



Gelation, thermal and pasting properties of pigeon pea (*Cajanus cajan* L.), dolichos bean (*Dolichos lablab* L.) and jack bean (*Canavalia ensiformis*) flours



Belén A. Acevedo^{a,b}, María V. Avanza^a, María G. Châves^a, Felicidad Ronda^{b,*}

^a Faculty of Natural Sciences and Surveying, National Northeast University (UNNE), Av. Libertad 5450, 3400 Corrientes, Argentina

^b College of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid 57, 34004 Palencia, Spain

ARTICLE INFO

Article history:

Received 24 March 2013

Received in revised form 4 May 2013

Accepted 12 May 2013

Available online 18 May 2013

Keywords:

Gels

Legumes

Rheological properties

Thermal properties

Pasting properties

ABSTRACT

This study evaluated the rheological, thermal and pasting properties of pigeon pea (PP), dolichos bean (DB) and jack bean (JB) legume flours and gels. Starch and protein contents were also measured and its molecular weight distribution was determined by electrophoresis. PP and DB showed the highest viscosities while JB had the highest pasting temperature. The minimum flour concentrations for gel formation were estimated at 6–8% for DB and PP and 10% for JB. Above these concentrations all flour suspensions heated to 95 °C led to gels with a solid-like behavior. Differential scanning calorimetry showed two endothermic peaks in all flours at 80–89 °C and 96–100 °C. Avrami model was successfully fitted to the hardening kinetics of PP and DB gels stored at 4 °C. The half-life times were 22 and 6 h for PP and DB respectively. PP and DB flours were able to form self-supporting gels and could be applied in the formulation of gel-like foods.

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1. Introduction

Legumes are members of the Leguminosae that are widely cultivated in the tropics and subtropics areas. The efforts geared towards legumes are predicated on the fact that legumes have been established to be cheap but abundant sources of protein (Adebowale and Lawal, 2004). This explains the growing interest in the study of flours from different types of legumes.

Pigeon pea (*Cajanus cajan* L.) is one of the oldest food crops in India and the country contributes over 90% of the world production. Additionally, it is also a food crop in many other tropical countries and it is commercially important in East Africa, the Caribbean and Latin America (Akande et al., 2010). Dolichos bean (*Dolichos lablab* L.) is an indigenous legume of the arid and semi-arid area of the South and East Asia. Besides, this legume is cultivated in arid lands of Africa, Middle East and South America (Osman, 2007). Jack bean (*Canavalia ensiformis*) is fairly widely distributed, being cultivated in Africa, Asia, the West Indies, Latin America and India (Bressani et al., 1987).

The three legumes studied in this work have a particular nutritional value as they have low concentration of fat, high content of protein and carbohydrate, moderate content of crude fiber and a

reasonably balanced range of all dietary essential minerals, important amino acids (glutamic acid, lysine and tryptophan) and vitamin B and E (Osman, 2007; Kaushal et al., 2012).

Functional properties of legume flours need to be studied as they determine their successful performance as food ingredients (Kaushal et al., 2012). Gelation is one of the most important functional properties. It determines the suitability of incorporation of a particular substance into food products as it affects the capacity of gels in retaining water, lipids, sugars, flavors and other ingredients, besides conferring consistency to the final product (Adebowale and Lawal, 2004).

Protein and starch are the major macromolecules that contribute to the gelation of legume flour. When starch suspensions are subjected to high temperature, its granules swell and rupture due to the disruption of amylopectin double helices (hydrogen bonds dissociation) while amylose preferentially leaches out from the swollen granules (gelatinization). Starch retrogradation, caused by re-crystallization of the polymer (dispersed amylose and amylopectin) chains, occurs in foods when gelatinized starch is cooled down and subsequently stored at low temperature (Biliaderis, 2009). It is important to distinguish between the short-term development of a gel structure via amylose crystallization and long-term reordering of amylopectin which is a much slower process involving recrystallization of the outer branches of this polymer (Morris, 1990; Kalichevsky et al., 1990). On the other hand, protein gelation is a process involving the initial denaturation of proteins

* Corresponding author. Tel.: +34 979108339; fax: +34 979108302.

E-mail addresses: belenapovolo@gmail.com (B.A. Acevedo), vavanza@yahoo.es (M.V. Avanza), chavesgm@yahoo.com.ar (M.G. Châves), fronda@iaf.uva.es (F. Ronda).

into unfolded polypeptides and attractive forces and thermodynamic conditions are then gradually associated to form the gel matrix. Upon cooling, the uncoiled polypeptides associate to make a network. Cross linking may involve multiple hydrogen bonds, ionic attractions, disulfide bonds, hydrophobic associations or a combination of them (Wang and Damoradan, 1991). When all these changes occur, important variations of viscoelasticity and texture of gel can be measured.

Several studies were performed on the gel properties (rheological, texture, thermal and pasting) of rice flours (Kapri and Bhat-tacharya, 2008; Katekhong and Charoenrein, 2012) and wheat flours (Singh et al., 2011). Besides, some studies have been reported on the least gelation concentration (LGC) of legumes flours. Adebowale and Lawal (2004) investigated the effect of pH, ionic strength and addition of carbohydrates on LGC of JB flours, obtaining the best gelation property at pH = 4, 0.1 M of ionic strength and the addition of maltose (25% w/W). Kaushal et al. (2012), reported LGC values of 12% (w/v) for PP flours. However, to our knowledge, there is very scarce information about gel properties of legumes flours, mainly rheological properties. This information is helpful and necessary to incorporate legume flours into existing food formulations in order to modify their functional and nutritional quality, as well as to create new products such as gel-like products.

The aim of the present work was to study the gelation capacities, thermal and pasting properties of pigeon pea, dolichos bean and jack pea flours, and evaluate the viscoelastic and textural properties of the gels.

2. Materials and methods

2.1. Material

Pigeon pea (PP) (*Cajanus cajans* L.), dolichos bean (DB) (*Dolichos lablab* L.) and jack bean (JB) (*Canavalia ensiformis*) seeds were obtained from Estación Experimental “El Sombrero-Corrientes” (Instituto Nacional de Tecnología Agropecuaria-INTA) Argentina, (crop 2011). Shrunken, discolored and insect infested seeds were eliminated, sun dried and then the legumes were kept in a hermetic vessel stored at 10 °C until use.

2.2. Preparation of seed flours

Legumes (with seed coat) were ground in an electric mill (KSM2 Braun, Naucalpan de Juárez, México) and subsequently sieved through 80 ASTM (177 μ m). In the case of JB, seeds were previously crushed to smaller fragments using a mill (F-4 Quaker City, Philadelphia, USA).

2.3. Chemical composition

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). Carbohydrates were determined according to Roe (1955). Starch content was performed using the method of Fraser et al. (1956) and amylose with the Megazyme amylose/amylopectin kit (Megazyme Bray, Ireland). These assays were made in duplicate.

2.4. Sodium dodecil sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

All gels were run in minislabs (Bio-Rad Mini Protean II Model). SDS-PAGE was performed according to Laemmli's method (1970) using continuous gels (12%). Flours samples (1%, w/v) were dissolved in 0.125 M Tris-HCl, pH 6.8, 0.02% (v/v) glycerol, 0.1% (w/

v) SDS, 0.05% (w/v) bromophenol blue, and centrifuged at 15,800 \times g for 5 min at 4 °C. Supernatants were loaded onto the gel (30–40 μ g of protein per lane). Samples to be run under reducing conditions were boiled for 1 min in 0.005% (v/v) 2-mercaptoethanol (2-ME) buffer before centrifugation. Electrophoresis was conducted for 1 h at a constant voltage of 200 V. The following molecular weight standards were used to estimate the molecular masses of polypeptides: phosphorylase b (94 kDa); bovine serum albumin (67 kDa); ovalbumin (45 kDa); carbonic anhydrase (30 kDa); trypsin inhibitor (20.1 kDa); α -lactalbumin (14.4 kDa), (Pharmacia Hepar Inc, Franklin, OH, USA).

2.5. Thermal properties

Thermal characteristics of flours were determined using a differential scanning calorimeter (DSC-822e Mettler Toledo AG, Schwerzenbach, Switzerland) calibrated for temperature and heat flow using Indium. Samples of flour were weighed into medium pressure steel pans (120 μ L). Flour weights were around 12 mg and distilled water was added to make 70% moisture content. The samples were scanned from 25 to 130 °C at 5 °C/min using an empty pan as reference. After heating, samples were cooled at 20 °C/min to 25 °C and reheated under the same previous conditions to assess the reversibility of thermal transitions. Starch retrogradation was evaluated in samples stored in the pans at (4 \pm 2) °C for 7 days. These samples were scanned from 0 to 150 °C at a heating rate of 5 °C/min. The enthalpy (ΔH), onset temperature (T_o) and peak temperature (T_p) of endotherms were measured. The reported values are the means of duplicated measurements.

2.6. Pasting properties

Pasting properties were studied by using Rapid-Visco-Analyzer (RVA) (RVA-4 Newport Scientific Pty Limited, Warriewood, Australia) controlled by the ThermoLine software (Newport Scientific Pty Limited, Warriewood, Australia). Viscosity profiles of flours were recorded using flour suspensions (20%, w/w; 28.5 g total weight). The samples were heated from 50 to 95 °C at 6 °C/min after equilibrium time of 1 min at 50 °C and a holding time of 5 min at 95 °C. The cooling was carried out from 95 to 50 °C at 6 °C/min with a holding for 2 min at 50 °C. Parameters recorded were pasting temperature (PT) (temperature at which starch granules begin to swell and gelatinise due to water uptake), peak viscosity (PV) (maximum paste viscosity achieved), trough viscosity (TV) (minimum paste viscosity achieved after holding at 95 °C), final viscosity (FV) (viscosity at 50 °C), breakdown viscosity (BV) (difference between peak viscosity and trough viscosity) and setback viscosity (SV) (difference between final viscosity and trough viscosity). Each sample was analyzed in triplicate.

2.7. Oscillation measurements

Suspensions of different concentrations (8, 10, 12 and 15%, w/w, of PP and DB flours and 12, 15, 17 and 20%, w/w, of JB flour, with 28.5 g total weight in all cases) were prepared in duplicate by using the RVA. The suspensions were stirred at 160 rpm, heated from 50 to 95 °C at a rate of 6 °C/min and held at 95 °C for 15 min. Dynamic test of the gels were carried out with a RheoStress 1 rheometer (Thermo Haake, Karlsruhe, Germany) with parallel plate geometry (60 mm diameter) of serrated surface and with 1 mm gap. Hot paste from the RVA was quickly transferred to the parallel plates. The excess of suspension was removed and vaseline oil was applied to cover the exposed sample surfaces. Before the measurement, the suspension was rested for 700 s to allow relaxation. Preliminary tests of time sweeps (at 1 Hz and 1 Pa) allowed to assure a stable response after 450 s. Frequency sweeps were

carried out from 10 to 0.1 Hz in the linear viscoelastic region (LVR), previously established for each sample by means of stress sweeps from 0.1 to 1500 Pa at 1 Hz of frequency. The limit of the LVR (τ_{\max}) was located by the decrease of G' modulus above 10%, that coincided with the sudden increase of $\tan \delta$. Samples were characterized by rheological assays at 90 and 25 °C. The RheoWin 4 software (Thermo Haake, Karlsruhe, Germany) was used for data analysis. Each gel was prepared twice and measured in duplicate. Power law model was fitted to frequency sweep data as in previous works (Ronda et al., 2011):

$$G'(\omega) = G'_1 \cdot \omega^a$$

$$G''(\omega) = G''_1 \cdot \omega^b$$

$$\tan \delta(\omega) = \frac{G''(\omega)}{G'(\omega)} = \left(\frac{G''_1}{G'_1}\right)_1 \cdot \omega^c = (\tan \delta)_1 \cdot \omega^c$$

The coefficients G'_1 , G''_1 and $(\tan \delta)_1$ 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 these moduli and the loss tangent with the oscillation frequency.

2.8. Least gelation concentration

Least gelation concentration (LGC) of flours was determined by the method of Sathe et al. (1981). Test tubes containing 5 ml of 4, 6, 8, 10, 12, 14, 16, 18 and 20% (w/w) of flour in distilled water were heated for 1 h in boiling water, followed by rapid cooling under running water. The tubes were further cooled in a water bath at 4 °C for 2 h. LGC is the concentration above which the sample did not fall down or slip when the test tube is inverted.

2.9. Firmness of fresh and aged gels

The firmness of flour gels was evaluated using a TA-XT2 Texture Analyzer (Stable Microsystems, Surrey, UK) equipped with the software Texture Expert. Gels were prepared from a 15% (w/w) suspension for PP and DB flours and 25% (w/w) for JB flour (28.5 g total weight) by using the RVA. The suspensions were stirred at 160 rpm, heated from 50 to 95 °C at a rate of 6 °C/min and held for 15 min at 95 °C, followed by rapid cooling under cold running water (1 min). The tubes were further cooled in a water bath at 4 °C for 2 h. Subsequently, samples were stored at (4 ± 2) °C for 0, 2, 24, 48, 96 and 192 h in order to evaluate the gel hardening kinetics. A compression test with an aluminum 75 mm diameter (SMSP/75) probe was used. The force to penetrate to 50% depth, at 1 mm/s was recorded. Gels (2 cm diameter and 2 cm of height) were analyzed at (20 ± 2) °C. The Avrami equation (Ronda and Roos, 2011) was fitted to gel firmness kinetics data:

$$\frac{F_\infty - F_t}{F_\infty - F_0} = e^{-kt^n}$$

where F_∞ is the leveling-off value of firmness, obtained at infinite time, F_0 is the firmness at initial time, F_t is the firmness at time t , k is a constant of velocity and n is the Avrami exponent. The values of the constants k and n were used to calculate the value of half-life, $t_{1/2}$, which is defined as the time required to achieve 50% of leveling-off extent of firmness (Ronda and Roos, 2011):

$$t_{1/2} = \left(-\frac{\ln 0.5}{k}\right)^{1/n}$$

Half-life time gives an idea of the rate of the gel firmness change from its initial value, F_0 , to the leveling-off point, F_∞ . Fitting of the Avrami equation to the data was carried out using a least squares minimization, fitting routine with four adjustable parameters (F_0 ,

F_∞ , k , n) with help of the “solver” tool of the software Excel (Microsoft Corporation, Washington, USA) to minimize the sum of squared residuals.

2.10. Statistical analysis

Experimental data were analyzed using one-way analysis of variance (ANOVA) followed by the least significant difference test LSD Fisher ($\alpha = 0.05$). Statistical analysis was performed using InfoStat software (InfoStat Group, Córdoba, Argentina).

3. Results and discussion

3.1. Proximate composition

The composition of flours is reported in Table 1. The protein content of the three legume flours were significantly different ($p < 0.05$), varying from 23 to 32.4% in dry basis (db) and following the order: JB > DB > PP. These values are in the range of those reported by other authors (Sridhar and Seena, 2006; Osman, 2007; Akande et al., 2010). The highest ash and the lowest fat contents were observed in DB flours. The highest carbohydrate content was obtained for JB flour ($62 \pm 1\%$) (db) and the lowest for DB flour ($50 \pm 1\%$) (db); however, JB flour had the lowest starch content. Amylose contents in starch ranged from 14.2 to 23.2% representing 11.6%, 7% and 4.8% of amylose in PP, DB and JB flours (db) respectively.

3.2. Protein characterization

The SDS-PAGE of JB, DB and PP flours, obtained under reducing and non-reducing conditions, are presented in Fig. 1. High molecular weight aggregates were observed under non-reducing conditions, in all flours (arrow 1, Fig. 1A). Under reducing conditions, a number of bands of elevated molecular weights appeared (Fig. 1B) indicating that the aggregates were formed by intermolecular interactions through disulfide bridging. Under non-reducing conditions, polypeptides of 58–60 kDa (arrow 2) and 45 kDa (arrow 3) were observed in the three flours. These polypeptides were not dissociated by 2-ME reducing agent in DB and PP flours. On the contrary, the reduction of 58–60 kDa polypeptide was observed in JB flour (arrow 4, Fig. 1B). In the same conditions, a polypeptide of 94 kDa (arrow 5) was observed in JB flour in greater proportion than in DB flour, being absent in PP flour. In 2-ME presence this band disappeared in JB flour, denoting the presence of disulfide bridges and evidencing the different nature of JB polypeptides with respect to the other two legumes. The main polypeptides present in most legumes, 7 s (45 and 58–60 kDa) (Tang, 2008) and 11 s globulins (60 kDa) (Meng and Ma, 2001), were observed in different ratios in the studied flours.

3.3. Thermal properties

The DSC thermograms of the three studied flours exhibited two endothermic peaks of different characteristics (Fig. 2A). JB showed two clearly differentiated peaks at (89 ± 1) °C and (100 ± 1) °C with transition enthalpies of (6.6 ± 0.3) J/g of solids and (3.1 ± 0.1) J/g of solids respectively. PP presented a peak at (83.6 ± 0.1) °C with a transition enthalpy of (9.0 ± 0.4) J/g of solids and a very small peak, hardly visible, at (96 ± 2) °C with a transition enthalpy of (1.7 ± 0.1) J/g of solids. Kaur et al. (2007) reported a single peak for PP flour at 82 °C with a transition enthalpy of 4.79 J/g. The presence of the second peak in our work, could be attributed to the different geographic origin of these legumes. DB flour had the first peak at the lowest temperature, (80 ± 2) °C, and a transition enthalpy of

Table 1
Chemical composition of legume flours (% on dry basis, except amylose).

Flour	Moisture (%)	Proteins (% w/w)	Ash (% w/w)	Fat (% w/w)	Carbohydrates (% w/w)	Starch (% w/w)	Amylose (% of starch)
PP	10.2 ± 0.1a	23.0 ± 0.1a	3.44 ± 0.02a	3.35 ± 0.01b	56 ± 2b	50.0 ± 0.1b	23.2 ± 0.1c
DB	11.6 ± 0.1b	29.0 ± 0.6b	4.95 ± 0.02b	1.62 ± 0.01a	50 ± 1a	49.3 ± 0.3b	14.2 ± 0.2a
JB	10.3 ± 0.1a	32.4 ± 0.1c	3.49 ± 0.04a	5.38 ± 0.02c	58 ± 1b	35.7 ± 0.4a	17.0 ± 0.1b

PP: pigeon pea, DB: dolichos bean, JB: jack bean.

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

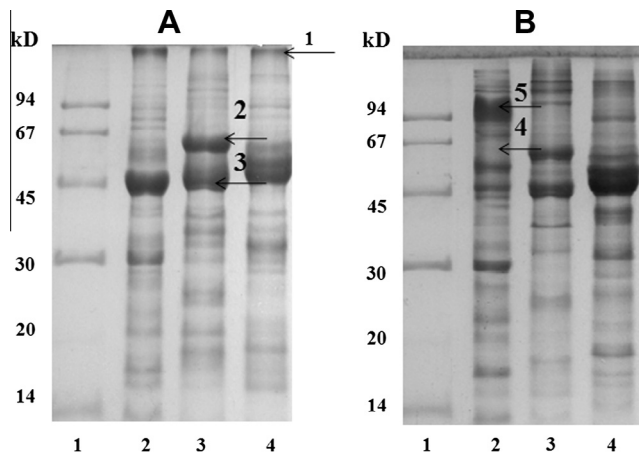


Fig. 1. SDS-PAGE electrophoresis of flours. (A) non-reducing conditions; and (B) reducing conditions. (1) Molecular weight standards; (2) JB: jack bean flour; (3) PP: pigeon pea flour; (4) DB: dolichos bean flour. Arrow 1: high molecular weight aggregates; Arrow 2: 58–60 kDa; Arrow 3: 45 kDa; Arrow 4: 58–60 kDa; Arrow 5: 94 kDa.

(7.7 ± 0.8) J/g of solids, and the second peak at a similar temperature to the rest of flours (98 ± 4)°C, with a transition enthalpy of (3.3 ± 0.9) J/g of solids. The differences observed in flour thermal transitions may be attributed to differences in several aspects such as, size, shape and distribution of starch granules in the flours, amylose:amylopectin ratio, internal arrangement of starch fractions within the granule, different protein fractions and the interaction between starch, protein and lipids (Liu et al., 2006). The peak that appeared at the lowest temperature could be attributed to changes involved in starch gelatinization, denaturation of proteins and changes associated to protein-starch interactions (Henshaw et al., 2003). In DB and PP flours, the first thermal transition occurred in a wide temperatures range, while a narrow peak was observed in JB flour (Fig. 2A). This was probably due to an overlap of transitions (related to starch gelatinization and denaturation of some protein) in DB and PP flours. The second peak, obtained between 95 and 100 °C in all flours, could be attributed to denaturation of proteins like 11 s globulins (Meng and Ma, 2001).

The reversibility of phase transitions within the range of 25–150 °C was investigated by re-scanning the samples. In all cases no thermal transitions were seen in the second scan. After 7 days of storage at 4 °C, flour samples were reheated in the DSC oven to investigate starch retrogradation. Endotherms (Fig. 2B) showed a single peak for all flours. The transition temperatures (peak temperatures) for retrograded starches were found at (63 ± 5) °C, (59.2 ± 0.2) °C and (55.9 ± 0.8) °C for PP, JB and DB, respectively. The transition temperatures were lower than starch gelatinization temperatures in the original flour. Similar data were reported by Liu et al. (2006) in legumes and tubers flours after 2 weeks of storage at 5 °C. This behavior suggests that retrogradation results in reassociation of the gelatinized starch molecules, but in less ordered manner and hence less perfect or stable forms and more heterogeneous in stability than those existing in the native

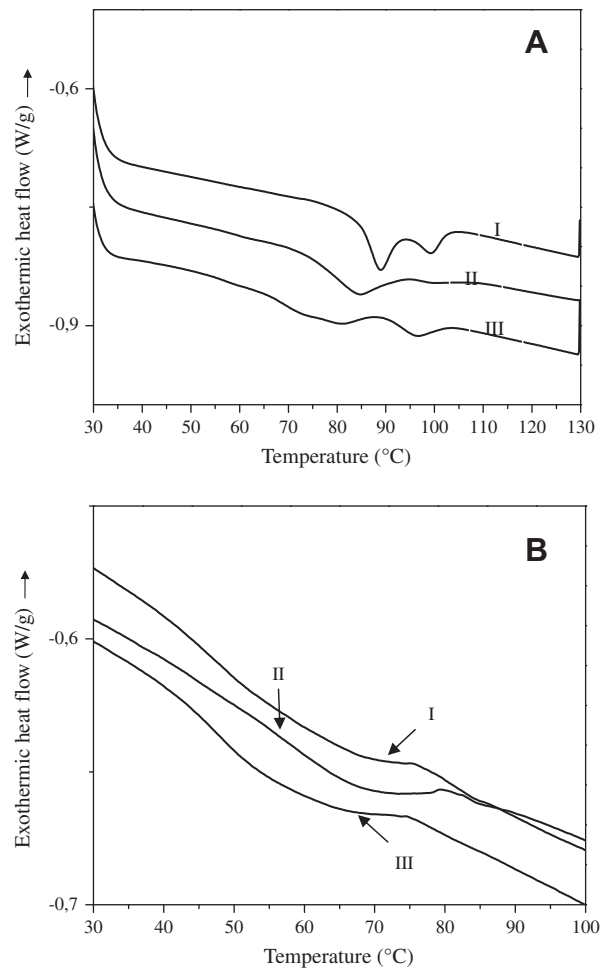


Fig. 2. DSC thermograms of the flours (flour/water ratio: 30:70) (A) First temperature scan and (B) Second temperature scan after 7 days of storage at 4 °C. I: JB (jack bean); II: PP (pigeon pea); III: DB (dolichos bean).

starch granules (Ronda and Roos, 2008). The phase transition enthalpies obtained in 7 day-stored samples varied in the range (5.2–6.1) J/g of solids without significant differences between the three legume flours. Lawal and Adebawale (2005) obtained lower enthalpies than we did for starch isolated from *Canavalia ensiformis* after 7 days of storage at 30 °C. This difference confirms that amylopectin retrogradation depends on the temperature of storage, i.e., slower at higher temperatures (Ronda and Roos, 2011).

3.4. Pasting properties

The RVA results of legume flours are summarized in Table 2. The highest PT was observed for JB flour while the lowest one was obtained for DB flour (86 °C versus 76 °C). The PT value obtained for PP flour, 82 °C, was the same to that obtained by Kaur et al. (2007)

Table 2
Pasting properties of legume flours.

Flours	PV (mPa·s)	TV (mPa·s)	BV (mPa·s)	FV (mPa·s)	SV (mPa·s)	PT (°C)
PP	5605 ± 4b	3869 ± 7c	1702 ± 7b	7555 ± 7c	3593 ± 9b	81.62 ± 0.03b
DB	5733 ± 4c	3649 ± 11b	2124 ± 5c	7436 ± 5b	3757 ± 6c	75.62 ± 0.02a
JB	1356 ± 6a	604 ± 5a	756 ± 7a	969 ± 4a	374 ± 6a	85.52 ± 0.27c

PP: pigeon pea, DB: dolichos bean, JB: jack bean, 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 column are not significantly different ($p < 0.05$).

for PP flours from India. The highest PT value of JB flour indicates the presence of starch highly resistant to swelling and rupturing. This is coherent with the highest gelatinization temperature obtained for JB flour from DSC thermograms.

The PV represents the viscosity value in the equilibrium point between swelling and rupturing of starch granules. JB flour had the lowest PV value (1356 mPa·s) significantly different than that of DB (5733 mPa·s) and PP (5605 mPa·s) flours. Swelling power of flours is related to their protein and starch contents. The high protein content of JB flour might cause the starch granules to be embedded within a stiff protein matrix which limits the access of the starch to water and restricts the swelling (Kaushal et al., 2012). The lowest BV was obtained for JB flours (756 mPa·s) indicating the highest thermostability. Singh et al. (2011) pointed out that proteins could provide some protection against the breakdown due to the contribution of denatured proteins to support the structure of the matrix and inhibiting the thixotropic nature of the starch in flour. FV of PP (7555 mPa·s) and DB (7436 mPa·s) were higher than PV values. By contrast, the FV of JB flour (969 mPa·s) was lower than its PV. The lowest SV of JB flour (374 mPa·s) indicates its lower tendency to retrograde. This behavior could be related to the lower amylose content of this flour.

3.5. Viscoelastic properties of gels

The viscoelasticity of gels formed from legume flour suspensions of different concentrations was examined by oscillatory measurements at 90 and 25 °C. The LVR varied notably ($p < 0.05$) depending on the type of flour, the concentration and the temperature of measurements. The maximum stress (τ_{\max}) applicable within LVR, beyond which the gel structure was broken, increased with the gel concentration and decreased with temperature (Table 3).

The G'_1 , G''_1 and $(\tan \delta)_1$ coefficients and the exponents a , b and c obtained from fitting the power law model to frequency sweep data in the range 1–10 Hz are shown in Table 3. The R^2 values (0.959–0.999) obtained demonstrate the good adjustment of the systems studied to this model (data not shown). JB gels exhibited elastic and viscous moduli markedly lower than PP and DB gels. Both viscoelastic moduli increased significantly ($p < 0.05$) with the rise of flour concentration and, except for the lowest gel concentrations, decreased with temperature. The high consistency of gels at 90 °C can be related to the formation of cross-links among the protein molecules or ongoing incorporation of protein and rearrangements in the network structure (Renkema et al., 2001). The elastic and viscous moduli, which in general are higher at 25 °C than 90 °C, could be explained by the formation of hydrogen bonds that stabilize and strengthen the matrix causing the stiffness of gels at lower temperatures (O'Kane et al., 2004). The lower consistencies exhibited at 25 °C by the gels of low concentration (at 8% for PP and DB and below 20% for DB) could be due to the dilution of the systems lowering the extent of these bonds. The marked differences in gel rheological properties of the three legumes could be related to the previously commented differences in protein contents and compositions. Differences in starch content and amylose/amylopectin ratios could also affect these properties since the

flour gels are composite networks in which starch granules are embedded in a continuous matrix of physically cross-linked amylose chains (Ring, 1985).

For all dispersions, G'_1 was greater than G''_1 , therefore $(\tan \delta)_1 < 1$, which suggests a solid elastic-like behavior. Similar observations have been reported previously for rice flour dispersions of different concentrations (10, 14 and 18%) heated for 60 min (Kapri and Bhat-tacharya, 2008). The highest $(\tan \delta)_1$ values were observed for JB which indicates a higher ratio G''/G' and a softer gel behavior (Weipert, 1990). Low values of the a exponent, near zero, for all gels except for the JB ones at 25 °C, mean that the elastic modulus, G' , was not dependent on the frequency, indicating a stable gel structure. Similar results were obtained by Lu et al. (2007) for fermented and non-fermented rice gels. The greater values of the b exponent, mainly observed at the lowest concentrations and at 90 °C, imply a higher dependency of the viscous modulus on the frequency. The c exponent decreased with the increase of gel concentrations, except for JP gel. This means that the ratio G''/G' had a lower dependence on frequency at higher gel concentrations (Ronda et al., 2011). This is coherent with the effect of concentration on a and b exponents as c exponent could be estimated from $(b-a)$.

The concentration dependence of the storage modulus, which provides information about the gelation efficiency and the structure of the particle network, can be approximated by a power-law function: $G \propto C^y$ (Renkema and van Vliet, 2004). Power-law functions between concentration and G'_1 and G''_1 were obtained for the dispersions: $G'_1 = m \cdot C^n$ and $G''_1 = p \cdot C^q$, where m and p are the G' and G'' moduli values at a gel concentration of 1% and at a frequency of 1 Hz and where n and q are indexes that reflect the nature of the association behavior and the network structure (Avanza et al., 2005) (Table 4). Correlation coefficients, R^2 , that ranged 0.942–0.999, indicate the good fitting of the model to experimental results. The values of n and q at 25 °C were greater than those at 90 °C for the three flours, which show the formation of a more ordered gel matrix at 25 °C (Avanza et al., 2005). Power-law function between G' and the concentration were also shown for soybean protein isolates (Renkema and van Vliet, 2004) and amaranth protein isolates (Avanza et al., 2005). However, linear relationships between G' and concentration have been reported for potato, wheat, corn and rice starch gels (Biliaderis and Juliano, 1993). Therefore, the elastic modulus, G'_1 , variation with the concentration of the flours studied, is probably more related to the protein content than the starch content of the flours.

By using the power law equations at 90 and 25 °C (Table 4), we predicted the minimum concentration required for the gel formation. The minimum concentration was determined as the concentration value that equaled both moduli ($G' = G''$) (Avanza et al., 2005). The values achieved were between 6–8% for DB and PP flours and 10% for JB flours, which were close to those obtained by the traditional method of LGC, i.e., 8% for DB and PP flours and 12% for JB flour.

3.6. Gel firmness kinetics

The firmness kinetics of PP and DB flour gels stored at 4 °C during 8 days led to the curves presented in Fig. 3. The firmness

Table 3
Rheological properties of gels. The power law model was fitted to experimental measurements. $G'(\omega) = G'_1 \cdot \omega^a$; $G''(\omega) = G''_1 \cdot \omega^b$; $\tan \delta(\omega) = (\tan \delta)_1 \cdot \omega^c$. τ_{\max} was obtained from stress sweeps.

Flour	Temperature (°C)	Concentration (%)	G'_1 (Pa)	a	G''_1 (Pa)	b	$(\tan \delta)_1$	c	τ_{\max}
PP	90	8	94 ± 4a	0.06 ± 0.02a	15 ± 1a	0.31 ± 0.01a	0.17 ± 0.01a	0.24 ± 0.01c	39 ± 4a
		10	124 ± 12a	0.09 ± 0.01a	20 ± 2a	0.32 ± 0.02a	0.16 ± 0.01a	0.23 ± 0.01bc	49 ± 2ab
		12	175 ± 8ab	0.13 ± 0.01a	33 ± 1b	0.33 ± 0.01a	0.19 ± 0.01a	0.20 ± 0.01b	57 ± 3b
	25	15	288 ± 7b	0.13 ± 0.03a	58 ± 3c	0.29 ± 0.01a	0.20 ± 0.04a	0.15 ± 0.03a	108 ± 2c
		8	63 ± 6a	0.08 ± 0.01a	11 ± 1a	0.33 ± 0.01c	0.17 ± 0.04b	0.25 ± 0.03c	29 ± 4a
		10	129 ± 2b	0.09 ± 0.01a	18 ± 1b	0.33 ± 0.01c	0.14 ± 0.01ab	0.24 ± 0.01c	70 ± 4b
DB	90	12	587 ± 17c	0.06 ± 0.02a	63 ± 2c	0.18 ± 0.02b	0.11 ± 0.01a	0.12 ± 0.04b	392 ± 3c
		15	1558 ± 14d	0.05 ± 0.02a	169 ± 3d	0.11 ± 0.02a	0.11 ± 0.01a	0.06 ± 0.02a	892 ± 2d
		8	25 ± 5a	0.12 ± 0.07a	8 ± 1a	0.40 ± 0.04b	0.30 ± 0.03b	0.28 ± 0.03a	16 ± 3a
	25	10	85 ± 8b	0.09 ± 0.01a	15 ± 4ab	0.34 ± 0.03ab	0.18 ± 0.06a	0.25 ± 0.04a	47 ± 3b
		12	189 ± 8c	0.09 ± 0.01a	28 ± 2b	0.30 ± 0.01ab	0.15 ± 0.02a	0.21 ± 0.01a	92 ± 4c
		15	320 ± 10d	0.09 ± 0.01a	47 ± 2c	0.27 ± 0.01a	0.14 ± 0.01a	0.18 ± 0.01a	250 ± 3d
JB	90	8	24 ± 3a	0.12 ± 0.04a	5 ± 0.4a	0.41 ± 0.03d	0.21 ± 0.05b	0.29 ± 0.07b	26 ± 4a
		10	141 ± 4b	0.07 ± 0.01a	19 ± 0.2a	0.28 ± 0.01c	0.13 ± 0.01a	0.20 ± 0.01ab	88 ± 3b
		12	571 ± 30c	0.06 ± 0.01a	63 ± 7b	0.16 ± 0.01b	0.11 ± 0.01a	0.10 ± 0.01a	392 ± 2c
	25	15	1914 ± 55d	0.05 ± 0.01a	241 ± 7c	0.06 ± 0.01a	0.12 ± 0.01a	0.10 ± 0.02a	1337 ± 3d
		12	27 ± 0.1a	0.12 ± 0.02a	10 ± 0.4a	0.32 ± 0.02a	0.36 ± 0.01b	0.20 ± 0.01ab	14 ± 3a
		15	42 ± 1a	0.13 ± 0.01a	15 ± 2a	0.35 ± 0.01a	0.35 ± 0.03b	0.22 ± 0.01b	26 ± 3ab
PP	90	17	111 ± 11b	0.13 ± 0.01a	32 ± 4b	0.28 ± 0.02a	0.29 ± 0.01a	0.15 ± 0.01a	31 ± 4b
		20	175 ± 17c	0.14 ± 0.01a	43 ± 8b	0.30 ± 0.04a	0.25 ± 0.02a	0.16 ± 0.02a	69 ± 3c
		12	9 ± 1a	0.38 ± 0.04c	5 ± 0.4a	0.49 ± 0.02c	0.56 ± 0.01d	0.10 ± 0.02a	8 ± 2a
	25	15	21 ± 3a	0.26 ± 0.01b	10 ± 1b	0.43 ± 0.01bc	0.47 ± 0.01c	0.18 ± 0.01b	29 ± 3b
		17	60 ± 8b	0.20 ± 0.01ab	22 ± 2c	0.37 ± 0.02b	0.36 ± 0.01b	0.17 ± 0.01b	72 ± 3c
		20	232 ± 14c	0.14 ± 0.01a	58 ± 1d	0.29 ± 0.01a	0.25 ± 0.01a	0.15 ± 0.02ab	221 ± 1d

PP: pigeon pea, DB: dolichos bean, JB: jack bean.

Data are the mean ± standard deviation. Values with a letter in common in the same column for each temperature and flour are not significantly different ($p < 0.05$).

Table 4

Parameters correspond to the fitting of experimental measured of G' and G'' to power-law function ($G' = m \cdot C^n$; $G'' = p \cdot C^q$) at 90 °C and 25 °C.

Flour	T (°C)	m	n	R^2	p	q	R^2
PP	90	1.61 ± 0.02	1.90 ± 0.01	0.993	0.15 ± 0.01	2.17 ± 0.11	0.978
	25	$3.10^{-4} \pm 1.10^{-5}$	5.73 ± 0.05	0.984	$6.10^{-4} \pm 3.10^{-5}$	4.60 ± 0.09	0.970
DB	90	$8.10^{-3} \pm 2.10^{-4}$	3.99 ± 0.02	0.969	$2.10^{-2} \pm 1.10^{-3}$	2.90 ± 0.03	0.995
	25	$1.10^{-5} \pm 1.10^{-7}$	7.02 ± 0.08	0.992	$1.10^{-5} \pm 5.10^{-7}$	6.21 ± 0.05	0.999
JB	90	$7.10^{-4} \pm 3.10^{-5}$	4.18 ± 0.15	0.942	$4.10^{-3} \pm 2.10^{-4}$	3.11 ± 0.15	0.942
	25	$1.10^{-6} \pm 5.10^{-8}$	6.29 ± 0.09	0.957	$4.10^{-5} \pm 1.10^{-6}$	4.71 ± 0.22	0.965

PP: pigeon pea, DB: dolichos bean, JB: jack bean.

change over long storage periods is mainly related to amylopectin recrystallization (Morris, 1990; Kalichevsky et al., 1990; Jacobson et al., 1997) and the Avrami relationship is often used to model

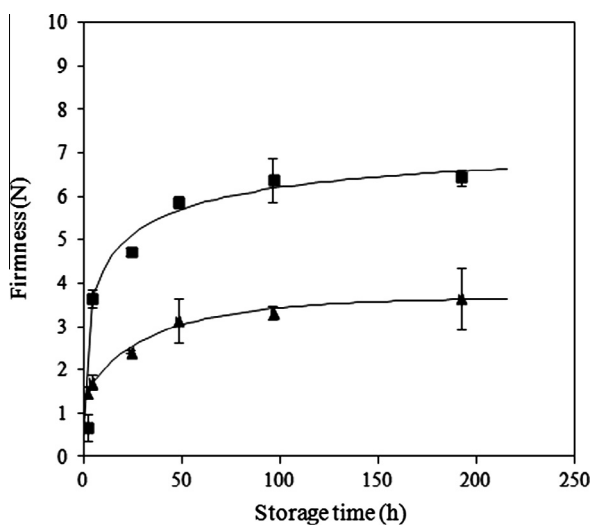


Fig. 3. Firmness kinetics of gels stored at 4 °C. The continuous lines resulted from fitting the experimental values to the Avrami equation. (■) DB: dolichos bean, (▲) PP: pigeon pea.

starch crystallization kinetics (Ronda and Roos, 2011). The Avrami relationship fitted to experimental data gave continuous lines shown in Fig. 3. In this study, gel firmness increased significantly during the first 48 h of storage, remaining practically constant during the rest of storage time. The correlation coefficients of the fittings, R^2 , were 0.989 and 0.990 for PP and DB, respectively. The values of the Avrami model parameters (F_0 , F_∞ , k , n) were (1.29 ± 0.34) N, (3.66 ± 0.22) N, (0.05 ± 0.08) h⁻ⁿ, 0.82 ± 0.39 for PP gels and (0.68 ± 0.21) N, (7.05 ± 0.29) N, (0.35 ± 0.12) h⁻ⁿ, 0.38 ± 0.04 for DB gels. The initial firmness, F_0 , of PP gels was notably higher than that of DB gels. However, the firmness kinetics at 4 °C was faster for the DB gels than for the PP one as can be seen in Fig. 3 and can be concluded from the Avrami constant, k , which was seven times higher for DB than PP. This can also be analyzed from the half-life time, $t_{1/2}$, that was 6 and 22 h for DB and PP, respectively. This means that DB gel took much less time to reach the 50% of the leveling-off firmness than the PP one. The gel leveling-off firmness, F_∞ , was also notably higher for DB than PP gels. This behavior was probably due to a faster and more extensive amylopectin recrystallization in DB than in PP gel. Moreover, gel firmness also depends on protein–protein interaction mainly the disulfide bonds (Avanza et al., 2005).

Data for gel JB firmness kinetics (data not shown) did not fit to Avrami model. In this case, the firmness of the gel showed large fluctuation during the first 24 h of storage showing no significant ($p > 0.05$) variations thereafter with the time of storage at 4 °C.

In conclusion, PP, DB and JP flours are capable of forming gels by heating. The minimum concentrations required for the gel formation were between 6–8% for DB and PP flours and 10% for JB flours. Gels obtained at different flour concentrations presented a solid elastic-like behavior in all flours. On the other hand, the differences in protein profile and the starch characteristics of JB flours with respect to DB and JP were reflected in their thermal, pasting and rheological properties and therefore in their gelation properties.

The results of this research provide important information on the rheological and thermal properties of the legume flours studied. PP and DB flours were able to form self-supporting gels and could be applied in the formulation of gel-like foods while JP flour showed poorer gelling properties and lower pasting viscosities.

Acknowledgements

The investigation was supported by the Spanish Institutions Ministerio de Economía y Competitividad (Projects AGL2012-35088) and Comunidad de Castilla y León (Project VA252A12-2) and National Agency for Science and Technology from Argentina (PICT-2010-2341). The stay of B. Acevedo at University of Valladolid was supported with a EuroTANGO grant. Authors thank A. Lazaridou and C. Biliaderis their comments on the phase transitions observed in DSC thermograms.

References

- Adebowale, K.O., Lawal, O.S., 2004. Comparative study of the functional properties of bambarra groundnut (*Voandzeia subterranean*), jack bean (*Canavalia ensiformis*) and mucuna bean (*Mucuna pruriens*) flours. *Food Research International* 37, 355–365.
- Akande, K.E., Abubakar, M.M., Adegbola, T.A., Bogoro, S.E., Doma, U.D., 2010. Chemical evaluation of the nutritive quality of pigeon pea [*Cajanus cajan* (L.) Millsp.]. *International Journal of Poultry Science* 9 (1), 63–65.
- AOAC, Official methods of analysis, 1990. Association of Official Analytical Chemists, 15th ed. Arlington, Virginia.
- Avanza, M.V., Puppo, M.C., Añón, M.C., 2005. Rheological characterization of amaranth protein gels. *Food Hydrocolloids* 19, 889–898.
- Biliaderis, C.G., 2009. Structural transitions and related physical properties of starch. In: BeMiller, J.N., Whistler, R.L. (Eds.), *Starch: Chemistry and Technology*. Academic Press, London, pp. 293–372.
- Biliaderis, C.G., Juliano, B.O., 1993. Thermal and mechanical properties of concentrated rice starch gels of varying composition. *Food Chemistry* 48, 243–250.
- Bressani, R., Brenes, R.G., Gacia, A., Elias, L.G., 1987. Chemical composition, amino acid content and protein quality of *Canavalia* spp seeds. *Journal of the Science of Food and Agriculture* 40, 17–23.
- Fraser, T.R., Brendon-Bravo, M., Holmes, D.C., 1956. Proximate analysis of wheat flour carbohydrates. 1. Methods and scheme of analysis. *Journal of the Science of Food and Agriculture* 7, 577–589.
- Henshaw, F.O., McWatters, K.H., Akingbala, J.O., Chinnan, M.S., 2003. Thermal properties of cowpea flour: a study by differential scanning calorimetry. *Nahrung* 47, 161–165.
- Jacobson, M.R., Obanni, M., BeMiller, J.N., 1997. Retrogradation of starches from different botanical sources. *Cereal Chemistry* 74, 511–518.
- Kalichevsky, M.T., Orford, P.D., Ring, S.G., 1990. The retrogradation of amylopectins from various botanical sources. *Carbohydrate Research* 198, 49–55.
- Kapri, A., Bhattacharya, S., 2008. Gelling behavior of rice flour dispersions at different concentrations of solids and time of heating. *Journal of Texture Studies* 39, 231–251.
- Katekhong, W., Charoenrein, S., 2012. The effect of rice aging on the freeze-thaw stability of rice flour gels. *Carbohydrate Polymers* 89, 777–782.
- Kaur, M., Sandhu, K.S., Singh, N., 2007. Comparative study of the functional, thermal and pasting properties of flours from different field pea (*Pisum sativum* L.) and pigeon pea (*Cajanus cajan* L.) cultivars. *Food Chemistry* 104, 259–267.
- Kaushal, P., Kumar, V., Sharma, H.K., 2012. Comparative study of physicochemical, functional, antinutritional and pasting properties of taro (*Colocasia esculenta*), rice (*Oryza sativa*) flour, pigeon pea (*Cajanus cajan*) flour and their blends. *Food Science and Technology* 48, 59–68.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature* 227, 680–685.
- Lawal, O.S., Adebowale, K.O., 2005. Physicochemical characteristics and thermal properties of chemically modified jack bean (*Canavalia ensiformis*) starch. *Carbohydrate Polymers* 60, 331–341.
- Liu, Q., Donner, E., Yin, Y., Huang, R.L., Fan, M.Z., 2006. The physicochemical properties and in vitro digestibility of selected cereals, tubers and legumes grown in China. *Food Chemistry* 99, 470–477.
- Lu, Z., Yuan, M., Sasaki, T., Li, L., Kohyama, K., 2007. Rheological properties of fermented rice flour gel. *Cereal Chemistry* 84 (6), 620–625.
- Morris, V.J., 1990. Starch gelation and retrogradation. *Trends in Food Science and Technology* 1, 2–6.
- Meng, G.T., Ma, C.Y., 2001. Thermal properties of *Phaseolus angularis* (red bean) globulin. *Food Chemistry* 73, 453–460.
- O’Kane, F.E., Happe, R.P., Vereijken, J.M., Gruppen, H., van Boekel, M.A., 2004. Characterization of pea vicilin. 2. Consequences of compositional heterogeneity on heat-induced gelation behavior. *Journal of Agricultural and Food Chemistry* 52, 3149–3154.
- Osman, M., 2007. Changes in nutrient composition, trypsin inhibitor, phytate, tannins and protein digestibility of *Dolichos lablab* seeds [*Lablab Purpureus* (L.) Sweet] occurring during germination. *Journal of Food Technology* 5 (4), 294–299.
- Renkema, J.M.S., Knabben, J.H.M., van Vliet, T., 2001. Gel formation by b-conglycinin and glycinin and their mixtures. *Food Hydrocolloids* 15, 407–414.
- Renkema, J.M.S., van Vliet, T., 2004. Concentration dependence of dynamic moduli of heat-induced soy protein gels. *Food Hydrocolloids* 18, 483–487.
- Ring, S.G., 1985. Some studies on starch gelation. *Starch/Stärke* 37, 80–83.
- Roe, J., 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. *Journal of Biological Chemistry* 212, 335–343.
- Ronda, F., Oliete, B., Gómez, M., Caballero, P., Pando, V., 2011. Rheological study of layer cake batters made with soybean protein isolate and different starch sources. *Journal of Food Engineering* 102, 272–277.
- Ronda, F., Roos, Y.H., 2008. Gelatinization and freeze-concentration effects on recrystallization in corn and potato starch gels. *Carbohydrate Research* 343, 903–911.
- Ronda, F., Roos, Y.H., 2011. Staling of fresh and frozen gluten-free bread. *Journal of Cereal Science* 53, 340–346.
- Sathe, S.K., Deshpande, S.S., Salunkhe, D.K., 1981. Functional properties of lupin seeds (*Lupinus mutabilis*) proteins and protein concentrates. *Journal of Food Science* 47, 491–502.
- Singh, S., Singh, N., MacRitchie, F., 2011. Relationship of polymeric proteins with pasting, gel dynamic- and dough empirical-rheology in different Indian wheat varieties. *Food Hydrocolloids* 25, 19–24.
- Sridhar, K.R., Seena, S., 2006. Nutritional and antinutritional significance of four unconventional legumes of the genus *Canavalia* – A comparative study. *Food Chemistry* 99, 267–288.
- Tang, C.H., 2008. Thermal denaturation and gelation of vicilin-rich protein isolates from three *Phaseolus* legumes: A comparative study. *Food Science and Technology* 41, 1380–1388.
- Wang, C.H., Damoradan, S., 1991. Thermal gelation of globular proteins: influence of protein conformation on gel strength. *Journal of Agricultural and Food Chemistry* 39, 433–438.
- Weipert, D., 1990. The benefits of basic rheometry in studying dough rheology. *Cereal Chemistry* 67, 311–317.