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Original article

Effect of different treatments on the microstructure and functional and pasting properties of pigeon pea (*Cajanus cajan* L.), dolichos bean (*Dolichos lablab* L.) and jack bean (*Canavalia ensiformis*) flours from the north-east Argentina

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Summary The effect of germination (G; 5 days), soaking-cooking (SC; 6 h–20 min, 6 h–40 min, 6 h–60 min) and microwave (M; 50%, 70%, 100%) treatments on pigeon pea (PP), dolichos bean (DB) and jack bean (JB) seeds was studied. Microstructure of seeds and functional (protein solubility, water-holding capacity, oil-holding capacity, emulsion stability) and pasting properties of flours were determined. Germination and microwave treatments modified the protein matrix of cotyledon cells preserving the shape of the starch granule, whereas the SC treatment (6 h–60 min) affected both. The soaking-cooking is the most influential treatment on the functional properties of PP, DB and JB flours, as increased water absorption capacity (73–96%), decreased protein solubility (>80%) and the tendency to retrogradation of amylose (69–85%) also improved emulsion stability.

Keywords Cream destabilisation percentage, emulsion stability, microstructure, pasting properties, protein solubility.

Introduction

In the north-east of Argentina, legumes like pigeon pea (PP; *Cajanus cajans*), dolichos bean (DB; *Dolichos lablab*) and jack bean (JB; *Canavalia ensiformis*) are cultivated by small and medium farmers and they are used primarily for human consumption (regional meals). These pulses have high protein content (23–32 g per 100 g) and carbohydrates (50–58 g per 100 g), low fat, vitamins and minerals (Acevedo *et al.*, 2013).

To achieve the usage of these legume flours in the food industry, especially for coeliac diets, and to promote their growth in the region, their functional properties must be studied. These properties play an important role in the physical behaviour of food during preparation, processing and storage, thereby altering the sensory characteristics of food (Cheng & Bhat, 2016). Generally, functional properties are contributed by the protein components of foods and are affected by composition, structure, conformation, interactions with other food components, and the environment (Boye *et al.*, 2010). On the other hand, the starch fraction and other components such as pectins and mucilages may also contribute to the overall effect observed (Hasan *et al.*, 2015).

Several authors have studied these legume species from elsewhere (Adebowale & Lawal, 2004; Tiwari *et al.*, 2008; Olalekan & Bosede, 2010; Kaushal *et al.*, 2012; Hasan *et al.*, 2015). Considering that functional and pasting properties may vary with the type, location and climate farming, it is necessary to know the functional properties of legumes grown in Argentina.

The treatments that are applied on legumes in the food industry (soaking, cooking, germination, autoclave, microwave, etc.) significantly enhance their nutritional value by inactivating the antinutritional factors. However, these treatments affect starch and

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protein structures, thereby altering their functional properties.

Thus, the aim of this study was to evaluate the effect of treatments (germination, soaking-cooking and microwave) on the microstructure of legume seeds (PP, DB, JB), and functional and pasting properties of PP, DB and JB flours obtained from treated seeds.

Materials and methods

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 2014). Shrunken, discoloured and insect-infested seeds were eliminated and sun–dried, and then, the legumes were kept in a hermetic vessel stored at 10 °C until use.

Processing methods

Germination (G)
Seeds were treated with sodium hypochlorite solution (0.7 mL per 100 mL) for 30 min using a seed-to-liquid ratio of 1:10 (g:mL). Then, the seeds were submerged in water for 11 h 30 min, using a seed-to-distilled water ratio of 1:10 (g:mL). Seeds were drained and placed in a tray with a damp cloth with sodium hypochlorite during 5 days.

Soaking-cooking (SC)
Seeds were soaked for 6 h in sodium bicarbonate solution (0.02 g per 100 mL; pH 8.3) using a seed-to-liquid ratio of 1:10 (g:mL). Seeds were drained and rinsed with distilled water and then were cooked in boiling water temperature for 20, 40 and 60 min in a beaker having a condenser, using a seed-to-distilled water ratio of 1:10 (g:mL).

Microwave (M)
Seeds were submerged in distilled water, using a seed-to-distilled water ratio of 1:10 (g:mL), for 10 min at different potencies (50%, 70% and 100%) (output power 800 Watt), in a microwave oven (Whirlpool WMD20SB model; Tierra del Fuego, Argentine).

All the treated seeds were washed with distilled water and dried in a hot air oven (San-Jor, Argentina) at 55 °C to a constant weight (24 h). Processing methods were performed in triplicate.

Scanning electron microscopy

A thin layer of each raw and treated seeds was obtained with a razor blade. Then, the samples were prepared following Sorrivas de Lozano & Morales (1983) and observed under a scanning electron microscope (model JEOL, 5800 LV, Japan) at 15 kV.

Preparation of seed flours

Raw and treated seeds (with seed coat) were ground in an electric miller (Braun KSM2 model, coffee grinder, México) and subsequently sieved through an 80 ASTM (177 μm). In the case of jack bean, seeds were previously crushed to smaller fragments using a Quaker City mill (model F-4, Philadelphia, PA USA).

Protein solubility

Protein solubility was determined according to the method of Bera & Murkherjee (1989). The protein content of the supernatants was measured by the Lowry *et al.* (1951) using UV–Vis spectrophotometer (JASCO V-630 bio, Japan). Protein solubility was expressed as the percent ratio between water-soluble protein determined by Lowry and total protein content determined by the Kjeldahl method (AOAC, 1990). Bovine serum albumin was used as standard protein.

Water/oil-holding capacity

Water/oil-holding capacity was determined according to the method of Beuchat (1977). Results were expressed as grams of water or oil retained per gram of flour.

Preparation of emulsions and dynamic light scattering measurements

All samples (1 mg protein per 1 mL) were dispersed in water and stirred for 1 h at room temperature. Emulsions were prepared by homogenising 4 mL of refined sunflower oil and 16 mL of the protein suspension (20% oil v/v) with a rotary homogeniser Ultra-Turrax T25 (IKA Labortechnik; Staufen, Germany) using a S25 N10 G dispersing element at 20 000 rpm for 90 s.

The emulsions stability was determined through the use of a vertical scan analyser Quick Scan (Beckman–Coulter inc., Danvers, MA, USA). Samples were loaded into a cylindrical glass measurement cell, and the backscattering percentage profiles (% Bs) along the tube were immediately monitored every 1 min for 1 h as a function of the sample height (total height, 60 mm approximately). Then, cells were still stored for 24 h at room temperature and another individual % Bs measurement was carried out. These measurements were used to plot the kinetics of the mean % Bs in the lower part of the tube (10–15 mm height) and the upper part of the tube (40–50 mm height).

The $K_{0.1}$ values indicate the stability of the emulsion with respect to the creaming process. The increment of $k_{0.1}$ suggests a decrease in the emulsion stability because of the increase in the speed of clarification. $k_{0.1}$ was calculated as follows:

$$K_{0.1} = (\%\mathrm{Bs}_{\mathrm{in}} \times t_{0.1})^{-1} \quad (1)$$

based on the mean values of % Bs in the lower part of the tube (10–15 mm height), where $t_{0.1}$ is the time to diminish 10% the % $\mathrm{Bs}_{\mathrm{in}}$ (initial value of % Bs).

The cream destabilisation percentage (% CD) was also calculated as follows:

$$\%\mathrm{CD} = 100 \times (\%\mathrm{Bs}_{\max} - \%\mathrm{Bs}_{24\mathrm{h}})/\%\mathrm{Bs}_{\max} \quad (2)$$

based on the mean values of % Bs in the upper part of the tube (40–50 mm height), where % $\mathrm{Bs}_{\max}$ is the maximum value of % Bs and % $\mathrm{Bs}_{24\ \mathrm{h}}$ is the value of % Bs at the 24 h after the first measure.

Pasting properties

Pasting properties were studied using Rapid Visco-Analyzer (RVA) (RVA-4 Newport Scientific Pty Limited, Warriewood, Australia) controlled by the Thermocline software (Newport Scientific Pty Limited, Warriewood, Australia). Viscosity profiles of flours were recorded using flour suspensions (20 g per 100 g, 28.5 g total weight). The samples were heated from 50 to 95 °C at 6 °C min^{-1} 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^{-1} with a holding for 2 min at 50 °C. Parameters recorded were pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), final viscosity (FV), breakdown viscosity (BV) and setback viscosity (SV).

Data analysis

The results of determination described in items 2.5 to 2.8 were expressed as mean values ± standard deviation of three true replicates. For each parameter evaluated, differences between treatments and legumes species were analysed by analysis of variance (ANOVA) followed by the least significant difference LSD Fisher's test (α=0.05). Statistical analysis was performed using Infostat software (Balzarini *et al.*, 2008).

Results and discussion

Scanning electron microscopy

The legume species studied in this work had similar changes in the microstructure during the treatments applied. For that reason, only the microstructural characteristics of the PP seeds are shown in Fig. 1. The cotyledon cells are the main storage reservoir of seeds, and they are formed by starch granules covered by a protein matrix (Fig. 1). In raw seeds, starch granules have a smooth and oval surface. The average starch granule measures observed were 24.1 × 17 μm for PP (Fig. 1a), 20.6 × 14.2 μm for DB and 36.9 × 26.3 μm for JB.

The micrographs of germinated seeds are shown in Fig. 1b. The cell wall did not show changes in comparison with its native state. Most starch granules retained their shape, size, and smooth surface; however, in some of them, a slight amylolysis was observed as a result of an increased activity of endogenous enzymes (Benítez *et al.*, 2013). Additionally, it was observed a granular protein matrix surrounding the starch granules that could be related to the degradation of storage proteins during germination. (Van Der Wilden *et al.*, 1980).

Soaking-cooking (6 h–20 min) maintained the form of the starch granule, but their surface flattened and the protein matrix exhibited contraction as a result of the heat applied. With the most aggressive treatment (SC 6 h–60 min), a dissemination of compartmentalisation was observed between starch granules and the matrix protein (Fig. 1c). Aguilera *et al.* (2009) obtained similar results, studying the microstructure of lentils and peas exposed to heat treatments.

Seeds treated by microwave did not show size variations in their starch granules, but compact structure of the protein matrix was affected (Fig. 1e and f). This could be due to protein denaturalisation caused by microwave heating.

Protein solubility

Figure 2 shows the percent of protein solubility depending on the pH samples (legume flours) studied. All the native flours studied showed a similar behaviour exhibiting the lowest value of solubility at pH 4 (10–13%), which is in coincidence with the isoelectric point of the proteins present in the samples tested (Barac *et al.*, 2015). In contrast, higher solubility values were observed at alkaline pH (70–95%), and PP flour exhibited the highest values. Germination improved the DB protein solubility, both acidic and basic pH. The increase in protein solubility could be due to the degradation of storage proteins by proteases that convert the insoluble proteins into soluble polypeptides during germination after imbibition process. (Urbano *et al.*, 2005; Mishra *et al.*, 2016) However, it did not show significant differences ($P \geq 0.05$) for PP and JB at the pH tested. Soaking-cooking significantly reduced the protein solubility, and it was irreversible when the thermal treatment applied was severe. The proteins solubility of the flours obtained from treated seeds (SC 40 min and SC 60 min) varied

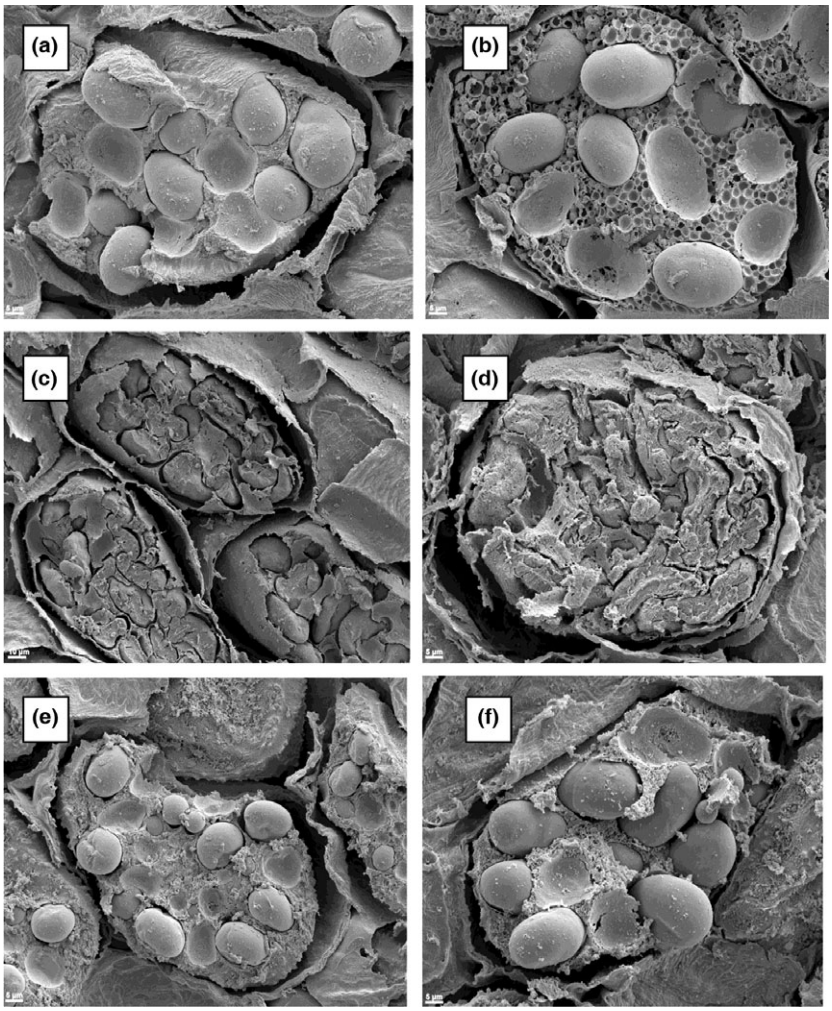

Figure 1 Scanning electron microscopy of PP (pigeon pea) seeds: (a) raw, Bar=5 μm; (b) G, Bar = 5 μm; (c) SC (6 h–20 min), Bar = 10 μm; (d) SC (6 h- 60 min), Bar = 5 μm; (e) M 50%, Bar = 5 μm; (f) M 100%, Bar = 5 μm.

in small proportion in the pH range tested. This behaviour can be attributed to the exposure of hydrophobic groups and aggregation of unfolded proteins by heat treatment (Aguilera *et al*., 2009). Several authors reported similar solubility results applying soaking-cooking to chickpeas, lentils and peas seeds (Ma *et al*., 2011). Microwave treatment decreased ($P < 0.05$) the protein solubility at pH 3, 7, 9 and 10, in comparison with native flours. The most important decrease was observed in the PP flour. The decrease in protein solubility could be explained by the effect of heating by microwaves, which increased surface hydrophobicity of protein due to unfolding of the helical secondary structure, exposure of hydrophobic amino acids and the formation of disulphide bonds. (Ashraf *et al*., 2012)

Water-holding capacity

The WHC of native and treated flour is presented in Table 1. The JB native flour exhibited the highest WHC value (1.5 g/g). This could be due to the high protein content of JB flour (32.4 ± 0.1%), regarding to the other flours studied (DB: 29.0 ± 0.6%; PP: 23.0 ± 0.1%) (Acevedo *et al*., 2013). Furthermore, carbohydrates and fibre are also hydrophilic constituents; therefore, they can interact with food water and contribute to WHC. In previous studies, Acevedo *et al*. (2013) informed that the highest carbohydrate content was obtained for JB flour (62 ± 1) % (db) and the lowest for DB flour (50 ± 1) % (db); however, JB flour had the lowest starch content (35.7 ± 0.4% (db)).

The good WHC of JB flour may prove useful in products where good viscosity is required, such as in soups and gravies.

Germination improved WHC of PP flour (50%); however, no significant differences ($P > 0.05$) were observed for DB and JB flours. Soaking-cooking increased ($P < 0.05$) WHC values of PP (73%), DB (96%) and JB (94%) flours. This may be due to

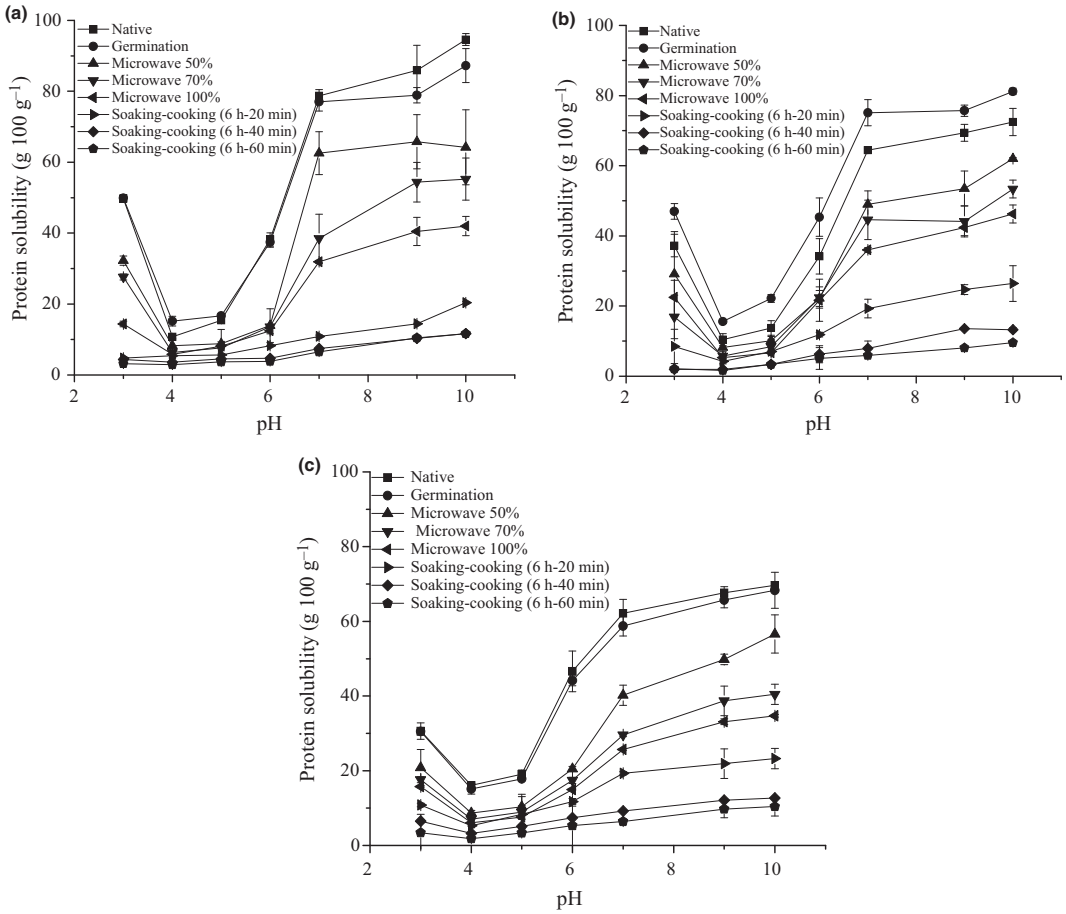

Figure 2 Protein solubility of treated and nontreated flours. a) PP (pigeon pea), b) DB (dolichos bean), c) JB (jack bean).

conformational changes of proteins during the treatment applied (Singh, 2001). Instead, microwave treatment increased WHC for DB (33%) and JB (40%), but it did not affect PP, which may be due to uncoiling and more exposure of the hydrophilic domains of proteins (Ashraf *et al.*, 2012). Water retention improvement of legumes flour by heat treatment has been reported by Aguilera *et al.* (2009).

Oil-holding capacity

The OHC is an important functional property, as it helps to improve mouth feel and the retention of flavour (Du *et al.*, 2014). OHC has been attributed to physical entrapment of oil within the protein, and noncovalent bonds such as hydrophobic, electrostatic and hydrogen bonds are the forces involved in lipid–protein interaction (Lawal, 2004).

The DB native flour showed the highest value of OHC (1.50 g/g), while PP exhibited the lowest (1.11 g/g) (Table 1). Variations in the particle sizes, starch and protein contents, protein types (Butt & Batool, 2010), and nonpolar amino acid side chain ratios on the protein molecule surface (Adebowale & Lawal, 2004) possibly explain differences in the oil-binding capacity of the flours. According to the obtained results, DB flour would be the most suitable for use in bakery products or meat, meat extenders, doughnuts, bread, cakes, baked goods and soup mixes.

Pigeon pea and DB flours obtained from treated seeds did not show significant differences of OHC values ($P > 0.05$) in comparison with their native flour. In contrast, JB flours from soaked-cooked seeds increased this property by 47%. This could be due to changes in hydrophobic proteins as these proteins show superior binding of lipids, implying that nonpolar amino acid side chains bind paraffin chains of fats (Boye *et al.*, 2010).

Emulsion stability

An emulsion is a thermodynamically unstable system, which tends to be destabilised by different mechanisms (cremated, flocculation, disproportion and coalescence

Table 1 Water-holding capacity (WHC) and oil-holding capacity (OHC) of native and treated flours[a]

	WHC (g H_2O per g flour)			OHC (g oil per g flour)		
Treatment	**PP**	**DB**	**JB**	**PP**	**DB**	**JB**
Native	1.00 ± 0.01^{a}	1.25 ± 0.00^{a}	1.50 ± 0.01^{ab}	1.11 ± 0.01^{a}	1.50 ± 0.01^{ab}	1.18 ± 0.09^{a}
Germination	1.5 ± 0.00^{b}	1.31 ± 0.09^{ab}	1.62 ± 0.01b^{c}	1.14 ± 0.00^{a}	1.31 ± 0.09^{a}	1.44 ± 0.09^{ab}
Soaking-cooking (6 h–20 min)	1.72 ± 0.01^{c}	2.36 ± 0.18b^{c}	2.87 ± 0.19e	1.13 ± 0.00^{a}	1.50 ± 0.01^{ab}	1.75 ± 0.01^{c}
Soaking-cooking (6 h–40 min)	1.73 ± 0.00^{c}	2.62 ± 0.17b^{c}	2.88 ± 0.18^{d}	1.13 ± 0.01^{a}	1.50 ± 0.01^{ab}	1.81 ± 0.08^{c}
Soaking-cooking (6 h–60 min)	1.74 ± 0.02^{c}	2.37 ± 0.18^{c}	2.99 ± 0.01^{d}	1.11 ± 0.01^{a}	1.37 ± 0.17^{ab}	1.63 ± 0.18b^{c}
Microwave 50%	0.99 ± 0.02^{a}	1.62 ± 0.18^{d}	1.37 ± 0.00^{a}	1.13 ± 0.01^{a}	1.61 ± 0.16^{b}	1.37 ± 0.18^{ab}
Microwave 70%	0.98 ± 0.00^{a}	1.62 ± 0.18^{d}	1.50 ± 0.00^{ab}	1.12 ± 0.04^{a}	1.38 ± 0.17^{ab}	1.37 ± 0.17^{ab}
Microwave 100%	1.00 ± 0.01^{a}	1.75 ± 0.01^{d}	1.75 ± 0.01^{c}	1.11 ± 0.03^{a}	1.50 ± 0.00^{ab}	1.25 ± 0.00^{a}

^{a,b,c} Means with the same letters within a column do not differ significantly ($P > 0.05$).

[a]Mean ± standard deviation for three determinations from each of the three processed samples.

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

phase inversion) resulting in partial or complete separation of the immiscible phases (Wagner, 2000).

Table 2 shows $K_{0.1}$ values that were obtained from the kinetic of the lower part of the cell (10–20 mm) (Fig. 3a), and cream destabilisation percentage (% CD) of emulsions was calculated from the kinetic of % Bs at the upper part of the pipe (40–50 mm) (Fig. 3b) for emulsions prepared from native and treated flours. The $K_{0.1}$ and % CD values are only shown for soaking-cooking (6 h–60 min) and microwave treatments (100%) as no differences were observed in the other conditions studied.

Figure 3a shows the kinetic of % Bs of the emulsions of native and treated flours of PP in the lower part of the pipe of emulsion (10–20 mm). Emulsions prepared from treated flours (soaking-cooking and microwave) retard the decrease of % Bs with regard to the native one, which means a delay in the process of cremating of emulsions.

The $K_{0.1}$ values are related to the speed of clarification of the lower part of the emulsion due to the cremating process that takes place because of the action of the gravity on the emulsion drops. The most stable emulsion was then prepared with native PP flour. Soaking-cooking treatment improves stability of emulsions, but increased the cream destabilisation percentage (%CD) (Table 2). This could be due to the dissociation and partial unfolding of proteins, leading to exposure of hydrophobic amino acid residues, which consequently increased the surface activity and adsorption at the oil and water interface (Ma *et al.*, 2011). On the other hand, the least stable emulsion was the one generated from JB native flour and the treatments did not modify this behaviour (Table 2).

Figure 3b shows the kinetic of % Bs at the upper part of the pipe (40–50 mm) of PP flour. It was observed that the cream was accumulated in the upper part of the pipe because of the gravity action. The emulsions prepared with flours from raw and germinated seeds presented the major % Bs of cream along the 60 min, in comparison with the emulsions prepared with treated flours (soaking-cooking and microwave). The emulsions of flours prepared from seeds treated by soaking-cooking and microwave presented the major percentage of destabilisation of the cream for PP, DB and JB (Table 2).

Table 2 $K_{0.1}$ values and cream destabilisation percentage (% CD) of emulsions of different native and treated flours[a]

Treatment	**PP**	**DB**	**JB**
Native	$K_{0.1}$= 0.0023 ± 1.1 × 10^{-4a}	$K_{0.1}$=0.0027 ± 1.3 × 10^{-4a}	$K_{0.1}$= 0.0033 ± 1. 6 × 10^{-4a}
	CD %=6.16 ± 0.30^{c}	CD %= 8.20 ± 0.40^{c}	CD %= 1.15 ± 0.04^{c}
Germination	$K_{0.1}$=0.0020 ± 1 × 10^{-4ab}	$K_{0.1}$=0.0023 ± 1.2 × 10^{-4ab}	$K_{0.1}$=0.0034 ± 1.7 × 10^{-4a}
	CD %= 8.73 ± 0.40^{b}	CD %= 9.10 ± 0.37b^{c}	CD %= 1.10 ± 0.03^{c}
Soaking-cooking (6 h- 60 min)	$K_{0.1}$=0.0015 ± 7.5 × 10^{-5c}	$K_{0.1}$=0.0019 ± 9.5 × 10^{-5b}	$K_{0.1}$= 0.0032 ± 1. 4 × 10^{-4a}
	CD %=10.23 ± 0.50^{ab}	CD % 10.50 ± 0.45^{ab}	CD %= 3.19 ± 0.12^{a}
Microwave 100%	$K_{0.1}$=0.0017 ± 8.5 × 10^{-5bc}	$K_{0.1}$=0.0021 ± 1.1 × 10^{-4b}	$K_{0.1}$= 0.0031 ± 1.5 × 10^{-4a}
	CD %=10.60 ± 0.51^{a}	CD %= 11.34 ± 0.51^{a}	CD %= 2.50 ± 0.10^{b}

^{a,b,c} Means with the same letters within a column do not differ significantly ($P > 0.05$).

[a]Mean ± standard deviation for three determinations from each of the three processed samples.

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

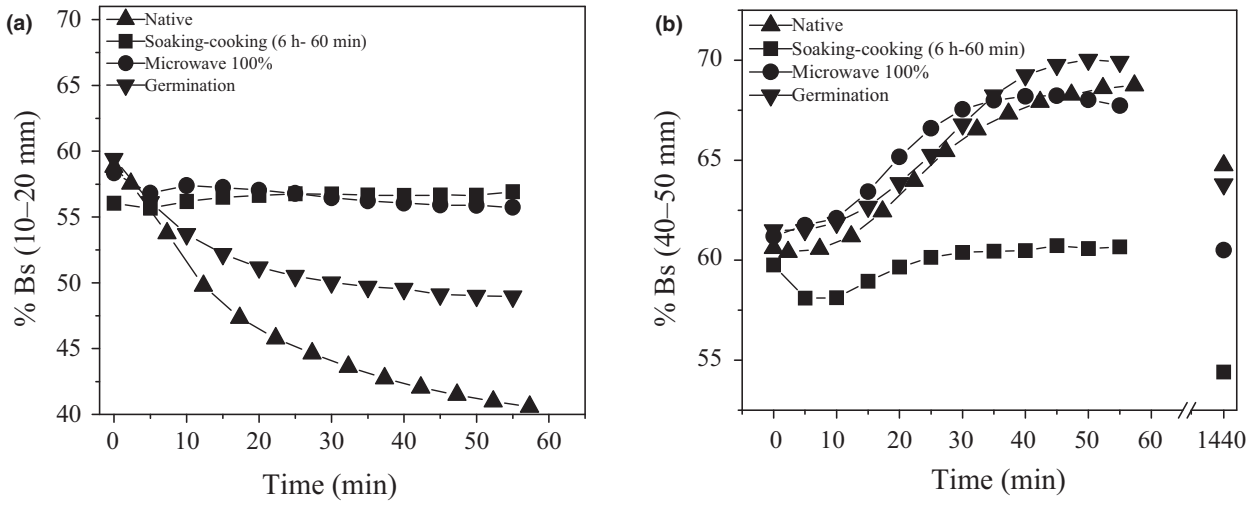

Figure 3 Kinetics of % Bs of PP (pigeon pea) flours. a) At the bottom (10–20 mm) and b) at the top (40–50 mm) of the measuring cell, obtained from Quick Scan curves of the emulsions of native and treated flours.

Pasting properties

The pasting characteristics play an important role in the selection of a variety to use in the industry as a thickener, binder or for any other use (Kaushal *et al.*, 2012). The values obtained for pasting properties of the native and treated flour are shown in Table 3.

Pasting temperature indicates the minimum temperature required to cook the flour. PT values of native flours ranged from 75.7 °C to 85.5 °C. These values

Table 3 Pasting properties of native and treated flours[a]

Flours	Treatment	PV (mPa.s)	TV (mPa.s)	BV (mPa.s)	FV (mPa.s)	SV (mPa.s)	PT (°C)
PP	Native	5892 ± 6^{d}	3801 ± 7^{c}	2091 ± 9^{f}	7950 ± 5^{e}	4149 ± 11^{f}	81.6 ± 0.1^{c}
	Germination	3997 ± 4^{b}	2455 ± 14^{a}	1542 ± 12^{d}	4431 ± 24^{a}	1976 ± 13^{c}	82.8 ± 0.1^{ef}
	Soaking-cooking (6 h–20 min)	3340 ± 12^{a}	3406 ± 23^{b}	358 ± 13^{a}	7739 ± 32^{d}	4333 ± 26^{g}	83.1 ± 0.2^{f}
	Soaking-cooking (6 h–40 min)	5550 ± 24^{c}	4225 ± 17^{f}	1325 ± 17^{c}	5856 ± 26^{b}	1631 ± 12^{b}	75.2 ± 0.1^{b}
	Soaking-cooking (6 h–60 min)	6372 ± 8^{f}	5205 ± 31^{g}	1167 ± 8^{b}	6493 ± 42^{c}	1288 ± 11^{a}	74.1 ± 0.2^{a}
	Microwave 50%	5948 ± 36^{d}	4069 ± 25^{d}	1879 ± 5^{e}	7889 ± 41^{e}	3820 ± 36^{e}	81.4 ± 0.1^{c}
	Microwave 70%	6023 ± 28^{e}	4131 ± 8^{e}	1892 ± 9^{e}	7977 ± 13^{e}	3846 ± 21^{e}	82.2 ± 0.1^{d}
	Microwave 100%	6324 ± 15^{f}	4267 ± 14^{f}	2058 ± 12^{f}	7942 ± 21^{e}	3675 ± 28^{d}	82.5 ± 0.1^{de}
DB	Native	6134 ± 27^{e}	3533 ± 18^{f}	2601 ± 18^{e}	7672 ± 16^{e}	4139 ± 17^{h}	75.7 ± 0.2^{c}
	Germination	1505 ± 18^{a}	955 ± 21^{a}	551 ± 6^{a}	1362 ± 6^{a}	408 ± 7^{a}	76.6 ± 0.2^{d}
	Soaking-cooking (6 h–20 min)	5122 ± 12^{b}	2445 ± 42^{b}	2678 ± 13^{f}	2930 ± 17^{b}	486 ± 6^{b}	68.9 ± 0.2^{b}
	Soaking-cooking (6 h–40 min)	5562 ± 31^{d}	2669 ± 16^{c}	2893 ± 24^{g}	3516 ± 16^{c}	848 ± 11^{d}	66.1 ± 0.2^{a}
	Soaking-cooking (6 h–60 min)	5350 ± 15^{c}	2925 ± 14^{d}	2425 ± 34^{d}	3550 ± 21^{c}	625 ± 9^{c}	65.4 ± 0.1^{a}
	Microwave 50%	6280 ± 23^{f}	3047 ± 23^{e}	3233 ± 14^{h}	6298 ± 41^{d}	3252 ± 13^{e}	75.3 ± 0.1^{c}
	Microwave 70%	6634 ± 13^{h}	4592 ± 22^{g}	2042 ± 13^{c}	8111 ± 52^{f}	3519 ± 16^{f}	78.1 ± 0.1^{e}
	Microwave 100%	6403 ± 9^{g}	4591 ± 14^{g}	1812 ± 5^{b}	8308 ± 34^{g}	3717 ± 23^{g}	77.2 ± 0.2^{d}
JB	Native	1722 ± 5^{b}	774 ± 4^{b}	948 ± 18^{b}	1407 ± 8^{b}	642 ± 4^{b}	85.5 ± 0.1^{a}
	Germination	1340 ± 13^{a}	632 ± 11^{a}	763 ± 21^{a}	989 ± 18^{a}	352 ± 14^{a}	86.1 ± 0.3^{b}
	Soaking-cooking (6 h–20 min)	4562 ± 26^{h}	2964 ± 25^{e}	1598 ± 15^{e}	4731 ± 24^{f}	1767 ± 18^{g}	86.7 ± 0.1^{c}
	Soaking-cooking (6 h–40 min)	4412 ± 29^{g}	3245 ± 31^{f}	1167 ± 23^{c}	4841 ± 43^{g}	1596 ± 25^{f}	87.1 ± 0.1^{c}
	Soaking-cooking (6 h–60 min)	4292 ± 31^{f}	3540 ± 42^{g}	752 ± 17^{a}	5444 ± 13^{h}	1904 ± 13^{h}	88.9 ± 0.2^{d}
	Microwave 50%	2218 ± 8^{c}	884 ± 18^{c}	1334 ± 9^{d}	1584 ± 26^{c}	700 ± 12^{c}	85.6 ± 0.2^{ab}
	Microwave 70%	3465 ± 13^{e}	1635 ± 13^{d}	1830 ± 12^{f}	2978 ± 28^{e}	1343 ± 26^{e}	85.7 ± 0.1^{ab}
	Microwave 100%	2722 ± 9^{d}	1611 ± 4^{d}	1112 ± 19^{c}	2815 ± 17^{d}	1205 ± 11^{d}	85.7 ± 0.1^{ab}

[a]Data are the mean ± standard deviation. Means with the same letters within a column do not differ significantly ($P > 0.05$) for each species of legumes. PP, pigeon pea; DB, dolichos bean; JB, jack bean.

are similar to those obtained by other authors for different species of legumes (Du *et al*., 2014). The highest value of PT (85.5 °C) was observed for the native JB flour; this behaviour is in accordance with the high resistant to swelling and to rupturing of starch granules. Soaking-cooking modified the swelling ability of starch granules, decreasing the PT for PP and DB flours and increasing this parameter for JB flour.

The PV represents the viscosity value in the equilibrium point between swelling and rupturing of starch granules. PP and DB native flours exhibited a higher PV compared to native JB flour. This behaviour would be related to the proportion of native starch present in the PP (50%), DB (49.3%) and JB (35.7%) flours (Acevedo *et al*., 2013). Also, the amylose–amylopectin ratio and the flour protein content could be involved (Kaushal *et al*., 2012). Germination caused a decrease of PV in PP (32%), DB (75.5%) and JB (22%) flours that could be due to the hydrolysis of starch grains from within the grain (Harris, 1976) (see Figure 1B). Swelling capacity is related to the level of starch, especially with amylopectin chains that decrease as consequence of metabolic activity during germination (Benítez *et al*., 2013). Soaking-cooking increased this property in JB.

The highest TV was obtained for the PP flour (3801 mPa.s) and the lowest for JB flour (774 mPa.s). The differences in this parameter may be attributed to the difference in the extent of amylose leaching, amylose–lipid complex formation and granule swelling (Liu *et al*., 1997).

The FV values of native flours of PP (7959 mPa.s) and DB (7672 mPa.s) exhibit a better ability to form a viscous paste compared to native JB flour. SV shows how the viscosity of the paste of the flour suspensions recovered during the cooling period. The high SV values obtained for the PP and DB native flours indicate a high tendency to retrogradation of amylose. However, the treatments applied to these seeds decreased the process of retrogradation, and this decrease was more remarkable in treated DB flour. The smaller tendencies to retrograde are an advantage in food products such as soups and sauces, which undergo loss of viscosity and precipitation as a result of retrogradation (Adebowale & Lawal, 2003), and therefore, DB-treated flours and native JB flour may be suitable for products like soup mixes.

Conclusion

The treatments applied (G, SC and M) to seeds of the legumes studied (PP, DB and JB) modified seed microstructure and the functional properties of their obtained flours. Microstructural modifications of main components of seeds were consistent with the functional properties of flours. The extent of these changes depended on the type of legume and the process applied.

Soaking-cooking was more influential in the functional properties than microwave and germination treatments, as that provoked more changes in the protein structure, as well as it modified the starch granule. Consequently, it increased water absorption capacity but decreased protein solubility and the tendency to retrogradation of amylose. Soaking-cooking treatment improves stability of emulsions, although increased the cream destabilisation percentage (%CD). The above data support the conclusion that leguminous flours from treated and raw seeds could be considered as functional ingredients in food formulation.

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