

Starch enzymatic hydrolysis, structural, thermal and rheological properties of pigeon pea (*Cajanus cajan*) and dolichos bean (*Dolichos lab-lab*) legume starches

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Abstract

Cajanus Cajans and *Dolichos lab-lab* legume starches from Argentine cultivars were investigated under a technological and nutritional point of view. Their physico-chemical, structural, thermal and the rheological properties of their gels were evaluated. Rice (RS) and potato (PS) starches were included as references. *In vitro* digestibility from Englyst method was also evaluated. Legume starches had the highest amylose content and the most stable chemical structure. Their rapidly digestible starch and starch digestibility rate index were very low, similar to PS, and 5-fold lower than RS. They had a much higher slowly digestible starch content than PS. Legume starches showed the highest gel stability versus heating and stirring and an intermedium pasting temperature between RS and PS. They formed viscoelastic gels at 6% concentration with stronger elastic-like behavior and higher yield stress than references. Our results indicate these legumes represent an efficient starch source to provide tailor-made properties to food/industrial applications.

Keyword: legume starch; gel rheological properties; *in vitro* starch enzymatic hydrolysis; DSC, TGA, structural properties

1. Introduction

Starch is a macro-constituent in many raw materials and processed foods with particular importance from both nutritional and functional point of view. The role of starch in many formulated products is as texture modifier, contributing in many quality aspects of processed foods (Villanueva et al., 2018a). Starch selection for industrial use is made by considering its availability and its physicochemical characteristics and quantitative and qualitative make-up, which vary depending on the source of starch. The starches from cereals (corn, wheat and

various types of rice), tubers or roots (potato and tapioca) are frequently used by industries; however, the continuous expansion of this market demands new starches sources, with novel properties or additional applications. On the other hand, the global market for starch is projected to reach US\$ 75.4 billion by 2022 from a forecast of US\$ 53 billion in 2016 for the global and regional markets including North America, Europe, Asia-Pacific, Latin America and rest of world (Research & Markets, 2016).

Legumes are a potential source of starch with interesting features, little exploited up to now. The species of legumes like pigeon pea (PP; *Cajanus cajan*s) and dolichos bean (DB; *Dolichos lab-lab*) are cultivated in East and South Africa, East and South Asia and South America (Kaur & Shandu, 2010). In the north-east of Argentina, these legumes are cultivated by small and medium farmers and are primarily used for human consumption. PP and DB present a starch content of 49–50% and different amylose/amylopectin ratios (Acevedo et al., 2013). In general, legumes promote slow and moderate postprandial glucose and insulin responses, with low glycemic index values, which are quite relevant characteristics for patients diagnosed with diabetes, some types of cancer, colon disease, obesity and cardiovascular diseases (Nayak et al., 2014).

Rheological and thermal properties as well as nutritional and structural characteristics determine the potential application of a starch. Several works were developed to increase the potential use of legume starches from India (Singh et al., 2008; Kaur & Shandu, 2010) or Nigeria (Agunbiade & Longe 1999). However, to best of our knowledge, the characteristics of DB (DBS) and PP (PPS) starches from South American cultivars have never been studied so far. Thus, the aim of this paper was to study the structural, physicochemical, *in vitro* digestibility, thermal behavior (pasting profiles, phase transitions and thermal mass loss) and rheological properties of the gels made from starches isolated from Argentinean legumes in order to establish their technological and nutritional functionality. Rice and potato starches were used as references and analyzed in parallel to the legume starches.

2. Materials and methods

2.1 Materials

Pigeon pea (PP) (*Cajanus cajan*s L.) and dolichos bean (DB) (*Dolichos lab lab* L.) were supplied by the “Estación Experimental El Sombrero-Corrientes” (Instituto Nacional de Tecnología Agropecuaria-INTA) Argentina. Shrunken, discolored and insect infested legumes seeds were eliminated, sun dried and then the legumes were kept in a hermetic vessel stored at 10 °C until use. The commercial rice starch (RS) and the commercial potato starch (PS) were provided by Beneo-Remy n. v. (Leuven-Wigjmaal, Bélgica) and Ferrer Alimentation SA (Barcelona, Spain), respectively.

2.2 Starch isolation and its proximal analysis

Legume starches were extracted by using the method of Schoch and Maywald (1968). Samples were hermetically stored in polyethylene bags. Moisture content (Method 925.10), ash (Method 923.03), fat (Method 920.39) and protein content (Method 920.87) were measured with the standard methods of AOAC (AOAC, 1990). Amylose content was determined with the Megazyme assay kit (Megazyme International Ireland Ltd., Bray, Ireland).

2.3 Morphological properties

Dried, finely ground starch samples were mounted on an aluminum stub with double-sided tape and coated with a thin film of gold. The samples were observed under a scanning electron microscope (model JEOL, 5800 LV, Tokyo, Japan) at 15 kV.

2.4 X-ray diffraction

The diffraction assessment was developed using the method described by Villanueva et al. (2018b).

2.5 *In vitro* digestibility of starch

In vitro starch digestibility was measured according to Englyst et al. (1992) with modifications (Abebe et al., 2015). The glucose released at 20 min (G_{20}) and 120 min (G_{120}) and the total glucose (TG) were determined by the glucose oxidase colorimetric method. Rapidly digestible starch $RDS=0.9 \cdot (G_{20}-FGS)$, slowly digestible starch $SDS=0.9 \cdot (G_{120}-G_{20})$, resistant starch $ReS=0.9 \cdot (TG-G_{120})$, total starch $TS=0.9 \cdot (TG-FGS)$ and rapidly available glucose $RAG=G_{20}$ were calculated. Starch digestion rate index (SDRI), which expresses the amount of RDS in the sample as a percentage of the TS content, was also computed. Each sample was measured at least in triplicate.

2.6 Differential scanning calorimetry (DSC)

Thermograms of starch samples were determined using a differential scanning calorimeter (DSC-822e Mettler Toledo AG, Schwerzenbach, Switzerland) following the method described by Villanueva et al. (2018b). The retrogradation of starch was evaluated in the samples previously gelatinized and stored in the DSC pans for 7 days at (4 ± 2) °C following the same temperature scan. The enthalpy (ΔH), onset temperature (T_o), end-set temperature (T_e), peak temperature (T_p) and the difference T_e-T_o (ΔT) were established in both scans, at 0 and 7 days. Samples were run in duplicate.

2.7 Thermoviscous tests: Pasting profiles

Samples (6 g starch/100g) were analyzed following Acevedo et al. (2013) using a Kinexus Pro+ rheometer (Malvern Instruments Ltd, Malvern, UK) supplied with starch pasting cell

and controlled by rSpace software. The starch concentration was selected after determining that it was above the least gelation concentration (Sathe et al., 1981). Parameters recorded were pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), final viscosity (FV), breakdown viscosity (BV) and setback viscosity (SV).

2.8 Thermogravimetric analysis (TGA)

Thermogravimetric analyses were conducted with a TGA/SDTA-851e (Mettler Toledo, Schwerzenbach Switzerland) from 50°C to 600°C at a heating rate of 10°C/min under a nitrogen flow of 60 mL/min. A final isothermal step at 600 °C for 5 min under an air flow of 60 mL/min was performed. Samples (~10 mg) were weighed into aluminum oxide pans. Each run was repeated at least twice.

2.9 Rheological properties: Viscoelastic and flow behavior of starch gels

Flow and dynamic tests of starch gels were performed with Kinexus-Pro+ rheometer (Malvern Instruments Ltd, Malvern, UK) with parallel plate geometry (40mm diameter) of serrated surface and with 1mm of working gap. The gel samples were made following the same procedure described for thermoviscous tests. Just at the end of the pasting test the gels were removed from the canister and placed between the plates, the sample excess was removed and the sample was left to rest for 5 min to allow relaxation. Frequency sweeps were carried out from 20 to 0.1Hz in the linear viscoelastic region (LVR). The limit of the LVR (τ_{\max}) was determined according to Acevedo et al. (2013). Frequency sweep data were fitted to potential equations as described by Acevedo et al. (2013). The coefficients of the potential equation G'_l , G''_l , and $(\tan \delta)_l$, represent the elastic and viscous moduli and the loss tangent at a frequency of 1 Hz. The a , b and c exponents quantify the dependence degree of dynamic moduli and the loss tangent with the oscillation frequency.

Flow tests were carried out at 25°C and shear rates in the range 1 to 500 s⁻¹. To establish gels thixotropy shear rate cycles (up-down) were carried out and between the up and down phases, the maximum shear rate (500s⁻¹) was applied for 2 min. The thixotropy degree of the gels was established from the area of the hysteresis loop. The experimental data from both, the up and down shear rate scans, were fitted to Herschel-Bulkley model:

$$\tau = \tau_0 + K \cdot \gamma^n$$

Where: τ =shear stress (Pa), γ =shear rate (s⁻¹); τ_0 =yield stress (Pa), K =consistency coefficient (Pa·sⁿ) and n =flow behaviour index. Samples were measured in duplicate.

2.10 Statistical analysis

In order to check the statistical importance of observed variability, one-way ANOVA was applied and the least significant difference (LSD) was established at the importance level $p=0.05$. Statistical analysis was performed by using Statgraphics Centurion v.16 (Bitstream, Cambridge, MN, USA) software.

3. Results and discussion

3.1 Chemical composition of starch

The yield and the chemical composition of starches are shown in Table 1. The yield of DBS (35.3%) was lower than that of PPS (38.2%). Singh et al. (2004) reported the yield of several legume starches ranging from 18 to 45%. These differences could be associated to species and cultivars, starch granule associations with other constituents, physiological state of the seed, and the estimation methods (Wani et al., 2016). Legumes showed intermedium water contents between those of RS (9%) and PS (14%). Differences in the equilibrium water content of starches indicate the diversity in their chemical composition and the different number of hydrophilic sites, which influence their water holding ability (Wani et al., 2016). PPS and DBS showed higher values of fat (0.63–1.04%), protein (3.08–4.81%) and ash (1.03–1.23%) contents than RS and PS. These results, in particular the protein content, are related to the extraction method used. PPS showed the highest amylose/starch ratio (26 %) followed by DBS (21%), PS (20%) and RS (15.8%).

Table 1. Yield, chemical composition and starch fractions, TS, RAG and SDRI values of starches.

		PPS	DBS	RS	PS
Yield	(%)	38.24±0.14 ^b	35.32±0.28 ^a	-	-
Moisture	(%)	12.00±0.03 ^c	11.76±0.04 ^b	9.92±0.01 ^a	14.90±0.01 ^d
Fat	(% dm)	1.04±0.03 ^d	0.63±0.02 ^c	0.42±0.02 ^b	0.15±0.01 ^a
Ash	(% dm)	1.03±0.05 ^b	1.23±0.03 ^c	0.21±0.01 ^a	0.32±0.02 ^a
Protein	(% dm)	4.81±0.17 ^c	3.08±0.42 ^b	0.56±0.02 ^a	0.20±0.01 ^a
Amylose	(% starch)	26.24±1.07 ^c	21.18±0.13 ^b	15.81±1.18 ^a	9.60±0.46 ^b
TS	(% dm)	86.4±3.5 ^a	89.5±2.2 ^{ab}	98.3±1.9 ^b	95.8±2.8 ^{ab}
RDS	(% dm)	9.9±2.2 ^a	10.8±1.2 ^a	54.5±1.5 ^b	7.3±0.5 ^a
SDS	(% dm)	23.6±2.3 ^{bc}	19.9±2.3 ^b	28.0±1.7 ^c	7.4±0.8 ^a
R _e S	(% dm)	54.1±1.9 ^b	57.7±2.1 ^b	17.6±2.5 ^a	81.4±3.6 ^c
RAG	(% dm)	10.3±2.5 ^a	12.0±1.4 ^a	60.6±1.7 ^b	8.1±0.5 ^a
SDRI	(%)	11.4±0.9 ^b	12.1±1.2 ^b	55.4±2.0 ^c	7.6±0.4 ^a

PPS: pigeon pea starch, DBS: dolichos bean starch, RS: rice starch, PS: potato starch. dm: dry matter.

TS: total starch, RDS: rapidly digestible starch, SDS: slowly digestible starch, R_eS: resistant starch, RAG: rapidly available glucose, and SDRI: starch digestion rate index

Data are the mean ± standard deviation. Values with a letter in common in the same row are not significantly different (p<0.05)

3.2 Morphological properties

PPS and DBS, showed oval/elliptical shape and smooth surface, similar to PS (Figure S1). However, legume starches had remnants of protein matrix not present in PS due to the extraction method used. RS showed the well-known polyhedral morphology with the smallest granule size ($4.81 \times 4.51 \mu\text{m}$) while PPS and DBS showed an intermediate size ($14.4 \times 13.4 \mu\text{m}$ and $19 \times 14.3 \mu\text{m}$, respectively). PS presented the greatest heterogeneity of granule sizes ($46.5 \times 31.3 \mu\text{m}$ and $15.3 \times 13.8 \mu\text{m}$).

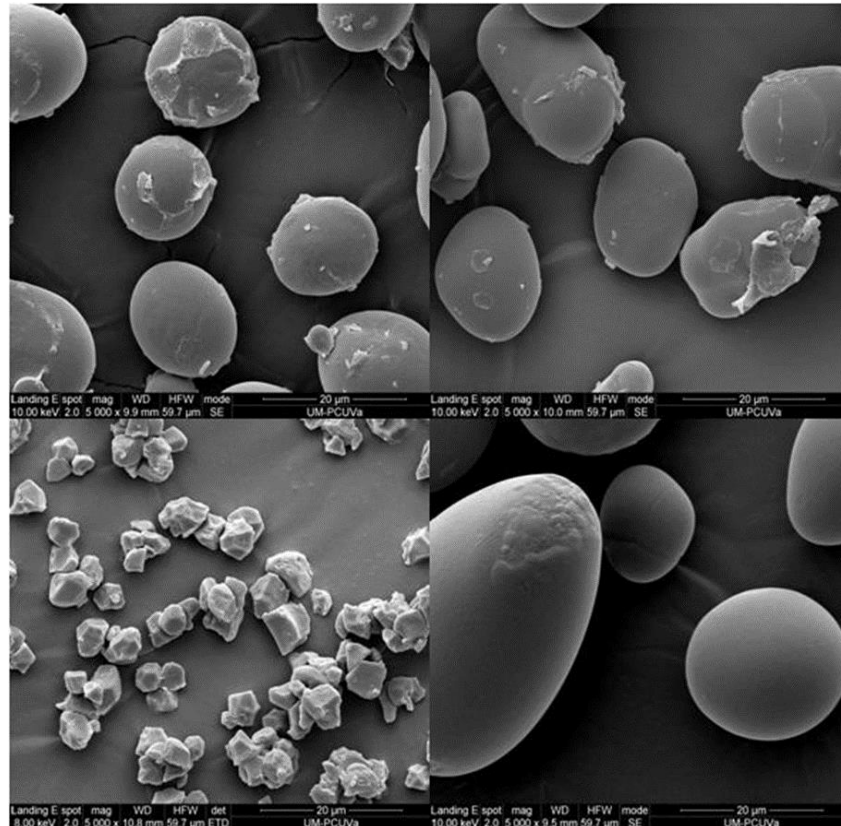


Figure S1. SEM pictures of starches at magnifications of 5000x. A) pigeon pea starch, B) dolichos bean starch, C) rice starch, D) potato starch.

3.3 X-Ray Diffraction

PPS and DBS showed C-type (mixture of A and B-type) diffraction pattern characteristic of legumes, with diffraction peaks at 5.6° , 15° , 17.2° , and 23.2° (Figure S2), consistent with earlier reports for legumes (Kaur & Sandhu, 2010). RS showed the typical A-type pattern ($2\theta = 15.2^\circ$, 17.0° , 18.0° , and 23.1°) while PS presented a B-type pattern ($2\theta = 5^\circ$, 16.7° , 22° and 24°). Both, A and B structures, are composed of ordered arrays of double helices, and the polymorphic variation is based on packing differences of the hexagonally arranged chain duplexes; the B structure is more spacious, accommodating larger amounts of water (Lawal & Adebawale, 2005) compatible with equilibrium water content values informed.

The relative crystallinity measured from diffraction intensity was 54% and 48% for PPS and BDS respectively, lower than that found in RS and PS (64.4% and 55.9%). This is related to the content of amylopectin and the crystal size of the starches (Singh et al., 2008).

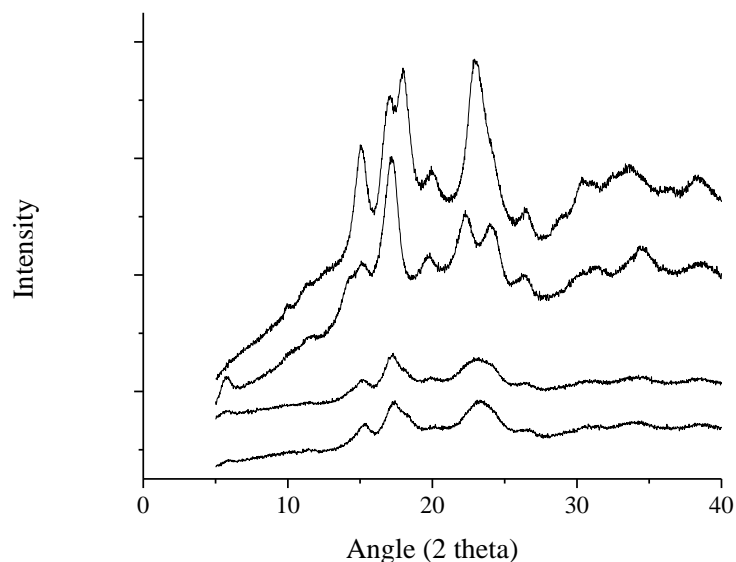


Figure S2. X-ray diffraction patterns of starches. PPS: pigeon pea starch, BDS: dolichos bean starch, RS: rice starch, PS: potato starch.

3.4 Starch fractions analysis

Table 1 summarizes the starch fractions obtained according to their ability to be attacked by digestive enzymes. The minor TS content found in PPS and DBS (86–90%) versus RS and PS (96–98%) agrees with their higher protein and fat contents. The RDS fraction of PPS (10%) and DBS (11%) was significantly ($p \leq 0.05$) lower than RS (55%) and similar to PS (7%). The low RDS content of these legume starches makes them particularly interesting for people suffering from type I diabetes. The larger granule diameter in legumes and potato than in rice starches, as assessed by SEM, would explain their lower specific surface area and their slower hydrolysis kinetics by the action of digestive enzymes. The data is in agreement with predictions that smaller starch granules result in a higher glycaemic index which is highly correlated with RDS content (Englyst et al., 2003). The highest SDS content was also found for RS (28%) followed by PPS (24%) and DBS (20%). The lowest value was for PS (7.4%). SDS is generally the most desirable form of dietary starch and it is completely, but more slowly, digested in the small intestine (Englyst et al., 1992). Legume starches combined the double advantage of having a low RDS content (similar to PS) and a high SDS content (similar to RS). SDS molecular structure is related to amylopectin unit chain length distribution. The resistant starch content (R_eS) found in starches was in the following order: $PS > DBS > PPS > RS$. R_eS has been defined as the sum of starch and the product of starch degradation that are not absorbed in the small intestine, but it is fermented in the large

intestine of healthy individuals (Englyst et al., 1992). Total digestibility (TD=RDS+SDS) of starches was 32.8%, 30.7%, 82.5% and 14.7% for PPS, DBS, RS and PS, respectively; RS was highly attacked by digestive enzymes, which could also be related to the small size of the starch granule that increases the specific surface for the substrate-enzyme contact (Abebe et al., 2015). In addition, the A X-ray diffraction pattern of RS also facilitates its enzymatic hydrolysis unlike the type B and C diffraction patterns (Sivoli et al., 2007). RAG and SDRI showed the same tendency than RDS (Table 1). Free glucose content of all starch samples was always below the detection limit.

3.5 Differential scanning calorimetry

Gelatinization and retrogradation thermal parameters of starches are shown in Table 2. PPS, DBS and RS showed similar values of $T_{p_{gel}}$ (67.6°C, 67.9°C and 67.1°C respectively) higher than PS (63.5°C), in agreement with the thermal stability of the starches measured by TGA. Differences in this transition temperature may be attributed to the degree of crystallinity of starch granules, which provides structural stability and makes the granule more resistant towards gelatinization (Ronda & Roos, 2008). An inverse relationship between starch gelatinization temperatures and short length chain amylopectin in starches from different Indian wheat varieties was reported by Singh et al. (2010). It seems that short chains of amylopectin are not able to form stable double-helical structures because of their shorter lengths, being easily disrupted by the heat at lower temperature. DBS exhibited the widest gelatinization peaks, with the highest ΔT_{gel} values (18°C), evidencing its major heterogeneity of crystalline regions within the starch granule (Kaur & Shandu, 2010). PPS and DBS had gelatinization enthalpies of 10.6 and 12.5 J/g, similar to RS but significantly lower than PS (16.9 J/g). This suggests that adjacent amylopectin chains are more strongly associated within PS native granule (Hoover et al., 1997). Starches with longer branch chain length, such as potato, display larger enthalpy changes, indicating larger amounts of energy were needed to gelatinize crystallites of longer chain lengths (Singh et al., 2010).

Starch retrogradation after storage of gelatinized samples at 4°C for 7 days was studied. The melting temperature of the recrystallized amylopectin decreased from 11.5°C to 3.5°C with respect to the initial gelatinization temperature of the samples, suggesting that smaller and/or lesser perfect crystalline regions were formed during storage (Ronda & Roos, 2008). PPS and DBS showed melting enthalpies of recrystallized amylopectin (8.1–7.4 J/g), similar to PS and significantly higher than RS (1.9 J/g), denoting a higher degree of retrogradation in legume than in rice starches after 7 days of storage. ΔH_{ret} reflects the thermal energy associated with crystallite melting and dissociation/unraveling of double helices formed during storage, influenced by the amylopectin chain length distribution (Singh et al., 2010). Additionally, RS presented a reversible second endotherm in both, gelatinization and retrogradation scans, at a temperature close to 97°C and with an enthalpy of 2.4 and 2.5 J/g starch, respectively. The peak appeared at the same temperature and with similar enthalpy than in the first/gelatinization scan. This peak has been related to the amylose–lipid complex

dissociation that happens at this temperature when the water content is high enough (>60–70 g/100g) (Villanueva et al., 2018b). This transition was not found in PS or either in the legume starches, which confirms the absence of monoacyl lipids in these starches. Singh et al. (2010) registered cooling cycles at -10°C/min after the heating scans of wheat starches and found exothermic peaks related to the amylose-lipid complex reassociation. Subsequent heating cycles showed an increase of ~5°C in the amylose-lipids dissociation temperature attributed to structural changes in amylose-lipids as result of successive heating/cooling cycles resulting in more stable crystalline polymorphs that dissociated at higher temperatures than amorphous amylose-lipids complex, more susceptible to degeneration. In our case, the dissociation temperature of the complex did not vary between successive cycles, demonstrating that the reassociation did not occur in a more stable crystalline structure.

Table 2. Temperatures and enthalpies of phase transitions (from DSC tests) and pasting properties (from thermoviscous tests) of the starches.

		Sample			
		PPS	DBS	RS	PS
To	(°C)	61.41±0.13 ^c	59.78±0.32 ^a	60.51±0.11 ^b	59.80±0.09 ^a
Tp	(°C)	67.56±0.16 ^{bc}	67.92±0.46 ^c	67.12±0.01 ^b	63.54±0.01 ^a
Te	(°C)	74.91±1.70 ^b	77.65±1.74 ^c	73.94±0.01 ^b	69.10±0.21 ^a
ΔT _{gel}	(°C)	13.50±1.83 ^a	17.87±1.72 ^b	13.43±0.10 ^a	9.31±0.30 ^a
ΔH _{gel}	(J/g)	10.58±0.81 ^a	12.47±0.57 ^b	11.27±0.75 ^{ab}	16.91±0.28 ^c
Tp _{am-lip}	(°C)	nd	nd	97.09±0.02	nd
ΔH _{am-lip}	(J/g)	nd	nd	2.41±0.16	nd
Tp _{ret}	(°C)	57.96±0.77 ^b	56.36±0.76 ^b	52.12±0.04 ^a	60.11±0.54 ^c
ΔT _{ret}	(°C)	30.27±1.50 ^b	24.09±1.70 ^{ab}	19.16±0.24 ^a	23.97±3.20 ^{ab}
ΔH _{ret}	(J/g)	8.07±0.13 ^b	7.41±0.79 ^b	1.93±0.53 ^a	9.21±0.14 ^b
PV	(mPa·s)	638±4 ^b	854±7 ^c	554±5 ^a	5779±57 ^d
TV	(mPa·s)	638±4 ^b	854±7 ^c	425±7 ^a	1667±102 ^d
BV	(mPa·s)	0±0 ^a	0±0 ^a	130±4 ^b	4112±45 ^c
FV	(mPa·s)	862±12 ^a	1018±7 ^b	863±2 ^a	2397±21 ^c
SV	mPa·s	224±8 ^a	164±14 ^a	445±1 ^b	730±81 ^c
PT	(°C)	83.9±0.2 ^c	80.6±0.4 ^b	91.2±1.1 ^d	67.9±0.6 ^a

PPS: pigeon pea starch, DBS: dolichos bean starch, RS: rice starch, PS: potato starch. All enthalpy values are referred to weight of dry matter.

To, Tp, Te: onset, peak and endset gelatinization temperatures; ΔT_{gel}: gelatinization temperature range (Te – To), ΔH_{gel}: enthalpy of gelatinization, Tp_{am-lip} and ΔH_{am-lip}: peak temperature and enthalpy of amylose-lipid complex dissociation measured during the gelatinization scan; Tp_{ret} and ΔH_{ret}: peak temperature and melting enthalpy of recrystallized amylopectin measured in the second scan (after 7 days of storage); ΔT_{ret}: retrogradation temperature range.

PV: peak viscosity, TV: trough viscosity, BV: breakdown viscosity, FV: final viscosity, SV: setback viscosity, PT: pasting temperature

Data are the mean ± standard deviation. Values with a letter in common in the same row are not significantly different (p<0.05).

3.6 Thermoviscous tests: Pasting profiles

Viscometric profiles are shown in Figure S3 and characteristic parameters are summarized in Table 2. Pasting properties of starch are affected by amylose, lipid, phosphorus contents and by branch chain-length distribution of amylopectin (Jane et al., 1999), as well also by starch granule size (Singh et al., 2010). Furthermore, the amylopectin chain-length and amylose molecular size produce synergistic effects on the viscosity of starch pastes (Jane and Chen, 1992). Viscometric profiles of PPS and DBS were both similar and significantly different from RS and PS. The PT of starches followed the order: PS(68°C)<DBS(81°C)<PPS(84°C)<RS(91°C), indicating that the resistance of legume starch granules to swell was intermediate between cereal and tuber starches. The low PT value of PS was related to the characteristic larger granule size and the absence of lipids and phospholipids of tuber starches compared to cereals and legumes (Jane et al., 1999; Singh et al., 2010). The PV followed the opposite order to PT: PS(5779mPa·s) > DBS(854mPa·s) > PPS(638mPa·s) > RS(554mPa·s), and increased in the same order than the granule size of starches, consistent with that observed by Singh et al. (2010). They demonstrated a positive correlation between pasting viscosities and the proportion of large size granules (>15 µm), and a negative correlation between pasting viscosities and the content of small granules (< 5 µm) in wheat starches of different Indian varieties. The presence of high phosphate monoester content and the absence of lipids and phospholipids in potato starch (Singh et al., 2004) as well as the high degree of reticulation of its structure may explain the great viscosity developed during pasting (Villanueva et al., 2018a). The lowest PV value of RS could be related to their major content of lipids and phospholipids linked to amylose and/or to the longer chains of amylopectin that restrict swelling of granules (McPherson & Jane, 1999). PPS and DBS showed not well-defined peaks with PV values higher than RS ($p < 0.05$) and showed the highest gel stability versus heating and stirring with BV values equal to zero (Figure S3). A low BV value is usually related to less disintegration of swollen granules or granule rigidity, starch purity and high lipid content (Singh et al., 2004). PPS and DBS showed also the lowest SV value while PS had the highest one. SV is mainly related to the amylose leached from starch granules and their tendency to reorganize after gelatinization (Abebe et al., 2015). However, the low SV value of PPS and DBS cannot be attributed to low amylose content (see Table 1) nor to the formation of the lipid-amylose complex, which would hinder its retrogradation, since DSC tests showed that this complex was not formed in these starch legumes (see Table 2). These results confirm that other factors should also affect the amylose entanglement extent and explain the limited increase in paste viscosity during the cooling phase of legume starch gels. Other authors have also suggested that amylose associations are not the only factor responsible for setback (Singh et al., 2010).

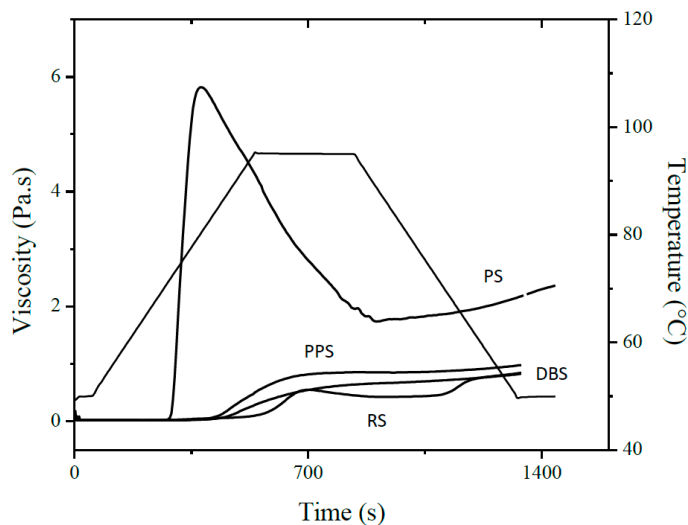


Figure S3. RVA curves of starches. PPS: pigeon pea starch, DBS: dolichos bean starch, RS: rice starch, PS: potato starch.

3.7 Thermogravimetric analysis

Figure S4 shows the derivative curves of weight loss (DTG) obtained from the mass loss versus temperature curves (TG trace) as result of the drying and thermal decomposition of starches. All starches showed a typical two-step weight loss pattern. The first stage corresponds to physical dehydration of starch while the second stage involves chemical dehydration and thermal decomposition. First mass loss (7%) took place in the range 49–182°C for all samples corresponding to the water loss by evaporation. The inflection point was close to 73°C for PPS, DBS and RS and 76.5°C for PS which means that water is more strongly bound in potato starch. The second and main step of mass loss occurred between 171°C and 598°C with an inflection point at 306°C (71.9% mass loss) for PPS and 310°C (72.4%) for DBS. The drastic loss in mass is due to the production of volatile compounds that could start with thermal condensation between hydroxyl groups of starch chains to form either segments and liberation of water molecules and other small molecular species and the formation of more carbonaceous residues (Malumba et al., 2016). Main degradation stage itself is quite complex, and may contain one sub-stage that could be defined by the appearance of a “shoulder” in the peak of DTG trace. RS and PS showed a shoulder, absent in legumes starches, which could represent two overlapping steps of thermal degradation. RS showed two inflection points at 290°C (9.63% mass loss) and 311°C (66.3%) and PS at 278°C (1.51%) and 302°C (67.9%). Therefore, PS chemical structure is thermally less stable than PPS, DBS and RS which could be related to the high content of phosphate groups in potato starch (Hoover, 2001).

The last degradation stage obtained at 600°C under oxidant conditions, attributed to the total oxidation of the organic matter (data not shown), led to similar mass losses for legume and

tuber starches (15.1–15.5%, for PPS, DBS and PS) that were higher than that found in RS (12.3%).

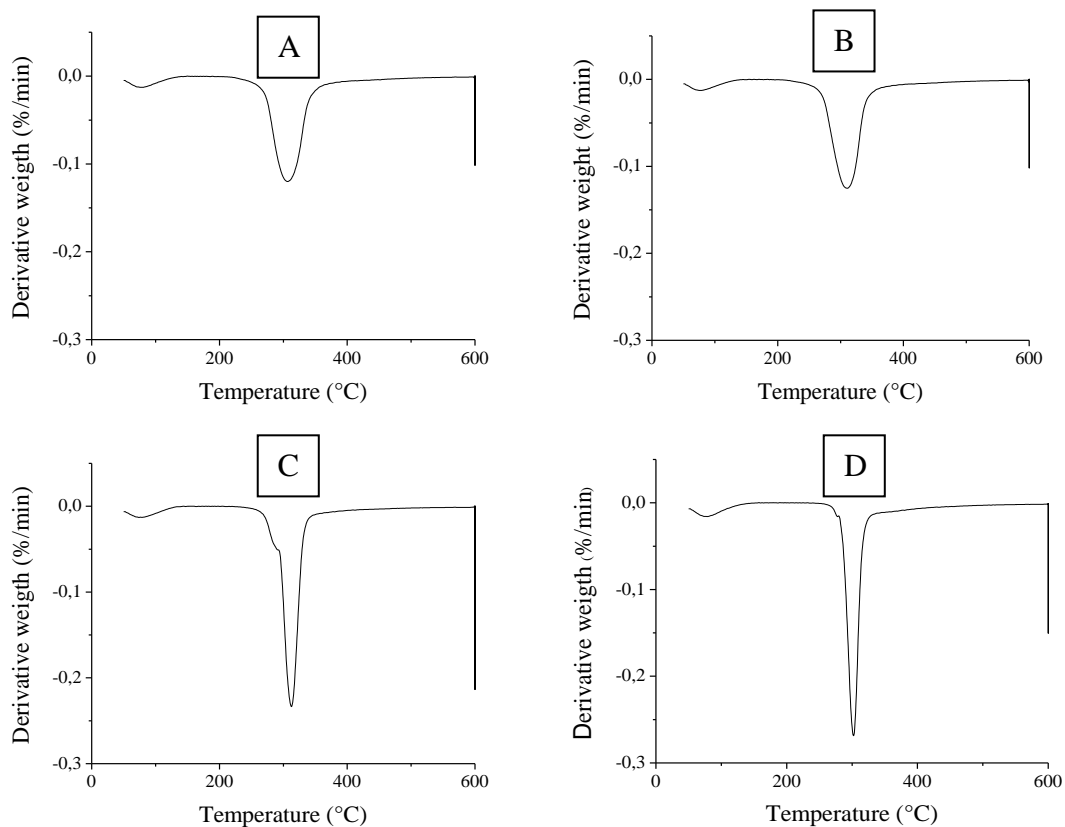


Figure S4. Derivative curves of weight loss (DTG) obtained from the mass loss versus temperature curves (TG trace). A: pigeon pea starch, B: dolichos bean starch, C: rice starch, D: potato starch.

3.8 Rheological properties of starch gels

Rheological parameters related to the viscoelastic and flow behavior of gels prepared with PPS, DBS, RS and PS are summarized in Table 3. PS gels presented the highest maximum stress value ($\tau_{\max}=252$ Pa) within the linear viscoelastic region denoting the greatest resistance to the rupture, similar to that of DBS gels (217 Pa), followed by PPS (95 Pa) and RS (48 Pa) gels. The viscoelastic behavior of gels formed was determined from frequency sweep assays. For all starches studied, the elastic modulus (G') resulted in higher values than the viscous modulus (G'') providing values of $\tan\delta\leq 1$, suggesting the solid elastic-like behavior of gels. The G'_I values of legumes starches were higher than references, PS and RS gels, indicating more structural strength of the formed network. Other authors also found that starches with lower amylose content, as happened in rice starch and particularly in potato starch, had weaker gel behavior (Singh et al., 2007). The higher increment in G' in starches with higher amylose content, as legumes, may be explained by the fact that amylose chains start to fold and crystallize, triggering the formation of a polymer network, while external

chains of high amylopectin starch will fold and reorder slowly (Singh et al., 2007). PPS and DBS showed also the lowest $\tan \delta$ (0.134 and 0.085) indicating stronger elastic gel behavior than RS (0.267) and PS (0.339).

Table 3. Rheological properties: Viscoelastic behaviour (from oscillatory tests) and flow properties (from steady tests) of starch gels (at 6% concentration). The values from oscillatory tests represent the fitting parameters of frequency sweep experimental data to the power law model ($G'(\omega)=G'_1 \cdot \omega^a$; $G''(\omega)=G''_1 \cdot \omega^b$; $\tan \delta(\omega)=(\tan \delta)_1 \cdot \omega^c$). τ_{\max} was obtained from stress sweeps. The flow properties represent the fitting parameters of shear rate scans (up and down) to Herschel-Bulkley model ($\tau=\tau_0+k \cdot \gamma^n$) and the hysteresis loop area that quantifies starch gels thixotropy.

		Sample			
		PPS	DBS	RS	PS
<i>Oscillatory tests: Viscoelastic properties</i>					
G'_1	(Pa)	106.25±0.66 ^b	160.90±5.13 ^c	28.60±0.45 ^a	38.02±1.09 ^a
a		0.019±0.004 ^{ab}	0.012±0.006 ^a	0.044±0.008 ^b	0.201±0.008 ^c
R ²		0.9992	0.9998	0.9985	0.9995
G''_1	(Pa)	14.27±0.50 ^b	13.69±0.79 ^b	7.62±0.19 ^a	12.89±0.06 ^b
b		0.103±0.007 ^b	0.099±0.002 ^{ab}	0.087±0.003 ^a	0.298±0.001 ^c
R ²		0.9999	0.9995	0.9988	0.9958
$\tan \delta_1$		0.134±0.004 ^b	0.085±0.002 ^a	0.267±0.011 ^c	0.339±0.011 ^d
c		0.084±0.007 ^b	0.088±0.002 ^b	0.043±0.005 ^a	0.097±0.008 ^b
R ²		0.9955	0.9992	0.9991	0.9981
τ_{\max}	(Pa)	95.38±0.38 ^b	216.65±18.31 ^c	47.80±0.94 ^a	251.71±0.01 ^c
<i>Steady tests: Flow properties</i>					
<i>Upward curve</i>					
t_0	(Pa)	49 ± 4 ^b	89 ± 8 ^c	13 ± 4 ^a	3 ± 3 ^a
K	(Pa·s ⁿ)	1.95 ± 1.10 ^a	1.79 ± 1.20 ^a	12.42 ± 0.04 ^b	59.56 ± 0.73 ^c
n		0.58 ± 0.05 ^b	0.61 ± 0.07 ^b	0.35 ± 0.01 ^a	0.36 ± 0.01 ^a
R ²		0.998	0.996	0.998	0.997
<i>Downward curve</i>					
t_0	(Pa)	15.45 ± 3.8 ^a	31.39 ± 1.2 ^b	7.98 ± 0.4 ^a	14.41 ± 3.6 ^a
K	(Pa·s ⁿ)	1.59 ± 0.04 ^a	3.69 ± 1.25 ^a	1.99 ± 0.17 ^a	19.31 ± 1.54 ^b
n		0.66 ± 0.03 ^c	0.56 ± 0.04 ^{ab}	0.63 ± 0.01 ^{bc}	0.52 ± 0.01 ^a
R ²		0.998	0.999	0.999	0.999
Hysteresis loop area	(Pa·s ⁻¹)	9408±1500 ^a	12751±2100 ^a	11329±1300 ^a	43802±1800 ^b

PPS: pigeon pea starch, DBS: dolichos bean starch, RS: rice starch, PS: potato starch.

Data are the mean ± standard deviation. Values with a letter in common in the same row are not significantly different ($p < 0.05$).

Low values of the a exponent, near zero, for PPS and DBS, mean that the elastic modulus (G'), was not dependent on the frequency, indicating a stable gel structure. The highest dependence of both moduli with frequency was obtained for PS.

The Herschel-Bulkley parameters for upward and downward flow curves of starches are shown in Table 3. The yield stress (τ_0), or stress required to achieve the gel flow, represents the breakdown of their structure under steady shear flow tests. The τ_0 values followed the order: DBS(89Pa)<PPS(49Pa)<RS(13Pa)<PS(3Pa). These results confirm legume starches form stronger gels than RS and PS. Flow behavior indices of all starches were significantly below 1, indicating a real plastic behavior. All the starch gels exhibited hysteresis loops indicating thixotropic behavior. The largest area was observed for PS which seems to be most susceptible to shear time. PPS and DBS had hysteresis loop areas four times smaller than PS, and similar to RS.

4. Conclusion

Legume starches showed nutritional, thermal and rheological properties that make them an attractive option to use in different food systems and industrial applications. PPS and DBS, with the characteristic C-type X-ray diffraction patterns, showed higher amylose contents than RS and PS, similar gelatinization temperature and enthalpy to those of RS, and a retrogradation extent equivalent to that of PS. PPS and DBS exhibited lower RDS than RS and higher SDS than PS. Because of that, these starches would be of potential use for patients with glucidic metabolism disorders. In addition, legume starches showed higher peak viscosity than RS and higher stability versus heating and stirring than RS and PS. Legume starch gels exhibited higher yield stress and higher solid-like behavior than RS and PS. Our results indicate that these legume starches provide alternative properties to that of cereal and tuber starches. Then, they represent an efficient and nontraditional starch source to provide tailor-made properties to food/industrial applications.

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Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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