 ± 0.25b	73.01 ± 0.70a	28.43 ± 0.77a	6.70 ± 0.65b
Pinto Durango	42.44 ± 0.49a	56.50 ± 0.37b	74.34 ± 0.35b,a	31.90 ± 0.84c,b	5.89 ± 0.51b

Data with the same letters in the same column are not significantly different ($P > 0.05$) by Tukey test. T_o: onset temperature, T_p: peak temperature, T_c: conclusion temperature, $T_c - T_o$: gelatinization and retrogradation temperature range, ΔH: gelatinization and retrogradation enthalpy.
[a] Values are mean ± SEM, $n = 3$.

Table 4
Pasting properties of bean starch from Black 8025 and Pinto Durango beans grown under irrigation and rain fed conditions measured by Rapid Visco Analyzer (RVA)[a].

Sample	Pasting temperature (°C)	Viscosity (RVU)			
		Peak	Breakdown	Final	Setback
Celaya: irrigation					
Black 8025	84.68 ± 0.02b	204.55 ± 3.88a	49.67 ± 2.17a	288.46 ± 5.71a	133.58 ± 4.00b
Pinto Durango	81.48 ± 0.78a	215.88 ± 4.30b	57.00 ± 2.50b	278.13 ± 11.13a	119.25 ± 4.33a
Ocampo: rain fed					
Black 8025	80.70 ± 0.05a	276.34 ± 3.41c	115.38 ± 4.38d	285.59 ± 1.33a	124.63 ± 2.30a
Pinto Durango	81.08 ± 0.48a	275.46 ± 3.71c	104.92 ± 5.16c	315.67 ± 0.25b	145.13 ± 1.20c

Data with the same letters in the same column are not significantly different ($P > 0.05$) by Tukey test.
[a] Values are mean ± SEM, $n = 2$.

Durango Bean starch from Ocampo showed the highest final viscosity, while the same starch grown in Celaya had the lowest value. This parameter is used to indicate the ability of starch paste to retrograde and form a strong gel after cooling (Ikegwo, Okechukwu, & Ekumankana, 2010).

3.6. Freeze–thaw stability and syneresis

Stability during freeze–thaw cycles is related to the ability of starch to resist physical changes during these phases (Takeiti, Fakhouri, Ormenese, Steel, & Collares, 2007). The freeze–thaw stability and syneresis of bean starches from Celaya and Ocampo are presented in Tables 5 and 6, respectively. The syneresis of bean starches slightly increased with the number of freeze–thaw cycles (days of storage). A similar pattern was observed by Huang et al. (2007) in three legume starches. The extent of syneresis of bean starch gels after five freeze thaw cycles was lower in Black 8025 bean starch from Celaya (Table 5). The low extent of syneresis suggests that the interaction between starch chains occurred slowly during the storage of starch gels in the freezer (Hoover & Ratnayake, 2002). When the samples were stored at 4 °C for 5 days, the syneresis values ranged from 12.64 to 15.64% (Table 6). During the second day the syneresis nearly doubled to 28.25–39.29%. Pinto Durango bean starch from Celaya showed the lowest syneresis and Black 8025 bean starch from Ocampo had the highest syneresis. The increasing of syneresis in bean starches, at refrigeration temperatures (4 °C), is due to amylose retrogradation. The structural differences such as the amylopectin chain length and proportion of short chains (Table 8) among starches could have effect on this parameter.

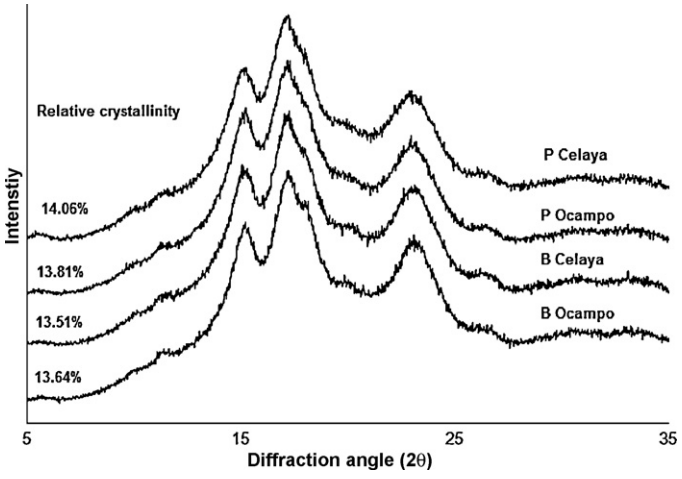

Fig. 5. X-ray diffraction pattern of bean starches. **BOcampo**: Black 8025 bean grown in Ocampo, **BCelaya**: Black 8025 bean grown in Celaya, **POcampo**: Pinto Durango bean grown in Ocampo and **PCelaya**: Pinto Durango bean grown in Celaya.

3.7. X-ray diffraction in bean starch

The X-ray diffractograms of bean starches and their relative crystallinity are depicted in Fig. 5. Black 8025 and Pinto Durango bean starches from both Celaya and Ocampo presented the characteristic C type pattern (mixture of A and B type starches) of legume starches. The X-ray diffraction pattern of bean starches showed peaks at diffraction angles 2θ of 5.4°, 15°, 17°, 20°, 23.2° and 26.6°. There were no observed differences in the position of these peaks

Table 5
Freeze–thaw stability of Black 8025 and Pinto Durango bean starches grown under rain fed and irrigation conditions (%)[a].

Starch	Freeze–thaw stability (cycles)				
	1	2	3	4	5
Celaya: irrigation					
Black 8025	45.51 ± 4.11a	49.00 ± 1.87a	52.74 ± 2.64a	56.07 ± 0.88b,a	47.31 ± 3.52a
Pinto Durango	46.67 ± 2.25a	61.61 ± 3.45c	57.98 ± 1.95b,a	55.51 ± 2.23b,a	53.05 ± 1.33b
Ocampo: rain fed					
Black 8025	44.40 ± 2.43a	53.28 ± 0.71b	52.21 ± 1.61a	52.24 ± 0.84a	54.92 ± 0.59b,a
Pinto Durango	57.49 ± 3.98b	59.99 ± 4.72c	55.98 ± 2.11a	51.21 ± 5.78a	53.36 ± 3.25b,a

Data with the same letters in the same column are not significantly different (P>0.05) by Tukey test.
[a] Values are mean ± SEM, $n = 2$.

among all starches. It has been reported that the peak intensity at $2\theta = 5.4$ is representative of the B polymorphic form. Pinto Durango bean starches presented higher intensity in this peak than Black 8025 bean starches. According to Hoover and Ratnayake (2002), this suggests the presence of a high proportion of B unit cell in Pinto Durango bean starch. The peak at $2\theta = 20$ corresponds to the amylose–lipid complex (Chakraborty et al., 2004). In this case, Black 8025 bean starches showed higher intensity than Pinto Durango bean starches. This could be because Black 8025 bean starches had the lowest amylopectin content (Table 1). With regard to relative crystallinity, bean starches followed the order: Pinto Durango Celaya > Pinto Durango Ocampo > Black 8025 Ocampo > Black 8025 Celaya. The degree of crystallinity of bean starches did not present significant differences between bean varieties and locality.

3.8. Starch molecular weight distribution

The molecular structure of starch plays a decisive role in its nutritional and technological functionality (Creek, Benesi, Runt, & Ziegler, 2007). The distribution of weight-averaged molecular weight of bean starch measured using HPSEC-RI system is shown in Table 7. Investigating the weight-averaged molecular weight of starch polymers is complicated for several reasons, including difficulties of solubilization of native starch, broad size distribution, and problems with degradation of starch during analysis. Size exclusion chromatography (SEC, also known as gel permeation chromatography – GPC), field-flow fractionation (FFF) and analytical ultracentrifugation (AUC) are common techniques for size separation of starch molecules. A range of detection (singly or as multiple detection) accompanies separation techniques, for example differential refractive index (DRI), multiple-angle laser light scattering (MALLS), in-line viscometry, quasi-elastic light scattering, fluorescence and in-line osmometry have been used in the literature (Gidley et al., 2010). In this study, weight-averaged molecular weights of bean starches were calculated using a series of GPC grade dextrans since this system was available in our laboratories. Therefore, we do not ignore the fact that if a different system (i.e., MALLS-RI) was used, different molecular weight values could be determined. The weight-averaged molecular weight of HMW-AP did not show differences between bean starches grown in both localities, except for Black 8025 bean starch from Celaya, which had the highest value (4.42×10^6 Da). Pinto Durango bean starch from Ocampo showed higher weight-averaged molecular weight for LMW-AP (2.93×10^6 Da) and Pinto Durango starch from Celaya had lower value (2.69×10^6 Da). However, bean starches did not present significant differences among localities and rain fed or irrigation conditions. The weight-averaged molecular weight distribution of amylose was not different among starches, except for Pinto Durango from Ocampo, which had the highest molecular weight (2.14×10^5 Da). The weight-averaged molecular weight was not affected by the bean variety or environmental conditions.

3.9. Amylopectin chain length distribution

To distinguish the unit chain composition of amylopectin, the branch chains were classified according to their degree of polymerization as A-chains (DP 6–12), B1-chains (DP 13–24), B2-chains (25–36) and B3-chains (DP >37) (Hanashiro, Abe, & Hizukuri, 1996). The amylopectin chain length distribution from Pinto Durango and Black 8025 bean starches grown in different localities is presented in Table 8. The bean starches had a high amount of chains with DP 13–24 followed by chains with DP 6–12. Also, they presented a low amount of chains with DP 25–36 and very small percent of chains with DP 37–40. No significant differences were observed in the chains with DP 6–12 and DP 13–24 between bean starches. Pinto Durango bean starch from Celaya had the highest proportion of chains with DP 6–12 (31.88%), while Black 8025 bean starch from this same locality had the highest proportion of chains with DP 13–24 (56.24%). However, bean starches grown in rain fed conditions (Ocampo) showed a slightly higher proportion of chains with DP 37–40 and average chain length (CL), indicating an effect of the locality. There is a lack of information regarding the effect of environmental factors on the chain length distribution of amylopectin of bean starches. Umemoto, Terashima, Nakamura, and Satoh (1999) reported that low temperatures during the grain filling caused an increased proportion of chains with DP 6–13 (short chains) and decreased the percent of chains with DP 20–27 and DP 44–54 (long chains) in rice. In the bean starch samples, the

Table 6
Syneresis of bean Black 8025 and Pinto Durango starches isolated from beans grown under irrigation and rain fed conditions (%)[a].

Starch	Syneresis (days)				
	1	2	3	4	5
Celaya: irrigation					
Black 8025	12.64 ± 0.40a	38.55 ± 0.77c	37.54 ± 0.77b	40.52 ± 0.95c	38.11 ± 0.51c
Pinto Durango	12.77 ± 0.49a	28.25 ± 0.44a	32.20 ± 1.32a	32.70 ± 1.13a	33.07 ± 0.81a
Ocampo: rain fed					
Black 8025	14.09 ± 1.00b	39.29 ± 1.45c	40.29 ± 0.20c	41.69 ± 0.86c	41.23 ± 1.23d
Pinto Durango	15.64 ± 0.49c	31.80 ± 0.94b	33.30 ± 0.71a	35.97 ± 0.60b	35.86 ± 0.91b

Data with the same letters in the same column are not significantly different ($P > 0.05$) by Tukey test.
[a] Values are mean ± SEM, $n = 2$.

Table 7
Average molecular weight by high performance size exclusion chromatography of bean starches from Black 8025 and Pinto Durango grown under irrigation and rain fed conditions.

Sample	HMW-AP (10^6)	LMW-AP (10^6)	Amylose (10^5)
	(Averaged molecular weight, Da)		
Celaya: irrigation			
Black 8025	4.42	2.86	1.94
Pinto Durango	4.34	2.69	1.94
Ocampo: rain fed			
Black 8025	4.36	2.77	1.85
Pinto Durango	4.36	2.93	2.14

HMW-AP: high molecular weight amylopectin, LMW-AP: low molecular weight amylopectin.

Table 8
Branch chain length distribution and average chain length (CL) of native bean starches from Black 8025 and Pinto Durango beans grown under irrigation and rain fed conditions[a].

Source starch	Distribution (%)				
	DP 6–12	DP 13–24	DP 25–36	DP 37–40	CL
Celaya: irrigation					
Black 8025	29.97 ± 0.63a	56.24 ± 0.72b,a	13.34 ± 0.10a	0.48 ± 0.01[g]	16.80 ± 0.04b
Pinto Durango	31.88 ± 0.96b,a	54.06 ± 1.46a	13.28 ± 0.19a	0.52 ± 0.04[g]	16.56 ± 0.11a
Ocampo: rain fed					
Black 8025	30.05 ± 0.50a	54.40 ± 0.05a	14.52 ± 0.29b	0.99 ± 0.05b	17.07 ± 0.08c
Pinto Durango	30.12 ± 1.45a	54.59 ± 0.99a	14.31 ± 0.38b	0.98 ± 0.08b	17.05 ± 0.21c

Data with the same letters in the same column are not significantly different ($P > 0.05$) by Tukey test.
[a] Values are mean ± SEM, $n = 2$.

amylopectin short chains decreased at higher temperature, while the chains with DP 13–34 increased with the increase of maturation temperature. The differences in the chain length distribution could be due to differences in genetic background or genetic variation of starch biosynthetic enzymes (Matsuki, Yasui, Kohyama, & Sasaki, 2003; Umemoto et al., 1999). The amylopectin structure of bean starch changes during the biosynthesis of starch due to water stress, temperature or soil. The environmental factors (water stress and locality) increased the proportions of chains with DP 25–36, DP 37–40 and CL. The changes caused in the structure of amylopectin because of environmental factors will have great influence on the physicochemical and digestibility properties of bean starch.

3.10. *In vitro* starch digestibility

The starch fractions and estimated glycemic index of uncooked and gelatinized bean starches from Celaya and Ocampo obtained by *in vitro* starch digestion are summarized in Tables 9 and 10, respectively. Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) of uncooked bean starches ranged from 0% to 0.24%, 20.39% to 30.14% and 61.55% to 74.89%, respectively (Table 9). The RDS content in each bean starch did not show differences among localities and water regimes. Pinto Durango bean starch from both localities had lower SDS content than Black 8025 bean starch. SDS, the more desirable nutritional fraction of starch, was low compared to cereal starches (43–53%) (Zhang, Ao, & Hamaker, 2006). The bean starches showed high RS content compared to other authors (Chung, Liu, Pauls, et al., 2008; Zhang et al., 2006). Pinto Durango bean starch from Celaya had the highest RS content and Black 8025 bean starch from Ocampo showed the lowest value. The RS content decreased in bean starches from Ocampo (rain fed conditions). The hydrolysis index (HI) ranged from 25.05% to 41.41%, while estimated glycemic indices (eGI) were from 29.79 to 43.90. Pinto Durango starch from Celaya presented the lowest HI and eGI, which may be due to high crystallinity. The samples from Ocampo had higher HI and eGI values compared with the bean starches from Celaya. This trend could be attributed to the granule size (Table 2), because bean starches from Celaya had larger sized granules than starches from Ocampo. This may have caused the increase in the digestibility of bean starches from Ocampo, because small granules have higher surface area and susceptibility to hydrolysis by digestive enzymes. In general, the digestibility of uncooked starch was affected by the locality, and was dependent of the bean variety. Starchy foods are generally cooked before eating, so bean starches were gelatinized and analyzed for *in vitro* starch digestibility. The starch nutritional fractions, HI and eGI of gelatinized bean starches are presented in Table 10. RDS, SDS, HI and eGI content increased, while RS decreased after starch gelatinization.

Table 9
Starch nutritional fractions and estimated glycemic index of uncooked bean starches from Black 8025 and Pinto Durango grown under irrigation and rain fed conditions by *in vitro* starch digestion (%)[a,b].

Sample	RDS	SDS	RS	HI	eGI
Celaya: irrigation					
Black 8025	0.19 ± 0.19a	24.11 ± 1.29b	67.61 ± 1.19b	32.72 ± 2.90b	36.40 ± 2.50b
Pinto Durango	0.00 ± 0.00a	20.39 ± 0.61a	74.89 ± 0.61c	25.05 ± 1.25a	29.79 ± 1.08a
Ocampo: rain fed					
Black 8025	0.00 ± 0.00a	30.14 ± 0.68c	61.55 ± 0.68a	41.41 ± 3.06c	43.90 ± 2.64c
Pinto Durango	0.24 ± 0.24a	20.87 ± 1.34a	67.46 ± 1.26b	29.53 ± 0.86b	33.65 ± 0.74b

RDS: rapidly digestible starch, SDS: slowly digestible starch, RS: resistant starch, HI: hydrolysis index, eGI: estimated glycemic index was calculated from equation proposed by Granfeldt et al. (1992) (eGI = 0.862IH + 8.198). Data with the same letters in the same column are not significantly different ($P > 0.05$) by Tukey test.
[a] Values are mean ± SEM, $n = 3$.
[b] Nutritional fractions of native bean starch (without gelatinization process).

Table 10
Starch nutritional fractions and estimated glycemic index of gelatinized bean starches from Black 8025 and Pinto Durango beans grown under irrigation and rain fed conditions by *in vitro* starch digestion (%)[a,b].

Sample	RDS	SDS	RS	HI	eGI
Celaya: irrigation					
Black 8025	26.95 ± 0.74b	49.06 ± 1.18b	15.90 ± 1.06a	143.57 ± 0.58c,b	131.96 ± 0.50c,b
Pinto Durango	32.56 ± 1.78c	42.78 ± 1.93a	19.94 ± 2.02b,a	142.28 ± 4.40b	130.84 ± 3.79b
Ocampo: rain fed					
Black 8025	16.33 ± 0.56a	57.34 ± 3.85c	18.02 ± 3.49a	115.61 ± 1.15a	107.85 ± 0.99a
Pinto Durango	27.83 ± 1.33b	45.84 ± 1.99a	14.89 ± 0.91a	137.44 ± 4.31b	126.67 ± 3.72b

RDS: rapidly digestible starch, SDS: slowly digestible starch, RS: resistant starch, HI: hydrolysis index, eGI: estimated glycemic index was calculated from equation proposed by Granfeldt et al. (1992) (eGI = 0.862IH + 8.198). Data with the same letters in the same column are not significantly different ($P > 0.05$) by Tukey test.

[a] Values are mean ± SEM, $n = 3$.

[b] Nutritional fractions of bean starch gelatinized.

With respect to the effect of locality, starches from beans grown in Ocampo showed a decrease in the RDS content and an increase in the SDS content. The locality did not affect the RS content in the bean starches. The bean starch from Black 8025 grown in rain fed conditions had lower HI and eGI and starch from Black 8025 grown under irrigation had the highest HI and eGI. The eGI of starches from Ocampo was lower than starches from Celaya, the former showing a higher proportion of long chains (B2 and B3) and CL (Table 8). The differences in the digestibility among bean starches have been attributed to diverse factors such as starch source, granule size, amylose/amylopectin ratio, amylopectin chain length distribution, degree crystallinity and type of crystalline polymorphic forms. The digestibility also can be affected by the physicochemical properties of the starch, which are influenced by processing or storage conditions (Sandhu & Lim, 2008). Chung, Shin and Lim (2008) determined the *in vitro* starch digestibility of uncooked and gelatinized corn starch. They found that the uncooked corn starch showed lower eGI (75.3%) than gelatinized corn starch (93.5%). The uncooked starches are less susceptible to enzymatic hydrolysis due to high content of RS. However, gelatinized starch undergoes changes in structure due to disorganization, increasing the accessibility to the digestive enzymes and consequently its hydrolysis (Chung, Shin, et al., 2008).

4. Conclusions

The starches did not present large differences in chemical composition, thermal and pasting properties and distribution of weight-averaged molecular weight between localities. Starch granule morphology was more affected by growing location than bean variety. The type of bean and growing location had higher effect on the percentage of LMW-AP than on the HMW-AP and amylose. All bean starches showed high percentage of A-chains (DP 6–12) and B1-chains (DP 13–24), and a very low amount of B3-chains (DP 37–40). The growing location had higher effect than bean variety on degree of polymerization and average chain length. The digestibility of the starch was affected both by the type of bean and the growing conditions. The results of this research contribute to the knowledge of the chemistry of bean starch and how it is affected by the type of bean and growing conditions.

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