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Effect of amylose:amylopectin ratio and rice bran addition on starch films properties

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ABSTRACT

The influence of the amylose:amylopectin ratio on the properties of pea, potato and cassava starch films and the effect of the incorporation of rice bran of two different particle sizes were studied. The structural, mechanical, optical and barrier properties of the films were analyzed after 1 and 5 weeks. The high content of amylose gave rise to stiffer, more resistant to fracture, but less stretchable films, with lower oxygen permeability and greater water binding capacity. Although no changes in the water vapour permeability values of the films were observed during storage, their oxygen permeability decreased. Throughout storage, films became stiffer, more resistant to break, but less stretchable. Rice bran with the smallest particles improved the elastic modulus of the films, especially in high amylose content films, but reduced the film stretchability and its barrier properties, due to the enhancement of the water binding capacity and the introduction of discontinuities.

1. Introduction

Conventional plastics are synthetic polymers derived from petroleum whose residues are not easily assimilated in the environment. This fact has led to the increasing use of biodegradable raw materials to obtain biodegradable films as an alternative to petroleum-derived polymers in different sectors, such as agricultural, medical or pharmaceutical. Nowadays, the use of edible films and coatings based on biodegradable polymers is increasing because these materials are environmentally friendly (Chen, Liu, Chen, Chen, & Chang, 2008; Mehyar & Han, 2004) and exhibit properties which can become similar to those observed in conventional plastics (Famá, Goyanes, & Gerchenson, 2007; Jiménez, Fabra, Talens, & Chiralt, 2012a; Rindlav-Westling, Stading, Hermansson, & Gatenholm, 1998).

Materials intended biodegradable packaging are classified according to their molecular structure; being polysaccharides, proteins and fats the most widely used (Adebiyi, Adebiyi, Jin, Ogawa, & Muramoto, 2008; Falguera, Quintero, Jiménez, Muñoz, & Ibarz, 2011; Gnanasambandam, Hettiarachchy, & Coleman, 1997; Mehyar & Han, 2004; Nam, Scanlon, Han, & Izydorczyk, 2007). Among the polysaccharides, starch, cellulose and their derivates are very commonly studied as film-forming compounds (Chen, Liu, Chang, Cao, & Anderson, 2009a; Jiménez et al., 2012a).

Starch is a polysaccharide from cereals (corn, wheat or rice), legumes (pea) and tubers (potato or cassava). It has a granular structure and is composed of two macromolecules: amylose and amylopectin. Amylose is a lineal polymer formed by glucose units linked by α-(1,4) whereas amylopectin is a highly branched polymer of glucose units with ramifications in α-(1,6). The amylose:amylopectin ratio depends on the source of starch and this ranges from 15:85 to 35:65, except in waxy starch and high amylose corn starch whose amylose content is about 5% and 50–80%, respectively (Liu, 2005). It is known that both polymers are responsible for the starch crystallization which leads to changes in the mechanical response (increased stiffness) of starch products (Talja, Helén, Roos, & Jouppila, 2007).

Starch is used to obtain films because of its high availability and great ability to form an odourless, colourless and transparent (Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009) polymer matrix with low oxygen permeability, which is very interesting for food preservation (Dole, Joly, Espuche, Alric, & Gontard, 2004; Han, Seo, Park, Kim, & Lee, 2006; Jiménez et al., 2012a; Liu, 2005). It is also especially attractive because of its biodegradability and low cost (Chen et al., 2008; Han et al., 2006; Lafargue, Lourdin, & Doublier, 2007). Nevertheless, starch films present some drawbacks: unstable mechanical properties due to the retrogradation phenomenon and a relatively high water vapour permeability (Chen et al., 2008; Lafargue et al., 2007; Phan The, Debeaufort,

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Voilley, & Luu, 2009; Wu, Liu Ch Chen, Chen, Anderson, & Chang, 2010).

In starch films, the retrogradation phenomenon over time, can greatly affect not only their mechanical properties but also their barrier capacity. Hence, the study of changes occurring during their storage is necessary to ensure their functionality at different times after processing. Jiménez, Fabra, Talens, and Chiralt (2012b) studied the effect of re-crystallization on physical properties of corn starch films containing fatty acids and concluded that fatty acid incorporation did not notably improve water vapour permeability while the degree of crystallinity of the matrix increased during storage time. In order to improve properties of the starch films different strategies have been used by different authors. Da Matta, Silveira, de Oliveira, and Sandoval (2011) evaluated mechanical properties of edible films made from wrinkled pea starch rich in amylose combined with xanthan gum and glycerol and they observed that the increase in xanthan gum concentration did not affect the physical and mechanical properties of the films. For potato starch films, Zhang, Thompson, and Liu (2011) studied how cellulose fibre and potato pulp affected the properties of thermoplastic starch. The addition of fibre did not affect the film glass transition. Nevertheless, moisture content, and the hydrophilic character of films increased in line with the fibre content. Souza et al. (2011) stated that starch films containing glycerol and clay nanoparticles are interesting biodegradable materials for packaging.

Natural fibres from plant origin can be used to improve barrier and mechanical properties of starch films as reported by Chen et al. (2009a) for pea starch films with pea hull fibre nanoparticles. Film transparency, tensile strength, elongation at break and water barrier properties were improved due to the high content of cellulose crystalline regions and the interactions between the nanofibre and the starch matrix. Famá, Gerschenson, and Goyanes (2009) introduced wheat bran as filler in cassava starch matrices, thus improving their mechanical and water vapour permeability.

Bran rice is a by-product of rice which is obtained from its bleaching and it represents about 10% of the grain weight. Rice bran contains good quality biological proteins, fats and starch. Depending on the variety of rice and the type of processing, rice bran contains about 15–20% fat, 12–16% protein, 23–28% fibre and 7–10% ash (Sánchez, Quintero, & González, 2004). Bran also has a high vitamin B and E complex (as α-tocopherol) content (Carroll, 1990).

Despite its interesting composition, rice bran is not given the importance it deserves since it is only used in animal food. Nevertheless, in recent years, different bran applications have been developed. This has been evaluated as a source of oil (Nikolosi, Ausman, & Hegstead, 1996), protein concentrates (Gnanasambandam & Hettiarachache, 1995) or material for obtaining edible films (Adebiyi et al., 2008; Dias, Müller, Larotonda, & Laurindo, 2010; Gnanasambandam et al., 1997).

The aim of this work was to analyze the influence of the different amylose:amylopectin ratios on the properties of films obtained from three different starches (pea, potato and cassava), and the effect of the addition of rice bran with two different particle sizes, as a film filler. Structural, mechanical, optical and barrier properties of the films were analyzed at different storage times in order to compare their behaviour and functionality.

2. Materials and methods

2.1. Materials

Pea (PE) and potato (PO) starch were purchased from Roquette (Lestrem, France) and cassava starch (CAS) obtained from Asia Modified Starch CO; LDT (Kalasin, Thailand). Rice bran was provided from Arrocería Antonio Tomás, S.L. (Sollana, Valencia, Spain). Glycerol was provided by Panreac Química S.A. (Castellar de Vallès, Barcelona, Spain).

2.2. Amylose–amylopectin ratio

Amylose–amylopectin ratio in each starch (pea, potato and cassava) was determined in triplicate, by using an Amylose/Amylopectin Assay Procedure enzymatic kit (Megazyme International Ireland, Bray Business Park, Bray, Co. Wicklow, Ireland).

2.3. Rice bran particle size

Two different bran fractions were obtained by sieving. The smallest fine particle size fraction (*F*), which passed through a 100 mm mesh; and the coarse fraction (*C*), which contained the fraction between 250 and 100 mm mesh.

The rice bran particle size, surface weighted mean diameter ($D_{3,2}$, Eq. (1)) and volume weighted mean diameter ($D_{4,3}$, Eq. (2)) were determined in bran aqueous dispersions and with ultrasonic homogenization, in triplicate, with a laser light scattering instrument (Malvern Instruments Ltd, Worcestershire, U.K.). Particle size measurements were taken for two different fractions. The differences between diameters indicate that particle size distributions are wide or that they are irregularly-shaped.

$$D_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (1)$$

$$D_{3,2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (2)$$

2.4. Compositional analysis of rice bran

Moisture content (*MC*) was determined from sample weight loss when samples were introduced into a convection oven at 100 °C for 24 h and, afterwards, equilibrated in desiccators with P_2O_5 for 2 weeks until constant weight.

Ash content was obtained by applying the gravimetric method 104/1 of the International Association for Cereal Science and Technology (ICC, 1990). The rice bran was introduced into a muffle "Select-Horn" (J.P. Selecta; Abrera, Barcelona, Spain) at 910 °C for 15 min.

Protein content was obtained by means of the Kjeldahl method, by using a digestion unit "Bloc-digest" (J.P. Selecta; Abrera, Barcelona, Spain) and a Kjeldahl distiller "Pro-Nitro M" (J.P. Selecta; Abrera, Barcelona, Spain); a conversion factor of $F = 5.95$ was used.

Fat content was obtained by using the Soxhlet method 30-20 (ICC, 1967). Samples were firstly dried at 103 °C and then the fat was extracted by an oil extractor "Det-GrasasN" (J.P. Selecta; Abrera, Barcelona, Spain).

Starch content of the rice bran was determined using the enzymatic Kit "Starch Assay Kit", (Sigma, Saint Louis, Missouri, USA).

Fibre content was estimated by means of the difference in terms of component percentages.

2.5. Preparation of films

Film forming dispersions contained 2% w/w of starch (pea, potato or cassava) and a starch:glycerol ratio of 1:0.25, on the basis of previous studies (Jiménez et al., 2012a). For starch films containing rice bran as filler, six formulations were obtained by adding fine (*F*) or coarse (*C*) rice bran to the starch dispersions. The bran was incorporated in a starch:rice bran ratio of 1:0.1.

Starch aqueous dispersions were maintained at 95 °C for 30 min to induce starch gelatinization. Then, glycerol was added and the

dispersion was homogenized using a rotor-stator homogenizer (Ultraturrax D125, Janke and Kunkel, Germany) at 13,500 rpm for 1 min and 20,500 rpm for 3 min at 95 °C under vacuum. For starch films containing rice bran, this was incorporated prior to the homogenization step. The film-forming aqueous dispersions (equivalent to 1.5 g) were cast into a levelled Teflon casting plates (15 cm diameter) and dried at 25 °C and 45%RH for 48 h. Then, they were peeled intact from the plates and conditioned at 53% RH and 25 °C in a chamber with magnesium nitrate-6-hydrate saturated solution (Panreac química, S.A., Castellar del Vallés, Barcelona, Spain). Film thickness was measured at six random positions with a Palmer digital micrometer to the nearest 0.0025 mm. All films were analyzed after one or five storage weeks, according to previous studies (Jiménez et al., 2012a), which revealed that notable changes can be observed in this period in starch films.

2.6. Characterization of films

2.6.1. X-ray diffraction spectra

X-ray diffraction spectra were obtained using a Diffractometer D8 Advance (Bruker AXS, 230 V, 50 Hz and 6.5 KVA, Karlsruhe, Germany). For this analysis conditioned samples were cut into squares of 4 cm and mounted on a carbon base. Spectra were obtained at 2θ between 5 and 30, using $K\alpha$ Cu radiation (λ: 1542 Å), 40 kV and 40 mA with a step size of 0.04982.

2.6.2. Microstructural properties

Microstructural analysis of films was carried out using a scanning electron microscope (SEM) (JEOL®, model JSM-5410, Japan) and an atomic force microscope (AFM) (Multimode 8, Bruker AXS, Inc. Santa Barbara, California, USA) with a NanoScope® V controller electronics. To this end, films were equilibrated in desiccators with P_2O_5 to eliminate some water present in the samples.

SEM observations were carried out on the film surface and in their cross section. To prepare the samples, films were frozen in liquid N_2 and cryofractured to observe the cross section. Samples were fixed on copper stubs, gold coated, and observed using an accelerating voltage of 11 kV. Three replicates per formulation were observed.

AFM with the PeakForce QNM (Quantitative NanoMechanics) was used to analyze surface film nanostructure. Measurements were taken from small areas of the film surface ($20 \times 20\ \mu m$) and the resulting data were transformed into 2D image and their scale is expressed as Log DMT modulus. Three images were captured per formulation, for samples stored for 1 and 5 weeks.

2.6.3. Moisture content

To determine film moisture content, five replicates by formulation were dried in a vacuum oven at 60 °C for 24 h, and then they were equilibrated with P_2O_5 until constant weight.

2.6.4. Water vapour permeability

The water vapour permeability (WVP) of films was determined following the gravimetric method ASTM E96-95 (1995) by using Payne permeability cups (Payne, elcometer SPRL, Hermelle/sd Argenteau, Belgium) of 3.5 cm diameter. The temperature was 25 °C and the relative humidity gradient was 53–100%, which was obtained using magnesium nitrate-6-hydrate and pure water, respectively. Cups were introduced into desiccators and these into a temperature-controlled chamber at 25 °C. Control of cup weights was performed every 2 h using an analytical balance (±0.00001 g). The water vapour transmission (WVTR) was determined from the slope obtained from the regression analysis of weight loss data versus time, once the steady state had been reached, divided by the film areas. For each type of film, WVP measurements were replicated four times.

2.6.5. Oxygen permeability

The oxygen permeability (*OP*) was obtained by using an Oxtran System (Mocon, Minneapolis, USA). Measurements were taken at 25 °C following the standard method (ASTM D3985-05, 2005) at 53% RH, using 50 cm^2 film samples. Oxygen permeability was calculated by dividing the oxygen transmission by the difference in oxygen partial pressure between the two sides of the film, and multiplying by the average film thickness. At least two replicates per formulation were considered.

2.6.6. Mechanical properties

Mechanical properties were measured with a Universal Test Machine (TA.XT plus, Stable Micro Systems, Haslemere, England), following the ASTM standard method D882 (ASTM, 2001), by using 50 mm/min. Equilibrated film samples (2.5 cm × 10 cm) were mounted in the film-extension grips (A/TG model), which were set 50 mm apart. Stress-strain curves were obtained and the tensile strength at break (TS), percentage of elongation at break (ε (%)) and elastic modulus (EM) were calculated. Eight replicates carried out per formulation.

2.6.7. Optical properties

The opacity of films was determined by applying the Kubelka–Munk theory of multiple dispersion to the reflection spectra (Hutchings, 1999; Judd & Wyszecki, 1975). Internal transmittance (*Ti*) of the films was quantified using equation (3). In this equation R_0 is the reflectance of the film on an ideal black background. Parameters a and b were calculated by Eqs. (4) and (5), where R is the reflectance of the sample layer backed by a known reflectance R_g. The reflection spectra on the white and black background was determined from 400 to 700 nm with a MINOLTA spectrocolorimeter CM.36000d (Minolta Co. Tokyo, Japan). Measurements were performed in the side of film which was in contact with air during drying and each formulation was analyzed in triplicate.

$$T_i = \sqrt{(a - R_0)^2 - b^2} \tag{3}$$

$$a = \frac{1}{2}\left(R + \frac{R_0 - R + R_g}{R_0 R_g}\right) \tag{4}$$

$$b = (a^2 - 1) \tag{5}$$

Gloss measurements were obtained according to the ASTM standard D523 method (ASTM, 1999), using a flat surface gloss meter (Multi-Gloss 268, MINOLTA) at an angle of 60° with respect to the normal to the film surface. Three films of each formulation were measured over a black matte standard plate. Results were expressed as gloss units, relative to a highly polished surface of standard black glass with a value close to 100.

2.7. Statistical analysis

The analysis of data was performed through variance analysis (ANOVA) using the Statgraphics Plus 5.1. software (Manugistics Corp., Rockville, MD). To discern between samples the Fisher least significant difference (LSD) ($p < 0.05$) was used.

3. Results and discussion

3.1. Characteristics of starches: Amylose–amylopectin ratio

Properties of starch films, such as mechanical behaviour, depend on the amylose:amylopectin ratio since the different behaviour of

amylose and amylopectin molecules contributes to film properties (Da Matta et al., 2011). The amylose content (w/w, %) of pea, potato and cassava starches were 24.9 ± 0.9, 17.9 ± 1.9 and 9 ± 2, respectively, with an amylose/amylopectin ratio of 1/3.0, 1/4.6 and 1/9.9, respectively. These values reflect an important difference between these starches, pea starch being the richest in amylose and cassava starch the poorest. Although the obtained values are in the reported range for the different starches, differences associated to origin or cultivar could be observed. Chen et al. (2008), Ma, Chang, and Yu (2008), Mehyar and Han (2004), and Zhang and Han (2006) reported higher amylose contents (30–40%) in pea starch. For potato starch the value obtained coincides with the result reported by Talja, Peura, Serimaa, and Jouppila (2008) whereas it was lower than that reported by Alvani, Qi, Tester, and Snape (2011) (25.2–29.1%). For cassava starch, higher amylose contents (19.7–22.5%) were found by Souza et al. (2011).

3.2. Properties of starch films

3.2.1. X-ray diffraction

Fig. 1 shows the X-ray diffraction spectra of pure starch films to analyze the re-crystallization progress in the films. For starch matrices, the crystalline structure was mainly attributed to the spontaneous recrystallization of amylose molecules after gelatinization (Forssell, Helleman, Myllärinen, Moates, & Parker, 1999; Myllärinen, Buleon, Lahtinen, & Forssell, 2002; Rindlav-Westling et al., 1998). This process occurs mainly during film drying when the chain mobility is still high due to the water content. Rindlav-Westling et al. (1998) reported that drying conditions at high relative humidity, or long drying times, greatly promote amylose crystallization, whereas amylopectin shows a retarded crystallization when the molecular mobility in the system is high enough.

After both 1 and 5 weeks of storage, pea starch films exhibited the highest crystallinity, as deduced from the greater intensity of its sharp peaks. On the contrary, the lowest crystallinity was found in cassava starch films, where an amorphous X-ray diffraction pattern was observed after both storage times. This behaviour can be related with the different amylose:amylopectin ratio and confirms that the crystallization progress in the films was faster as the amylose content increased. This was also observed by other authors in gelatinized starch (García, Martino, & Zaritzky, 2000), whereas for native starch the higher crystallinity in granules is associated with a greater content of amylopectin (Cheetham & Tao, 1998).

A typical C-type crystallinity pattern was found in pea starch films. This type of crystallinity is an intermediate form between A and B types, as reported by Carvalho (2008). In this sense, pea starch films showed peaks at 2θ 5.4° (characteristic of B-type polymorphs), 14.8° (characteristic of A-type polymorphs), 16.8° (characteristic of both A and B-type polymorphs) and 19.0° and 21.8° (characteristic of B-type polymorphs), in agreement with that reported by Chen et al. (2009a), Da Matta et al. (2011) and Wu et al. (2010). In the case of potato starch films, a typical C-type pattern can also be observed, with peaks at 5.1°, 11.7° and 17.2° of Bragg angle. Nevertheless, the peaks are smaller and less sharp as compared with those obtained in pea starch films, which indicates that the film exhibited a more amorphous character with smaller crystallites (Talja et al., 2008). Cassava starch films were mainly amorphous since no sharp peaks were found, as previously observed by other authors (Chen, Kuo, & Lai, 2009b).

Comparisons of difractograms after 1 and 5 storage weeks allow us to conclude that no significant changes in the crystallinity occur throughout the storage period in any case, probably due to the low moisture content of the films which inhibits the chain mobility to form crystalline associations for both amylose and amylopectin polymers. In this sense, several authors (Forssell et al., 1999; Myllärinen et al., 2002; Rindlav-Westling et al., 1998; Rindlav, Hulleman, & Gatenholm, 1997) have also found a fast crystallization of amylose during the film formation, whereas amylopectin crystallizes very slowly.

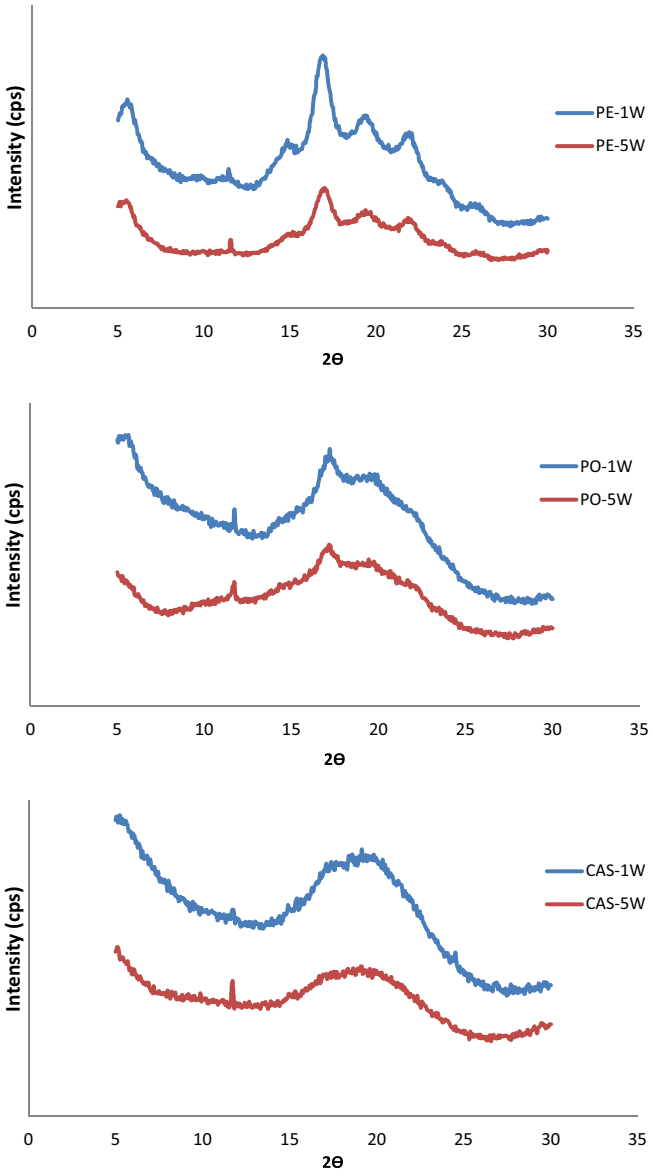

Fig. 1. X-ray diffraction pattern of pea (PE), potato (PO) and cassava (CAS) starch films at one (1*W*) and five (5*W*) storage weeks.

3.2.2. Microstructural features

SEM and AFM microstructure analyses provide information about the surface morphology and internal microstructure of the films. SEM images of the surface and cross section of the different starch films is shown in Fig. 2. In general, starch films showed a homogeneous aspect, thus indicating that the gelatinization step was enough to disrupt all the starch granules. Smooth film surfaces were also previously observed by other authors for starch films obtained by casting (Chen et al., 2009a,b; Wu et al., 2010). Nevertheless, in the cross section image, the presence of a heterogeneously-fractured layer on the film surface in the pea starch sample reveals the progress of crystallization in this zone, probably due to the greater molecular mobility associated to the water vapour diffusion near the film surface. The presence of microcracks in cassava starch films is remarkable. This may be due to the electron impact during observation, as explained by Jiménez, Fabra,

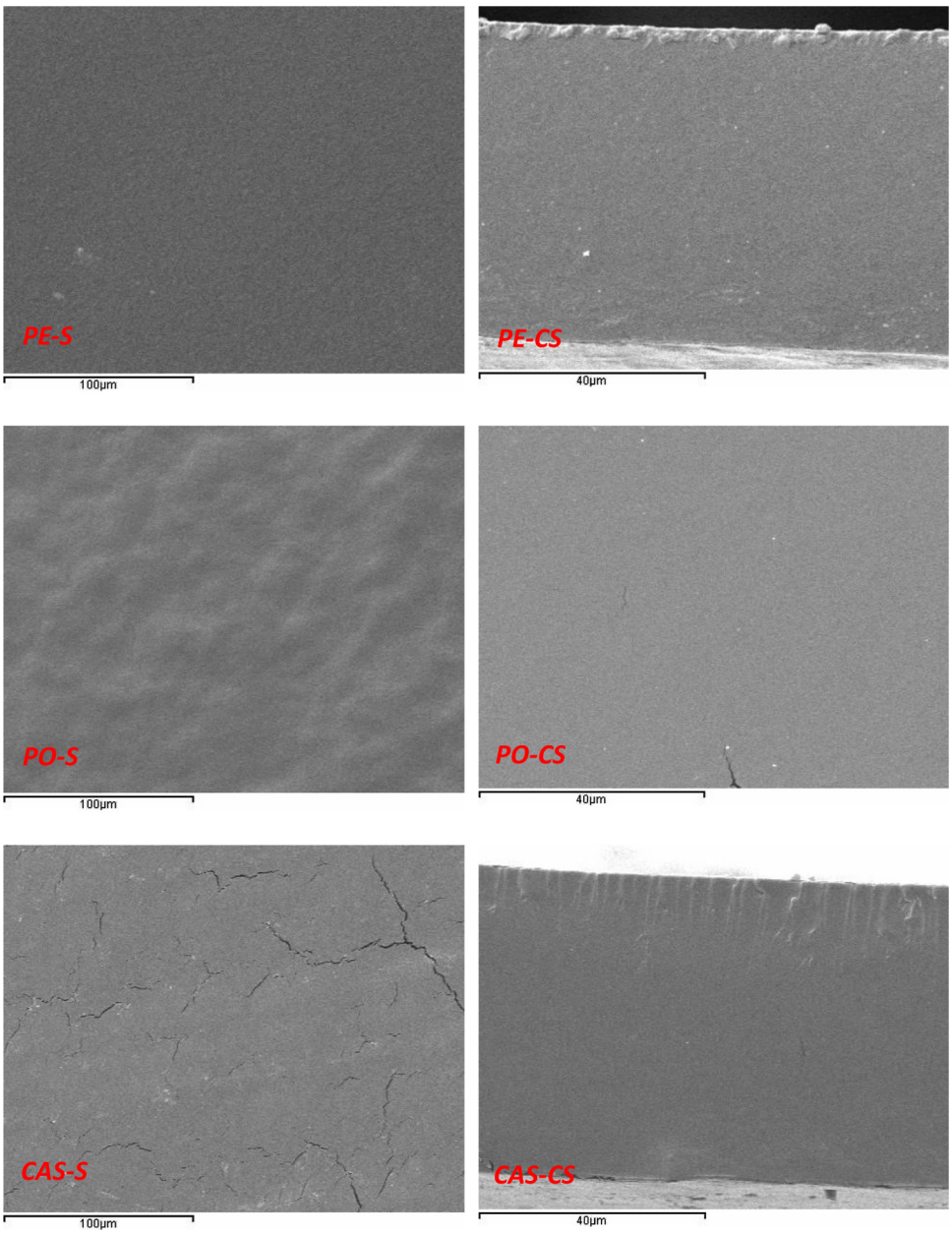

Fig. 2. SEM features of surface (*S*) and cross section (CS) of pea (PE), potato (PO) and cassava (CAS) starch films.

Talens, and Chiralt (2012c), and it reveals the lower mechanical resistance of this sample.

Fig. 3 shows AFM images of pea, potato and cassava starch films, obtained by using PeakForce QNM. Differences in the surface mechanical resistance can be observed in the samples at both 1 and 5 storage weeks. The surfaces of pea and potato starch films are rougher, which indicates the co-existence of crystalline (harder) and amorphous (softer) zones. It is remarkable that these zones are wider in pea starch than in potato starch samples, in agreement with the sharper peaks reflected in the X-ray spectra, associated with bigger crystals. No notable differences were appreciated between 1 and 5 storage weeks.

In cassava starch samples, lower values of DMT modulus can be observed, due to the more amorphous character of the films. It is remarkable that in these films, a harder surface was detected at 5 storage weeks (higher values of modulus). This reveals that films were significantly hardened during storage. Nevertheless, no crystallization was detected since the surface appears homogenous. The different behaviour of the starch matrices was coherent with the different amylose:amylopectin ratio, which is associated with a different recrystallization progress during film formation.

3.2.3. *Moisture content and barrier properties*

Table 1 shows the moisture content values of the studied films. The values ranged between 9.9 and 11.4%. Pea and potato starch films were the samples which exhibited the highest moisture content, as reported in previous studies (Kaisangsti, Kerdchoechuen, & Laohakunjit, 2012; Mehyar & Han, 2004). This can be associated with the higher degree of crystallization, since crystalline zones bond a greater amount of water than amorphous zones (Forssell et al., 1999; Myllärinen et al., 2002; Rindlav-Westling et al., 1998). Nevertheless, moisture content significantly ($p < 0.05$) decreased after 5 storage weeks, when more homogeneous values of moisture content were obtained for the different films. This indicates that films equilibrate slowly with the conditioning relative humidity, reaching a value closer to the equilibrium by losing water during

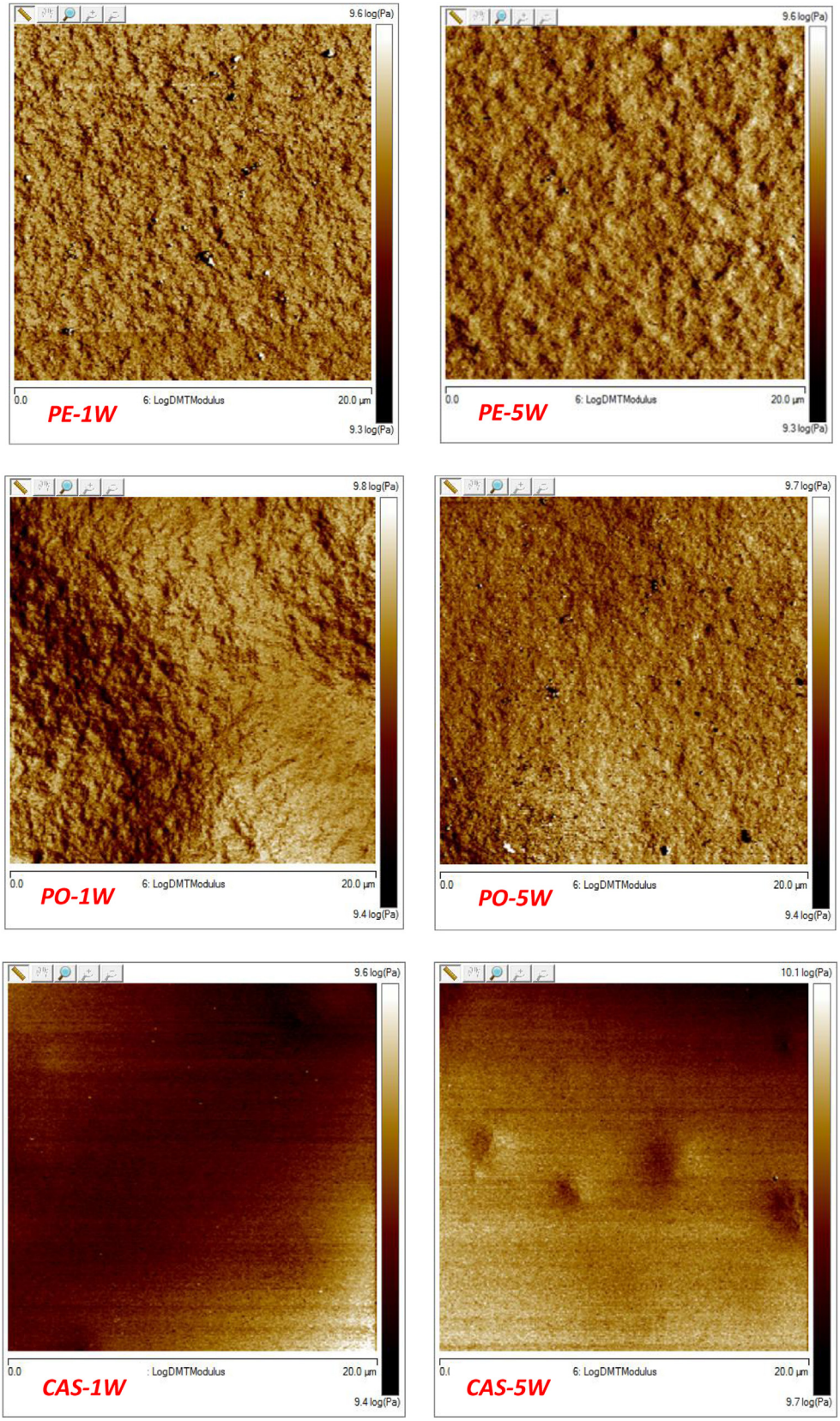

Fig. 3. Maps of Log DTM modulus obtained from AFM in surface of pea (PE), potato (PO) and cassava (CAS) starch films for samples stored for one (1*W*) and five (5*W*) storage weeks.

Table 1
Moisture content (*MC*), water vapour permeability (*WVP*) and oxygen permeability (*OP*) of pea (PE), potato (PO) and cassava (CAS) starch films at 1 and 5 storage weeks. Films with fine (*F*) and coarse (*C*) fractions incorporated to starch films were also included. Results are the mean ± standard deviation.

Film	*MC* d.b. (%)		*WVP* (g mm/kPa h m^2)		*OP* (10^{-14} cm^3/m s kPa)	
	1 week	5 week	1 week	5 week	1 week	5 week
PE	11.4 ± 0.4^{a1}	8.7 ± 0.4^{a2}	6.0 ± 0.3^{a1}	6.7 ± 0.7^{a1}	3.8 ± 0.3a^{1}	2.7 ± 0.2^{ab2}
PO	11.4 ± 0.7^{a1}	8.5 ± 0.3^{a2}	6.1 ± 0.5^{a1}	7.2 ± 0.2^{a1}	4.55 ± 0.07^{b1}	3.4 ± 0.3^{a2}
CAS	9.9 ± 0.9^{b1}	8.2 ± 0.3^{a2}	5.4 ± 0.4^{a1}	6.8 ± 0.5^{a2}	4.2 ± 0.4^{b1}	2.45 ± 0.12^{b2}
PE-*F*	9.99 ± 0.08^{a1}	9.53 ± 0.19^{b2}	6.35 ± 0.18^{a1}	6.3 ± 0.4^{a1}	4.5 ± 0.2^{1}	1.84 ± 0.08^{2}
PE-*C*	13.1 ± 0.9^{b1}	10.9 ± 0.8^{c2}	7.5 ± 0.9^{b1}	8.6 ± 0.4^{b2}		
PO-*F*	14.9 ± 1.4^{a1}	10.1 ± 0.9^{b2}	8.1 ± 0.9^{a1}	6.5 ± 0.7^{a2}	4.9 ± 0.2^{1}	3.06 ± 0.19^{2}
PO-*C*	16.1 ± 0.9^{a1}	11.4 ± 0.8^{c2}	10.3 ± 1.2^{b1}	9.2 ± 0.6^{b2}		
CAS-*F*	10.3 ± 0.5^{a1}	8.78 ± 1.09^{a2}	7.5 ± 0.4^{a1}	5.1 ± 0.8^{a2}	5.46 ± 0.07^{1}	3.56 ± 0^{2}
CAS-*C*	12.9 ± 1.2^{b1}	8.1 ± 0.3^{a2}	7.3 ± 0.9^{a1}	8.3 ± 0.2^{b2}		

Different letters (a, b, c) in the same column indicate significant differences among starch matrix and fine or coarse rice bran in the same matrix ($p < 0.05$).
Different numbers (1, 2) in the same row indicate significant differences among storage times for the same formulation ($P < 0.05$).

storage. The water loss will provoke a greater chain aggregation in the amorphous region which will imply an increase in the film compactness that will affect barrier and mechanical properties.

Water vapour permeability (*WVP*) values relate the final application of a film in contact with food systems and they must be as low as possible to avoid water transfer (Ma et al., 2008). Table 1 shows *WVP* values of starch films analyzed at 25 °C and a 53–100% RH gradient. No significant differences between *WVP* values of the different films were found at the different storage times, in agreement with results found by other authors (Han et al., 2006; Ma et al., 2008). The small changes in sample moisture content and the subsequent increase in the matrix compactness did not affect the water vapour barrier properties of the films.

Table 1 shows the mean values of oxygen permeability (*OP*) after the different storage times. For pea starch films, similar values have been reported by Mehyar and Han (2004). After one week, the *OP* values were significantly lower for films with the highest content of amylose (PE), which indicates that this polymer is mostly responsible for the oxygen barrier ability of the films. This coincides with that reported by García et al. (2000) for plasticized corn and amylomaize starch films. Likewise, Forssell, Lahtinen, Lahelin, and Myllärinen (2002) reported that unplasticized amylose films exhibited lower oxygen permeability than amylopectin films, regardless of their equilibration at different relative humidities. Nevertheless, the plasticizer content, in combination with the water content, had a great influence on the oxygen permeability values of starch films. *OP* values were significantly ($p < 0.05$) reduced after 5 storage weeks, coherently with the increment of the matrix compactness previously. In general, the *OP* values are very low and, as reported by Shen, Wu, Chen, and Zhao (2010), one great advantage of starch films is their ability to protect food products by forming an oxygen barrier.

3.2.4. Mechanical properties

Elastic modulus (*EM*), tensile strength at break (*TS*) and percentage of elongation at break (ε (%)) are the usual parameters with which to describe the mechanical behaviour of films, and they are closely related to the film microstructure (McHugh & Krochta, 1994). *TS* and ε (%) represent the film's resistance to elongation and its stretching capacity, respectively, whereas *EM* is a measure of the stiffness of films. Table 2 shows the mean values of these mechanical parameters for the starch films. The obtained values are similar to that reported by other authors for pea starch films (Chen et al., 2008; Da Matta et al., 2011), potato starch films (Cyras, Manfredi, Ton-That, & Vázquez, 2008) and cassava starch films (Famá, Rojas, Goyanes, & Gerschenson, 2005; Souza et al., 2011), respectively.

After one week of storage, the mechanical parameter values were significantly different ($p < 0.05$) for the three matrices. The pea starch films have the highest values in break strength and stiffness and the lowest in stretchability. On the contrary, cassava starch films exhibited the lowest break strength values and the highest in stretchability. This indicates the important role played by crystal formation in the mechanical behaviour of the matrix. After 5 weeks of storage, film stiffness and resistance to break increased for all the films, whereas film stretchability decreased, which coincides with the increase in the matrix compactness promoted by water loss. Nevertheless, the highest relative changes occurs for cassava starch films, which could indicate the formation of very small association zones of amylopectin chains between 1 and 5 weeks.

3.2.5. Optical properties

The optical properties of the films, gloss and transparency, are directly related with the film microstructure (previously described) and are affected by the surface and internal heterogeneity of the structure (Jiménez et al., 2012b). According to Hutchings (1999),

Table 2
Elastic modulus (*EM*), tensile strength at break (*TS*) and percentage of elongation at break (ε (%)) of pea (PE), potato (PO) and cassava (CAS) starch films at two storage time (1 week and 5 weeks). Films with fine (*F*) and coarse (*C*) fractions incorporated to starch films were also included Results are the mean ± standard deviation.

Film	*EM* (MPa)		*TS* (MPa)		ε (%)	
	1 week	5 week	1 week	5 week	1 week	5 week
PE	417 ± 41^{a1}	964 ± 88^{a2}	14.2 ± 1.3^{a1}	24 ± 2^{a2}	10 ± 2^{a1}	4.7 ± 0.9^{a2}
PO	40 ± 24^{b1}	430 ± 44^{b2}	3.04 ± 0.79^{b1}	11.6 ± 1.5^{b2}	29 ± 3^{b1}	9.4 ± 1.8^{b2}
CAS	20 ± 7^{b1}	771 ± 171^{c2}	1.7 ± 0.4^{c1}	12.5 ± 1.7^{b2}	48 ± 9^{c1}	1.8 ± 0.5^{c2}
PE-*F*	663 ± 229^{a1}	610 ± 72^{a1}	16 ± 7^{a1}	6.5 ± 0.9^{a2}	3.1 ± 0.9^{1a}	1.3 ± 0.2^{2a}
PE-*C*	618 ± 38^{a1}	579 ± 61^{a1}	13.7 ± 1.5^{a1}	6 ± 3^{a2}	4.3 ± 0.8^{1b}	1.2 ± 0.6^{2a}
PO-*F*	36 ± 9^{a1}	460 ± 98^{a2}	1.6 ± 0.4^{a1}	5.8 ± 1.4^{a2}	25 ± 13^{1a}	1.6 ± 0.6^{2a}
PO-*C*	108 ± 49^{b1}	478 ± 94^{a2}	1.8 ± 0.7^{a1}	5.9 ± 1.9^{a2}	9 ± 3^{1b}	1.5 ± 0.3^{2a}
CAS-*F*	33 ± 9^{a1}	543 ± 137^{a2}	1.2 ± 0.5^{a1}	6 ± 4^{a2}	42 ± 24^{1a}	1.2 ± 0.7^{2a}
CAS-*C*	43 ± 15^{a1}	387 ± 94^{a2}	1.5 ± 0.7^{a1}	3.57 ± 1.07^{a2}	16 ± 4^{1a}	1.11 ± 0.44^{2a}

Different letters (a, b, c) in the same column indicate significant differences among starch matrix and fine or coarse rice bran in the same matrix ($p < 0.05$).
Different numbers (1, 2) in the same row indicate significant differences among storage times for the same formulation ($p < 0.05$).

Table 3
Gloss values at 60° and internal transmittance (*Ti*) of pea (PE), potato (PO), and cassava (CAS) starch films at two storage times (1 week and 5 weeks). Films with fine (*F*) and coarse (*C*) fractions incorporated to starch films were also included. Results are the mean ± standard deviation.

Film	60°		*Ti* (450 nm)	
	1 week	5 week	1 week	5 week
PE	47 ± 17^{a1}	33 ± 8^{a1}	85.4 ± 1.6^{a1}	87.09 ± 0.12^{a1}
PO	9.9 ± 0.9^{b1}	9.7 ± 1.9^{b1}	85.9 ± 0.4^{a1}	85.09 ± 0.54^{a1}
CAS	18 ± 4^{c1}	16 ± 5^{c1}	84.9 ± 0.4^{a1}	86.6 ± 0.4^{b1}
PE-*F*	30 ± 4^{b1}	20 ± 5^{a2}	81.7 ± 0.2^{a1}	82.3 ± 0.5^{a1}
PE-*C*	14 ± 5^{a1}	13.5 ± 1.6^{b1}	81.8 ± 0.5^{a1}	81.5 ± 0.2^{a1}
PO-*F*	6.45 ± 1.07^{a1}	8.2 ± 0.7^{a2}	79.1 ± 1.4^{a1}	81.35 ± 1.02^{a1}
PO-*C*	8.75 ± 1.04^{b1}	6.9 ± 0.8^{b2}	80.8 ± 0.4^{a1}	80.6 ± 0.7^{a1}
CAS-*F*	16 ± 3^{a1}	11 ± 3^{a2}	81.7 ± 0.7^{a1}	82.0 ± 0.3^{a1}
CAS-*C*	13.5 ± 1.6^{a1}	15.4 ± 1.7^{a2}	81.3 ± 0.5^{a1}	81.09 ± 0.19^{a1}

Different letters (a, b, c) in the same column indicate significant differences among starch matrix and fine or coarse rice bran in the same matrix ($p < 0.05$).
Different numbers (1, 2) in the same row indicate significant differences among storage times for the same formulation ($p < 0.05$).

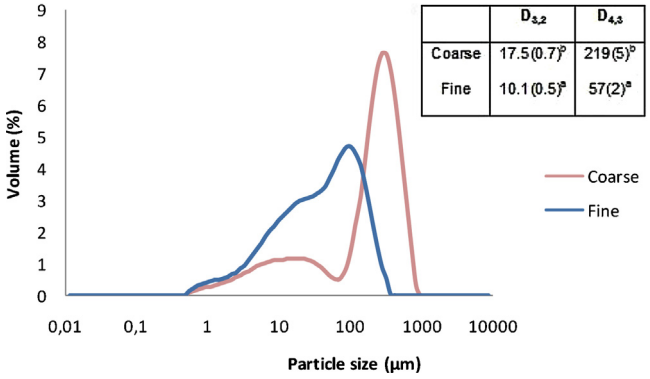

Fig. 4. Typical particle size distributions of the different bran fractions. $D_{3,2}$ and $D_{4,3}$, mean values ± standard deviation.

the above-mentioned parameters are the best optical properties with which to evaluate the appearance of the films. Table 3 shows the mean values of the internal transmittance (*Ti*) of films measured at 450 nm. The *Ti* of films is related to their degree of transparency and structural homogeneity: low *Ti* values are related to a high structural heterogeneity and with a greater opacity. After one week of storage, different starch based films did not show any significant differences ($p < 0.05$) as regards the *Ti* values, these being about 85%. These coincide with those reported in the case of corn starch films by Jiménez et al. (2012b). After five weeks, no significant changes in transparency occurred in the films. Table 4 also shows the mean gloss values of starch films at an incidence angle of 60° with respect to the normal to the film surface. At initial time, the gloss values of pea starch films were higher than those corresponding with cassava and potato starch films. The differences observed in the film gloss at initial time remained after 5 weeks of storage, since in no case did any significant changes in gloss occur during weeks of storage. The higher gloss of pea starch films could be due to the presence of crystalline structures at surface level, as deduced from the SEM micrographs and the higher surface modulus obtained by AFM.

Obtained results reveals that films obtained from amylose-rich starch are stiffer, more resistant to fracture, glossier, less permeable to oxygen and less extensible, than amylopectin-rich starch films. All the films became harder and more resistant during storage, and those richer in amylopectin become shorter (less stretchable). The oxygen permeability slightly decreased throughout storage time in every case.

3.3. Effect of rice bran addition on film properties

3.3.1. Rice bran characteristics

The bran particle size distribution curves for the two bran fractions (*F* and *C*) obtained by sieving are shown in Fig. 4. Mean diameters ($D_{3,2}$ and $D_{4,3}$) are also shown in Fig. 4. Differences between the particle size distributions of two fractions can be observed, although a certain degree of curve overlapping was obtained, since sieving only partially separates the particles by size. Significant differences ($p < 0.05$) between $D_{3,2}$ and $D_{4,3}$ were found for both fine and coarse fractions, thus indicating the presence of irregular particles of very different sizes. Fig. 5 shows the SEM micrographs of the powder of both bran fractions. Fraction *F* contains more spherical particles than fraction *C* which, in turn, contain composite (with different aggregated components) particles. The composition of both fractions is shown in Table 4. The mean values of the moisture, protein, fat and ash contents were very similar for *F* and *C* fractions, but significant differences were found for starch and fibre contents; the fine fraction was richer in starch (twice times richer) whereas the coarse fraction contained more fibre. The obtained composition of the two rice bran fractions coincides with data previously reported by Sánchez et al. (2004). The protein content is similar to that reported by Gnanasambandam et al. (1997) and Rodríguez (2007), while Pacheco, Peña, and Ortiz (2002) obtained a similar fat content. These authors observed that the varietal effect and smoothening method may cause significant differences in the ash, fat, protein, starch and fibre content of rice bran.

3.3.2. Properties of starch films containing bran

3.3.2.1. Effect on microstructural properties. Figs. 6 and 7 show SEM images of the surface and cross section of starch films containing rice bran. The cross section images show a continuous matrix with similar characteristics to those described for bran-free films, but with some dispersed particles, corresponding to proteins, lipid particles and fibres, incorporated by bran. Dispersed particles also appear at surface level in the film, thus indicating that flocculation and creaming occurred during film drying, leading particles to the film surface. It is remarkable that no starch granules were appreciated in the observed fields, which could be due to their gelatinization during the 4 min of hot homogenization with the gelatinized starch dispersions. Fat and proteins could also be well integrated in the matrix as a result of the thermal homogenization. In this sense, the particles observed will be mainly fibre.

In films with *C* bran fraction, big composite particles are observed in some film zones (Fig. 6). Incorporating bran particles led to some irregularities in the film thickness related with the presence of these very large particles. In fact, the mean thickness value of bran-free-films was 75.2 ± 1.1 μm, whereas for films with

Table 4
Moisture, protein, fat, ashes, starch and fibre percentage of fine (*F*) and coarse (*C*) rice bran. Chemical composition of rice bran, % dry basis. Results are the mean ± standard deviation.

	Moisture	Protein	Fat	Ashes	Starch	Fibre
Fine	7.2 (0.2)	15.3 (0.5)	16.2 (0.9)	9.84 (0.05)	27 (3)	24.23
Coarse	6.9 (0.2)	15.56	17.1 (1.3)	10.04 (0.02)	12.6 (0.8)	37.8

Fibre: estimated values.

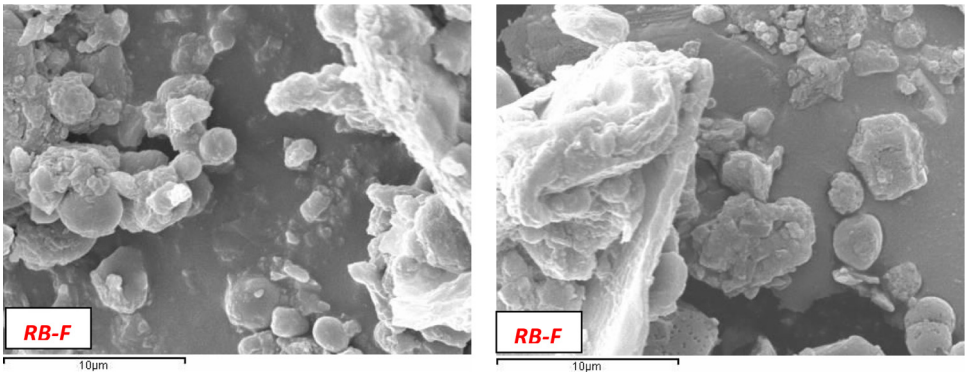

Fig. 5. SEM micrographs of fine (RB-F) and coarse (RB-C) rice bran fractions.

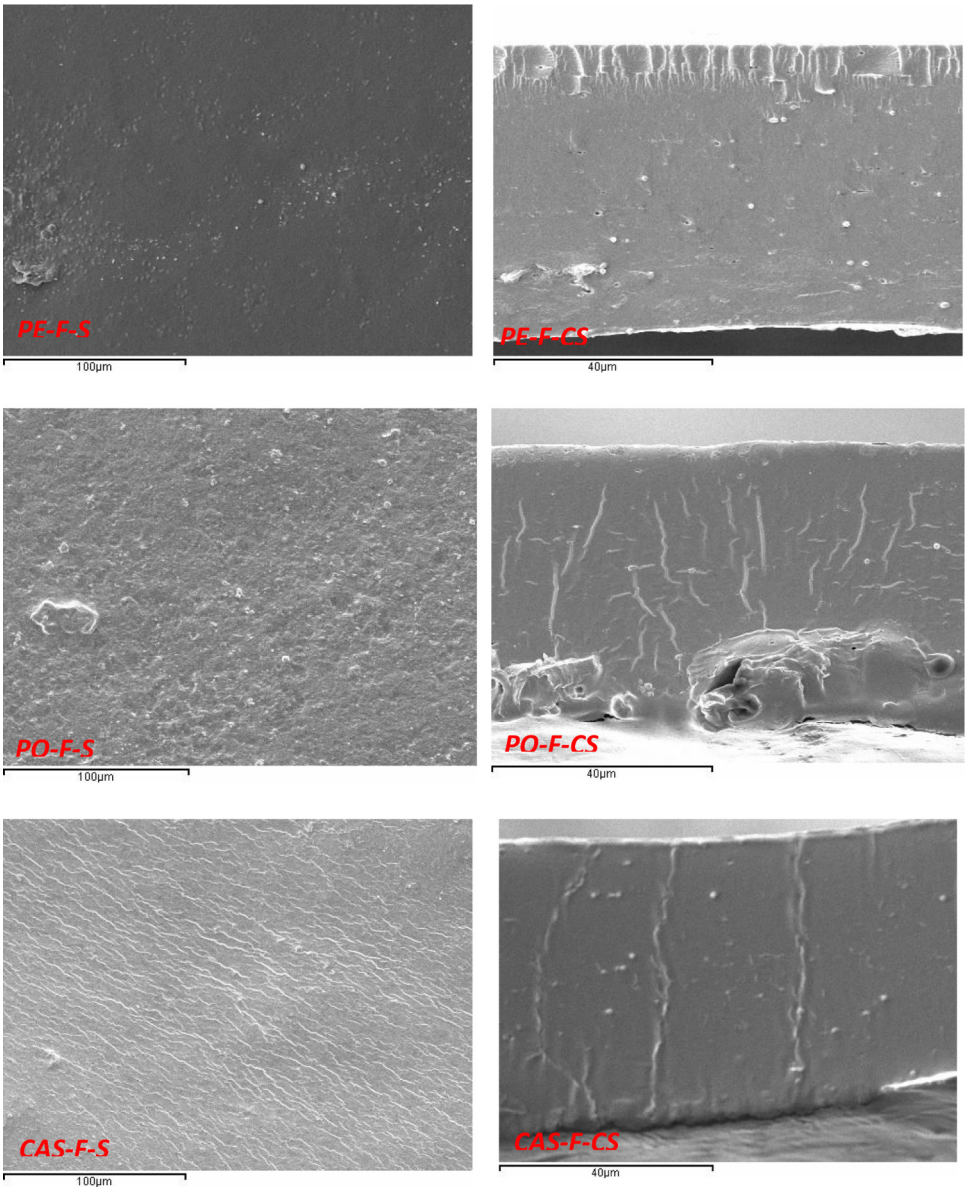

Fig. 6. SEM features of surface (*S*) and cross section (CS) of pea (PE), potato (PO) and cassava (CAS) starch films containing fine (*F*) rice bran.

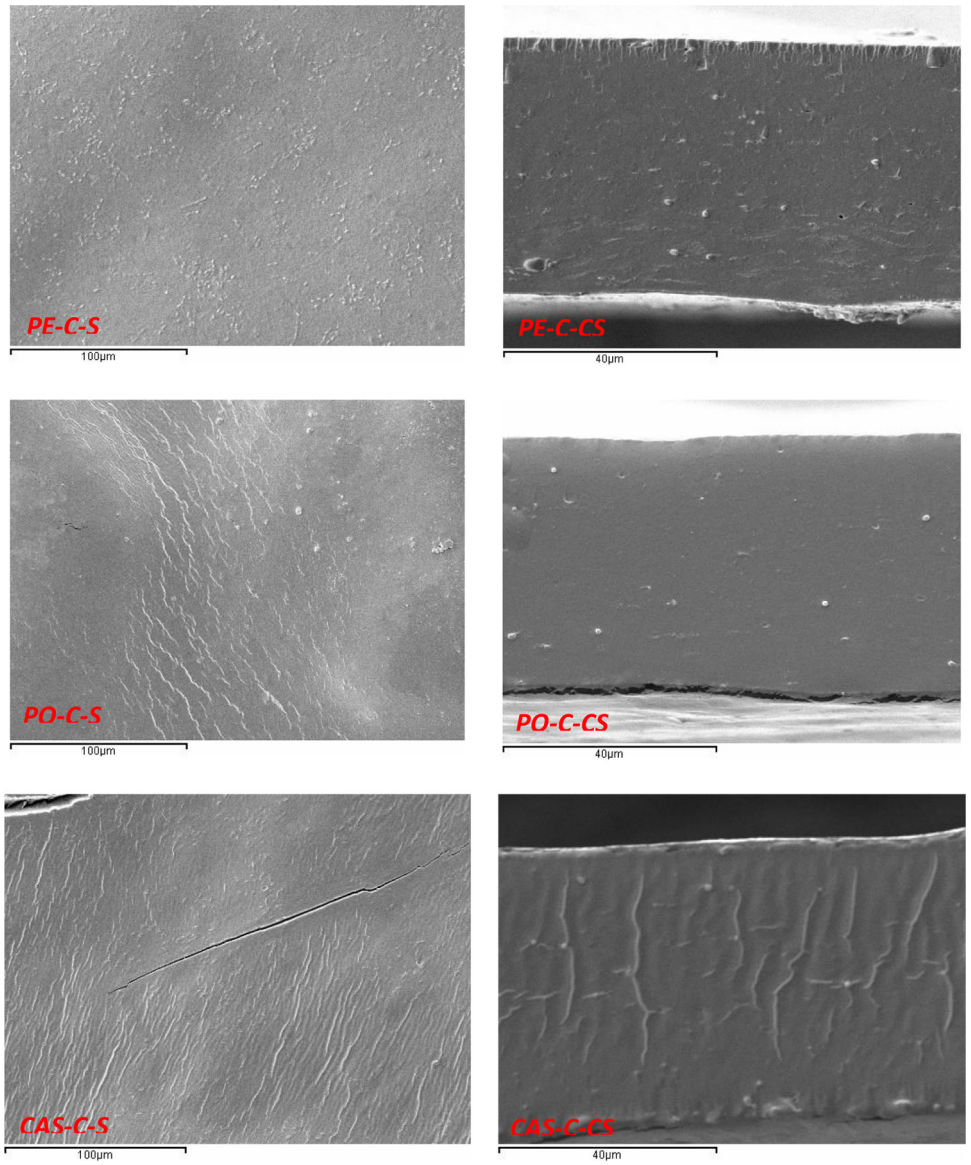

Fig. 7. SEM features of surface (*S*) and cross section (CS) of pea (PE), potato (PO) and cassava (CAS) starch films containing coarse (*C*) rice bran.

F and *C* fractions the values were 75.8 ± 0.8, and 98 ± 4 μm, respectively. The variation coefficients were 1.5, 1.1 and 4.1%, respectively. This indicates that the *C* fraction is not suitable to be incorporated into the films because it causes an irregular film formation with a non-constant thickness.

Fig. 8 shows the surface micrographs of films containing rice bran obtained by AFM using PeakForce QNM. The surface characteristics of the continuous matrix remain as in the bran-free films with notable differences between pea, potato and cassava films due to the differing extent of amylose crystallization. Crystalline zones appear lighter, whereas amorphous zones are darker. The incorporation of bran filler implied the appearance of a great number of hard particles on the film surface (white spots), as compared with the polymer background, which indicates that the dispersed material which migrates to the film's surface during the drying step is stiffer than the polymer matrix. Differences can be observed between the ratio and size of the hard particles on the film surface of the three different starch films. In pea starch films, smaller, free particles can be observed, whereas bigger aggregates appear in the potato starch films. In cassava starch films, the bran particles are not aggregated, but some very soft small spots appeared, which could be attributed to discontinuities on the film surface probably produced by the loss of particles, generating a surface void. This can also occur in the other films, but due to the natural surface roughness it was not easily appreciated.

Differences in the distribution of hard particles at surface level suggest that differing degrees of flocculation and creaming of these dispersed phase occurred during film drying. These phenomena are greatly dependent on the viscosity development (or gel formation) in the aqueous medium in line with water evaporation. Observed differences among samples could be explained in terms of the different development of thickening/gelling properties due to their different polymer composition. In this sense, it is remarkable that the amylose-richest pea starch could form gel more easily during the water evaporation step, thus inhibiting more efficiently the particle migration, as observed in the AFM images.

3.3.2.2. Effect on barrier properties. The moisture content of pure starch films ranged from 9.9 to 11.4 (Table 2). The incorporation of rice bran provoked an increase in the film's moisture content as can be observed in Table 1, mainly in films containing coarse fraction. This not only indicates that the addition of fibre leads to an increase

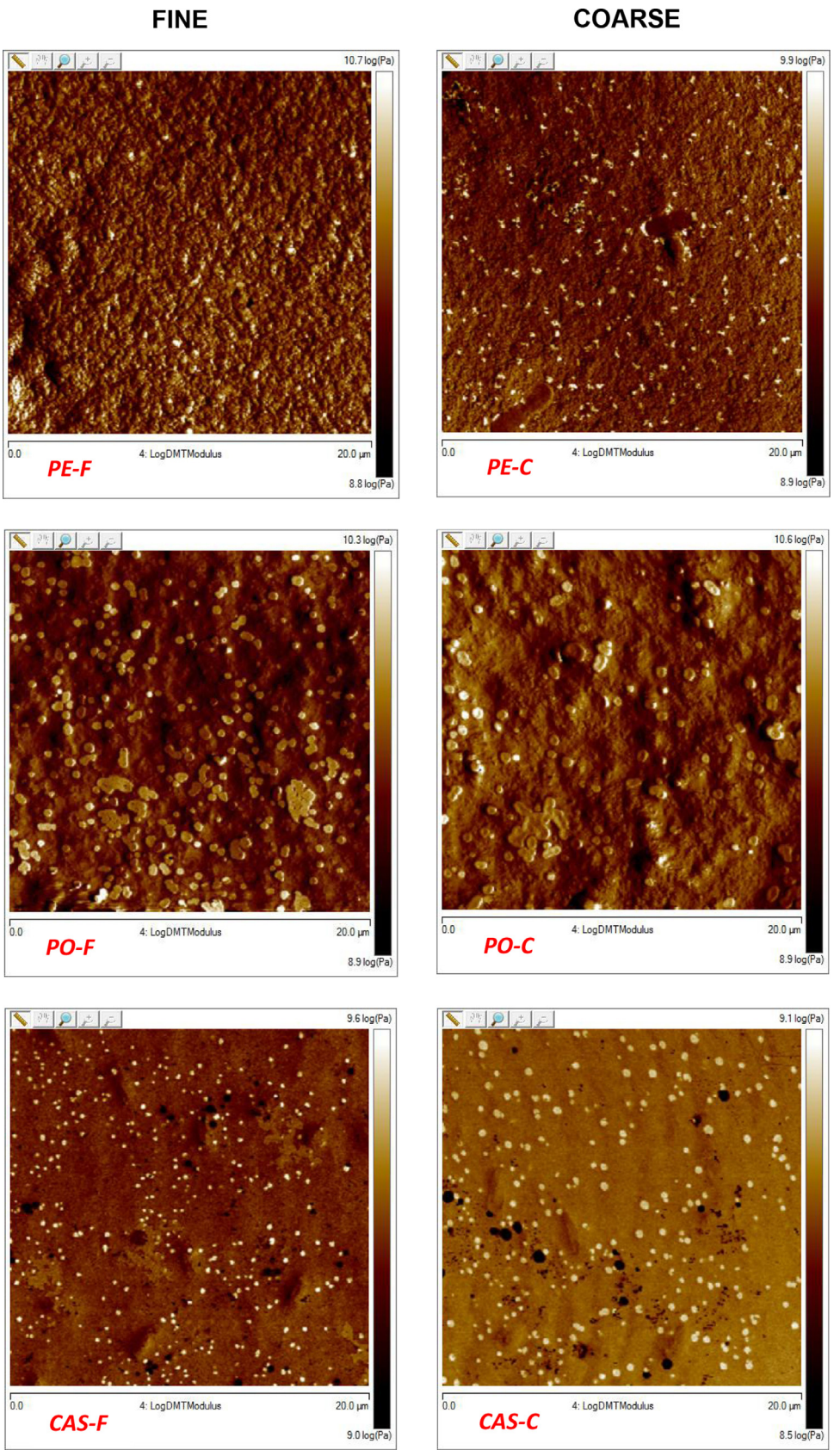

Fig. 8. Maps of Log DTM modulus obtained from AFM in surface of pea (PE), potato (PO) and cassava (CAS) starch films containing fine (*F*) and coarse (*C*) rice bran.

in the water retention capacity of the films, as suggested by Zhang et al. (2011). Besides, the introduction of mineral content (ashes) implies an increase in the water retention capacity of the matrix, especially from intermediate relative humidity. After 5 storage weeks, the moisture content significantly decreased ($p < 0.05$) in all the films, as was observed for bran-free films, which suggests their slow equilibration with the storage chamber relative humidity.

WVP analyzed at 25 °C and a relative humidity gradient 53–100%, are shown in Table 1. Whereas these values at initial time ranged between 5–6 g mm/kPa h m^2 for starch films without bran, in films containing rice bran these were significantly higher ($p < 0.05$), mainly for the films with the largest particles. This can be explained by the greater water content in the films, which plasticizes the matrix, as well as by the presence of large particles whose induced tension in the matrix can provoke associated channels that constitute preferential paths for mass transport. On the contrary, Famá et al. (2009) reported that wheat bran incorporation in cassava starch significantly reduced the *WVP*, although they use lower size particles (75–125 μm). The low particle size is essential as a means of improving the film matrix properties.

The influence of particle size on *WVP* values can be clearly observed in Table 1. Coarse fibres significantly increased *WVP* as compared with fine fibres, regardless of the type of starch. In this case, large particles seriously interrupt the continuity of the films, thus creating large channels for water diffusion. After storage 5 weeks, *WVP* values tend to decrease in films with fine particles, in line with the moisture content reduction in the matrix, whereas they tend to increase in films with the largest particles and greater fibre content.

The *OP* of films containing bran (Table 1) follows the same tendency as observed for bran-free films, increasing when the amylose content in the starch decreases. Nevertheless, for fine bran, fibres provoked a slight increase in the oxygen permeability of starch films, which can be due to increases in the water content. The incorporation of coarse rice bran gives rise to films with microcracks, associated to the tension in the dried film provoked by the largest particles. In these cases, it was not possible to measure the *OP* and this also contributed to the anomalous values of the *WVP*. After 5 storage weeks, mean values of *OP* significantly decreased, as observed in bran-free matrices, due to the reduction in moisture content.

3.3.2.3. Effect on mechanical properties. Table 2 shows the values of the mechanical parameters of the films with bran fractions after 1 and 5 weeks of storage. The incorporation of rice bran did not significantly affect the elastic modulus of potato and cassava starch films, but significantly increased the elastic modulus of pea starch films. The film resistance to fracture was not significantly affected by the addition of bran, although film extensibility was notably reduced, mainly for the coarse fraction in potato and cassava starch films. The presence of discontinuities in the matrix, associated to bran particles, affected the cohesion forces of the polymer network, giving rise to very brittle matrices. In pea starch films, the stretchability reduction is less appreciable due to their very low initial values. These results are coherent with those reported by Famá et al. (2009) for cassava starch films reinforced with wheat bran.

The effect of storage time is quite similar to that observed for bran-free films. The elastic modulus increased in all cases, except pea starch films, where no significant effect of storage time was observed and, in general, film resistance to break increased, while stretchability decreased. Nevertheless, films from pea starch showed a reduction of their mechanical resistance and no significant changes in elastic modulus, which suggest that bran addition seems to limit in this case the progress of chain aggregation throughout time.

3.3.2.4. Effect on optical properties. *Ti* values of starch films containing bran fibres are shown in Table 3. As expected, bran addition contributed to reduce film transparency due to the presence of a dispersed phase that leads to light scattering. Nevertheless, as observed in bran-free films, the transparency of films containing bran particles was not affected ($p > 0.05$) by the type of starch, nor by the bran particle size. Similar results were found by Famá et al. (2009).

Table 3 also shows mean gloss values of starch films containing rice bran. Bran addition decreased the film gloss with respect to the bran-free ones, except in potato starch based films, where gloss was very low, even without added bran. The effect of bran addition on gloss values was greater when coarse particles were added, in agreement with their greater particle size which largely contributes to the increase in the surface roughness and the subsequent gloss loss.

4. Conclusions

Properties of starch films were greatly affected by the amylose–amylopectin ratio. Amylose-rich films form amylose crystalline regions during film drying which give rise to stiffer, more resistant to fracture, but less stretchable films, with lower oxygen permeability and more water binding capacity. All the films develop changes throughout storage time, associated to water loss which leads to more compact matrices where polymer chains are more aggregated. This results in stiffer, more resistant to fracture and less extensible films, with lower oxygen permeability, but without changes in water vapour permeability. Rice bran with lower particle size ($D_{4,3}$ = 57 μm) improved the elastic modulus of the films, but reduced the film stretchability and worsened barrier properties. The reduction of the filler particle size is necessary to minimize the negative effect of large particles.

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