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Additional Information

# Effect of amylose:amylopectin ratio and rice bran addition on starchfilms properties.

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**Abstract**

The influence of the amylose:amylopectin ratio on the properties of pea, potato and cassava starch (with a high, intermediate and low amylose-amylopectin ratio, respectively) films and the effect of the incorporation of rice bran of two different particle sizes were studied. The structural, mechanical (elastic modulus, tensile strength and percentage of elongation at break), optical (gloss and internal transmittance) and barrier (water vapour permeability and oxygen permeability) properties of the films were analysed after 1 and 5 weeks under controlled storage conditions (25ºC and 53%RH). The properties of the films were affected by both amylose-amylopectin ratio and storage time. 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 (fibre particles) in the matrix.

**Keywords:** Pea, potato, cassava, rice bran, storage, microstructure.

## 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 plastics as an alternative to petroleum-derived polymers in different sectors, such as agricultural, medical or pharmaceutical. Nowadays, the use of films or edible coatings based on biodegradable polymers is increasing because these materials are environmentally friendly (Chen, Liu, Chen, Chen & Chang, 2008, Mehyar and Han, 2004) and exhibit properties which can become similar to those observed in conventional plastics (Jiménez, Fabra, Talens & Chiralt, 2012a; Famá, Goyanes & Gerchenson, 2007; Rindlav-Westling, Stading, Hermansson & Gatenholm, 1998).

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

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 (Jiménez *et al*., 2012a; Dole, Joly, Espuche, Alric & Gontard, 2004; Han, Seo, Park, Kim & Lee, 2006; Liu, 2005). It is also especially attractive because of its biodegradability and low cost (Han *et al*., 2006; Chen *et al*., 2008; 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 (Lafargue *et al*., 2007; Chen *et al*., 2008; Phan The, Debeaufort, Voilley & Luu, 2009; Wu *et al*., 2010).

In starch films, the retrogradation phenomenon over time, can greatly affect not only their mechanical properties but also their barrier capacity. So, the study of changes occurring during their storage is necessary to ensure their functionality at different times after processing. Different authors have studied the development of properties of starch films. Jiménez, Fabra, Talens, & 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 are used by different authors. Da Matta, Silveira, de Oliveira & 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 & 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, surface tension and the hydrophilic character of films increased in line with the fibre content. Souza, Benze, Ferrao, Ditchfield, Coelho, & Tadini (2011) stated that films based on

glycerol and clay nanoparticles as reinforcement are an interesting biodegradable alternative as packaging material. Famá, Gerschenson, & Goyanes (2009) also studied the influence of wheat bran on physicochemical characteristics of cassava starch films and concluded that the mechanical properties and water vapour permeability of starch-wheat bran composites improved when the fibre content rose.

One of the means of improving the barrier and mechanical properties of starch films is through the incorporation of natural fibres from plant origin as fillers. In this sense, Chen *et al*., (2009a) used pea hull fibre nanoparticles in pea starch films, which improved film transparency, tensile strength, elongation at break and water barrier properties due to the high content of cellulose crystalline regions and the interactions between the nanofibre and the starch matrix. Famá *et al*., (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 rice 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). In addition, bran 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, attempts have been to reappraise it by studying applications in different areas. In this way, rice bran has been evaluated as a source of oil (Nikolosi, Ausman, & Hegstead, 1990), protein concentrates (Gnanasambandam & Hettiarachache, 1995) and as a matrix of edible films (Dias, Müller, Larotonda, & Laurindo, 2010; Adebiyi *et al*., 2008; Gnanasambandam *et al*., 1997).

The aim of this work was to analyse the influence of the amylose:amylopectin ratio on the properties of films obtained from three different  (pea, potato and cassava)

starches, with different ratios of both polymers 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 analysed at different storage times (1 and 5 weeks) 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 obtained from Arrocería Antonio Tomás, S.L. (Sollana, Valencia, Spain). Glycerol, used as plasticizer, 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 which was purchased from Megazyme (Wicklow, Ireland).

### 2.3. Rice bran particle size

To select particle size, rice bran was sieved to obtain two different bran fractions. The smallest particle size fraction that pass through the 100 μm mesh and the coarse fraction contained between mesh 250 and 100 μm were obtained and used for film preparation. The smallest particle bran is named “Fine” (F) and the other bran fraction is called “Coarse” (C).

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, in triplicate, with a laser light scattering instrument (Malvern Instruments Ltd, Worcestershire, U.K.). Particle size measurements were taken for two different

fractions. To this end, bran fraction was dispersed in aqueous medium and measurements were taken with ultrasonic homogenization to maintain the sample homogeneity.

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

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

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 100ºC 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 910ºC for 15 min.

Protein content was obtained by means of the method of analysis 105/2 (ICC, 1994). The crude protein content was obtained by multiplying the nitrogen content, determined by the Kjeldahl procedure, using the factor F=5.95. A digestion unit “Bloc-digest” (J.P. Selecta; Abrera, Barcelona, Spain) and a Kjeldahl distiller “Pro-Nitro M” (J.P. Selecta; Abrera, Barcelona, Spain) were used.

Fat content was obtained by using the Soxhlet method 30-20 (ICC, 1967). Samples were firstly dried at 103ºC 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”, which was supplied by Sigma (Saint Louis, Missouri, USA).

193 Fibre percentage was estimated from the difference between the total percentage of
194 the rest of the analysed components and 100, assuming that starch is the only
195 carbohydrate.
196
197 2.5. Preparation of films
198 For preparation of starch films, three formulations based on distilled water, starch (pea,
199 potato or cassava) and glycerol were prepared. The dispersions contained 2% w/w of
200 starch whereas the plasticizer was added considering a starch:glycerol ratio of 1:0.25,
201 on the basis of previous studies (Jimenez et al. 2012a). In the preparation of starch
202 films containing rice bran as filler, six formulations were obtained by using fine (F) or
203 coarse (C) rice bran and starch (pea, potato or cassava) and glycerol. The film forming
204 dispersions were prepared in the same way and with the same glycerol ratio and bran
205 was afterwards incorporated in a starch:rice bran ratio of 1:0.1.
206 Starch aqueous dispersions were maintained at 95ºC for 30 min to induce starch
207 gelatinization. Then, glycerol was added and the dispersion was homogenized using a
208 rotor-stator homogenizer (Ultraturrax D125, Janke and Kunkel, Germany) at 13,500
209 rpm for 1min and 20,500 rpm for 3 min at 95ºC under vacuum. For starch films
210 containing rice bran, this was incorporated prior to the homogenization step. The film-
211 forming aqueous dispersions were cast into a levelled Teflon casting plates (15cm
212 diameter) and each film contained 1.5 g of total solids. Films were formed by drying at
213 25ºC and 45%RH for 48 h. Then, they were peeled intact from the plates and were
214 conditioned at 53% RH using magnesium nitrate-6-hydrate saturated solution (Panreac
215 química, S.A., Castellar del Vallés, Barcelona, Spain) at 25ºC until analysis. Their
216 thickness was measured at six random positions with a Palmer digital micrometer to
217 the nearest 0.0025 mm. All films were analyzed after one or five storage weeks.
218
219 2.6. Characterization of films
220 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 Kα Cu radiation (λ: 1,542 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 $P_2O_5$ for two weeks to ensure that no water was 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 analyse surface film nanostructure. Measurements were taken from small areas of the film surface (20x20 µm) and the resulting data were transformed into 2D image of the Log DMT modulus. Three images were captured per formulation, for samples stored for 1 and 5 weeks.

Measurements were also taken from several areas of the film surface (50 x 50 and 3 x 3 mm) using the tapping mode. The resulting data were transformed into a 2D image. Phase Imaging mode derived from Tapping Mode, that goes beyond topographical data to detect variations in composition, adhesion, friction, viscoelasticity, and other properties, including electric and magnetic, was also applied.

According to method ASME B46.1 (ASME, 1995), the following statistical parameters related with sample roughness were calculated: average roughness (Ra: average of the absolute value of the height deviations from a mean surface), root-meansquare roughness (Rq: root-mean-square average of height deviations taken from the mean data plane).

2.6.3. Moisture Content

To determine film moisture content, five replicates by formulation were dried in a convection oven at 60ºC for 24h, 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 25ºC 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 25ºC. 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) which determined the oxygen permeation. Measurements were taken at 25ºC following the standard method (ASTM D3985-05, 2005) at 53% RH. Film

samples (50 $cm^2$) were introduced into the equipment to perform the assay. Films were exposed to pure oxygen flow on one side and pure nitrogen flow on the other side. An oxygen sensor read permeation through the film and the rate of oxygen transmission was calculated taking into account the amount of oxygen and the area of sample. 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). Force-distance curves were obtained and transformed into stress-strain curves which allowed tensile strength at break (TS), percentage of elongation at break (%E) and elastic modulus (EM) to be obtained. Eight replicates carried out per formulation. Equilibrated film specimens (2.5 cm wide and 10 cm long) were mounted in the film-extension grips (A/TG model) which were set 50 mm apart. The speed of the testing machine during stretching was 50 mm $min^{-1}$ until breaking.

2.6.7. Optical properties

The opacity of films was determined by applying the Kubelka-Munk theory of multiple dispersion to the reflection spectra (Judd & Wyszecki, 1975; Hutchings, 1999). Internal transmittance (Ti) of the films was quantified using eq. (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 Rg. The reflection spectrum 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.

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

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

$$b = (a^2 - 1) \quad (5)$$

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 60º 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) at the 95% confidence level was used.

## 3. Results and discussion

### 3.1. Properties of the starches. Amylose:amylopectin ratio

Properties of starch films, such as mechanical behaviour, depend on the amylose:amylopectin ratio since the different behaviour of amylose (AM) and amylopectin (AP) molecules contributes to film properties (da Matta, 2011). The amylose content 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. Mehyar & Han, (2004); Chen et al. (2008); Ma, Chang, & Yu, (2008) and Zhang & Han (2006) reported amylose contents of between 30 and 40 % in pea starch, which is higher than the values obtained. For potato starch the value obtained coincides with the result reported by Talja, Peura, Serimaa, & Jouppila (2008) whereas it was lower than that reported by Alvani, Qi, Tester, & Snape, (2011). (25.2-29.1%). For cassava starch, higher amylose contents (19.7% and 22.5%) were found by Souza et al. (2011). The amylose content will affect the film properties since the phenomenon of recrystallization, which occurs during film formation and storage, has been mainly related with this polymer (Myllärinen, Buleon, Lahtinen, & Forssell, 2002 and Rindlav-Westling et al., 1998). This phenomenon is mainly responsible for changes in the mechanical behaviour (increase in the elastic modulus and decrease in the film stretchability) which make the films excessively brittle (Jimenez et al., 2012a).

### 3.2 X-ray diffraction

Figure 1 shows the X-ray diffraction spectra of pure starch films, which were equilibrated at 53% RH and 25ºC for 1 and 5 weeks in order to analyse 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 (Myllärinen et al., 2002; Forssell, Helleman, Myllärinen, Moates, & Parker, 1999 and Rindlav-Westling et al., 1998). This process occurs mainly during film drying when the chain mobility is still high due to the water content. Several authors (Rindlav-Westling et al., 1998) report 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 5.4º (characteristic of B-type polymorphs), 14.8º (characteristic of A-type polymorphs), 16.8º (characteristic of both A and B-type polymorphs) and 19.0º and 21.8º (characteristic of B-type polymorphs). Similar results have been observed by da Matta *et al*. (2011), Wu *et al*. (2010) and Chen *et al*. (2009a). In the case of potato starch films, a typical C-type pattern can also be observed, with peaks at 5.1º, 11.7º and 17.2º 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 difractogrames after 1 and 5 storage weeks allow us to conclude that no significant changes in the crystallinity occur throughout the storage period, 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. Different authors (Myllärinen et al., 2002) have pointed out that amylose crystallizes very fast during the film formation, whereas the crystallization of amylopectin is a slower process. In the

richest amylose starch (pea starch), crystallization had occurred at the first control time (1 week) and probably during the drying period, as reported by other authors (Myllärinen et al., 2002; Forssell, et al., 1999; Rindlav-Westling et al., 1998 and Rindlav, Hulleman, & Gatenholm, 1997). During storage, no notable changes in the X-ray diffraction pattern were observed in any case.

### 3.3. Microstructural features

SEM and AFM microstructure analyses provide information about the surface morphology and internal microstructure of the films. Figure 2 shows SEM micrographs of the surface and cross section of the different starch films. 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 (Wu et al., 2010; Chen et al., 2009a; Chen et al., 2009b). 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, Talens, & Chiralt (2012c), as a result of the lower mechanical resistance of this sample.

Figure 3 shows AFM images of pea, potato and cassava starch films, obtained by using PeakForce QNM. Raw data were converted into 2D images and their scale is expressed as Log DMT modulus. 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

412 the X-ray spectra, associated with bigger crystals. No notable differences were
413 appreciated between 1 and 5 storage weeks.
414 In cassava starch samples, more homogenous, but lower, values of DMT modulus can
415 be observed, due to the more amorphous character of the films. It is remarkable that in
416 these films, a harder surface was detected at 5 storage weeks which reveals that films
417 were significantly hardened (higher values of Modulus) during storage, although no
418 crystallization was detected since the surface appears homogenous. The different
419 behaviour of the starch matrices was coherent with the different amylose:amylopectin
420 ratio, which is associated with a different recrystallization progress during film
421 formation.
422
423 3.4. Moisture content and barrier properties
424 Table 1 shows the moisture content values of the studied films, stored for 1 and 5
425 weeks at 25ºC and 53% RH Humidity. The values ranged between 9.9 and 11.4% and
426 pea and potato starch films were the samples which exhibited the highest moisture
427 content, as reported in previous studies (Mehyar & Han, 2004; Kaisangsti,
428 Kerdchoechuen, & Laohakunjit, 2012). This can be associated with the higher degree
429 of crystallization, since crystalline zones bond a greater amount of water than
430 amorphous zones (Myllärinen et al., 2002; Forssell et al., 1999 and Rindlav-Westling et
431 al., 1998). Nevertheless, moisture content significantly ($p<0.05$) decreased after 5
432 storage weeks, when more homogeneous values of moisture content were obtained for
433 the different films. This development indicates that films equilibrate slowly with the
434 conditioning relative humidity, reaching a value closer to the equilibrium by losing water
435 during storage. The water loss will provoke a greater chain aggregation in the
436 amorphous region which will imply an increase in the film compactness that will affect
437 barrier and mechanical properties.
438 Water vapour permeability (WVP) values define the final application of a film in contact
439 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 25ºC 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.

The oxygen permeability (OP) was analyzed at 25ºC and 53% RH in films equilibrated under these conditions for 1 and 5 weeks. Table 1 shows the mean values of 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 *et al*. (2002) report 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. After 5 storage weeks, the oxygen permeability values of every film were significantly reduced, coherently with the increase in the matrix compactness, as commented on above. In general, the OP values are very low and, as reported byShen, Wu, Chen, & Zhao, (2010), one great advantage of starch films is their ability to protect food products by forming an oxygen barrier.

### 3.5. Mechanical properties

Film properties related to easy film-handling, their fragility or their stretchability, are very interesting from a technological point of view (Jiménez, 2009). Elastic modulus (EM), tensile strength at break (TS) and percentage of elongation at break (%E) are the usual parameters with which to describe the mechanical behaviour of films, and they

468 are closely related to the film microstructure (McHugh & Krochta, 1994). TS and %E
469 represent the film's resistance to elongation and its stretching capacity, respectively,
470 whereas EM is a measure of the stiffness of films. Table 2 shows the mean values of
471 these mechanical parameters for the films after 1 and 5 storage weeks at 25ºC and
472 53% RH. The mechanical behaviour of starch films was similar to that reported by other
473 authors for pea starch films (Chen *et al*., 2008, and Da Matta *et al*., 2011), potato
474 starch films (Cyras, Manfredi, Ton-That, & Vázquez, 2008 ) and cassava starch films
475 (Famá, Rojas, Goyanes, & Gerschenson, 2005.and Souza *et al*., 2011).
476 After one week of storage, the mechanical parameter values were significantly different
477 ($p<0.05$) for the three matrices. The pea starch films (with the highest amylose content)
478 have the highest values in break strength and stiffness and the lowest in stretchability.
479 On the contrary, cassava starch films (with the lowest amylose content) exhibited the
480 lowest break strength values and the highest in stretchability. This indicates the
481 important role played by crystal formation in the mechanical behaviour of the matrix.
482 After 5 weeks of storage, film stiffness and resistance to break increased for all the
483 films, coinciding with the increase in the matrix compactness promoted by water loss.
484 Nevertheless, the highest relative increase occurs for cassava starch films, which could
485 indicate the formation of very small association zones of amylopectin chains between 1
486 and 5 weeks. In the same sense, although all the films lost stretchability during storage,
487 it was cassava starch films which experienced the greatest losses due to the greater
488 extent of amylopectin association during the storage period. As previously commented
489 on, amylose rich starch crystallizes very fast during film drying and subsequent
490 conditioning, whereas amylopectin rich films crystallize more slowly, which is reflected
491 in the way that the different films develop mechanical behaviour. Nevertheless, it is
492 remarkable that the films that are richest in amylose (pea starch) showed the highest
493 values of stiffness and resistance to break after long storage times, whereas
494 intermediate amylose films (from potato starch) showed the greatest stretchability after
495 5 storage weeks.

### 3.6. 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), 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 with a greater opacity. Analyses were carried out in films previously equilibrated at 53% RH and 25 ºC. 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 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, which were measured at after 1 or 5 weeks' storage at 53%RH and at an incidence angle of 60º 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 occurduring 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.

In conclusion, films obtained from pea starch, richer in amylose, are stiffer, more resistant to fracture and glossier and less permeable to oxygen, although less extensible, than starch films with a lower amylose content. All the films become harder and more resistant during storage, and those richer in amylopectin become shorter

523 (less stretchable). The oxygen permeability slightly decreased throughout storage time
524 in every case.
525
526 3.7. Effect of rice bran addition
527 The two rice bran fractions obtained by sieving were analysed as to the particle size
528 distribution by dispersing them in water to the adequate obscuration rate in the laser
529 diffraction equipment. The bran particle size distribution curves are shown in Figure 4
530 for both the smaller particle fraction (F) and the bigger particle fraction (C). Differences
531 between the particle size distribution of two fractions (F and C) can be observed,
532 although a certain degree of curve overlapping was obtained, since sieving only
533 partially separates the particles by size. In Figure 4, the mean values of the bran
534 particle size in terms of $D_{3,2}$ and $D_{4,3}$ are also shown. The differences between these
535 diameters in a given sample indicate that particle size distributions are wide or that they
536 are irregularly-shaped. On the contrary, similar values are associated with narrow
537 particle size distributions and more spherical particles. As expected, significant
538 differences ($p < 0.05$) between $D_{3,2}$ and $D_{4,3}$ were found for both fine and coarse
539 fractions, thus indicating the presence of irregular particles of very different sizes.
540 Figure 5 shows the SEM micrographs of the powder of both bran fractions. Fraction F
541 contains more spherical particles than fraction C which, in turn, contain composite
542 particles (where components are not released). The composition of both fractions is
543 shown in Table 4. The mean values of the moisture, protein, fat and ash contentswere
544 very similar for F and C fractions, but significant differences were found for starch and
545 fibre contents; the fine fraction was richer in starch (twice) whereas the coarse fraction
546 contained more fibre. The obtained composition of the two rice bran fractions coincides
547 with data previously reported by Sánchez *et al*. (2004). The protein content is similar to
548 that reported by Rodríguez (2007), Gnanasambandam *et al*. (1997), whereas Pacheco,
549 Peña, & Ortiz, (2002) obtained a similar fat content. These authors observed that the

550 varietal effect and smoothening method may cause significant differences in the ash,
551 fat, protein, starch and fibre content of rice bran.
552
553 3.7.1. Effect of bran addition on microstructural properties
554 The microstructure analysis allows us to identify the arrangement of some components
555 of the film (mainly those non-miscible with the polymer) and the characteristics of the
556 polymer matrix. The microstructural features are also directly related with the film's
557 physical (mechanical, barrier and optical) properties. Figures 6 and 7 show the surface
558 and cross section micrographs of starch films containing rice bran obtained by SEM.
559 The cross section micrographs of films containing bran fractions show a continuous
560 matrix with similar characteristics to those described for bran-free films, but with some
561 dispersed particles, corresponding to proteins, lipid particles and fibres, incorporated by
562 bran. Dispersed particles also appear at surface level in the film, thus indicating that
563 flocculation and creaming occurred during film drying, leading particles to the film
564 surface. It is remarkable that no starch granules were appreciated in the observed
565 fields, which could be due to their gelatinization during the 4 minutes of hot
566 homogenization with the gelatinized starch dispersions. Fat and proteins could also be
567 well integrated in the matrix as a result of the thermal homogenization. In this sense,
568 the particles observed will be mainly fibre. Large composite particles are sometimes
569 observed (Figure 6), although in relatively low numbers for the fine fraction.
570 The presence of the large particles affected the film thickness. Incorporating bran
571 particles led to some irregularities in the film thickness related with the presence of
572 these very large particles. The mean thickness value of bran-films was 75.2±1.1 μm,
573 whereas for films with F and C fractions the values were 75.8±0.8, and 98±4 μm,
574 respectively. The variation coefficients were 1.5, 1.1 and 4.1%, respectively. This
575 indicates that while no differences were observed between bran-free films and those
576 containing the F bran fraction, the C fraction is, not suitable to be incorporated into the
577 films since it causes an irregular film formation with a non-constant thickness.

Figure 8 shows the surface micrographs of films containing rice bran obtained by AFM using PeakForce QNM. The data were converted into 2D images in terms of Log DMT modulus maps, where the darker colour means lower DMT modulus or soft areas. As can be observed, 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. 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. 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.

The fact that there are differences in the particle distribution at surface level indicates that differing degrees of flocculation and creaming occurred during film drying, which depends on the viscosity of the aqueous medium induced by starch. In this sense, pea starch, which has the highest amylose ratio, can form gel during film drying, thus inhibiting the particle migration. Cassava starch did not form gel, but the solutions exhibit very high viscosity. The potato starch film-forming dispersion is probably the one that shows the poorest stabilizing properties, thus promoting particle flocculation and migration towards the film surface.

606 3.7.2. Effect on barrier properties.

607 The moisture content of pure starch films ranged from 9.9 to 11.4 (Table 2). The
608 incorporation of rice bran provoked an increase in the film's moisture content as can
609 be observed in Table 1, mainly in films containing coarse fraction. This not only
610 indicates that the addition of fibre leads to an increase in the water retention capacity of
611 the films, as suggested by Zhang *et al* (2011), but also that the introduction of mineral
612 content (ashes) implies an increase in the water retention capacity of the matrix,
613 especially from intermediate relative humidity. After 5 storage weeks, the moisture
614 content significantly decreased ($p < 0.05$) in all the films, as was observed for bran-free
615 films, which suggests their slow equilibration with the storage chamber relative
616 humidity.

617 WVP, analyzed at 25ºC and a relative humidity gradient 53 - 100%, are shown in Table
618 1. Whereas these values at initial time ranged between 5-6 g mm/ kPa h $m^2$ for starch
619 films without bran, in films containing rice bran these were significantly higher ($p <$
620 0.05), mainly for the films with the largest particles. This can be explained by the
621 greater water content in the films, which plasticizes the matrix, as well as by the
622 presence of large particles whose induced tension in the matrix can provoke
623 associated channels that constitute preferential paths for mass transport. On the
624 contrary, Famá *et al*. (2009) reported that wheat bran incorporation in cassava starch
625 significantly reduced the WVP, although they use particles with a lower size range:
626 between 75 and125 µm. The low particle size is essential as a means of improving the
627 film matrix properties.

628 The influence of particle size on WVP values can be clearly observed in Table 1.
629 Coarse fibres significantly increased WVP as compared with fine fibres, regardless of
630 the type of starch. In this case, large particles seriously interrupt the continuity of the
631 films, thus creating large channels for water diffusion. After 5 weeks of storage, the
632 values of WVP slightly changed, but without any clear tendencies, depending on the
633 starch type and bran fraction. Nevertheless, whereas WVP values tend to decrease

634 with time in films with fine particles, in line with the moisture content reduction in the
635 matrix, they tend to increase in films with the largest particles and a greater fibre
636 content.
637 The OP of films containing bran (Table 1) follows the same tendency as observed for
638 bran-free films, increasing when the amylose content in the starch decreases.
639 Nevertheless, for fine bran, fibres provoked a slight increase in the oxygen permeability
640 of starch films, which can be due to increases in the films' water content. The
641 incorporation of coarse rice bran gives rise to films with micro-cracks, associated to the
642 tension in the dried film provoked by the largest particles. In these cases, it was not
643 possible to measure the OP and this also contributed to the anomalous values of the
644 WVP. After 5 storage weeks, the mean values of OP significantly decreased, as
645 observed in bran-free matrices, due to the reduction in moisture content.
646
647 3.7.3. Effect on mechanical properties
648 Table 2 shows the values of the mechanical parameters of the films with bran fractions
649 after 1 and 5 weeks of storage. The incorporation of rice bran did not significantly affect
650 the elastic modulus of potato and cassava starch films, but significantly increased the
651 elastic modulus of pea starch films. The film resistance to fracture was not significantly
652 affected by the addition of bran, although film extensibility was notably reduced, mainly
653 for the coarse fraction in potato and cassava starch films. In pea starch films, the
654 stretchability reduction is less appreciable due to their very low initial values. These
655 results are coherent with those reported by Famá *et al*. (2009) for cassava starch films
656 reinforced with wheat bran.
657 The effect of storage time is quite similar to that observed for bran-free films. The
658 elastic modulus increased in all cases, except pea starch films, where bran particles
659 partially inhibit the increase in the elastic modulus that occurred in bran-free films. This
660 may be due to the smaller water loss which occurred during storage when the films
661 contained bran. Film resistance to break increased in every case during storage,

except pea starch films where it diminishes, in agreement with that commented on above. Film stretchability also decreases in all cases, the values being very similar for all the films after 5 storage weeks. The presence of discontinuities in the matrix, associated to bran particles, affected the matrix cohesion forces, giving rise to very brittle matrices.

3.7.4. 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). These authors also compared the yellow index (YI) of cassava starch samples with different wheat bran contents and observed that YI values rose as the bran content increased.

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 throughout storage time, mainly due to water loss which leads to more compact matrices: stiffer, more

690 resistant to fracture and less extensible, with lower oxygen permeability, but without
691 changes in water vapour permeability. Rice bran with lower particle size ($D_{4,3}$= 57μm)
692 improved the elastic modulus of the films, especially in high amylose content films (pea
693 starch), but reduced the film stretchability and worsened barrier properties, due to the
694 enhancement of the water binding capacity of the films and the introduction of fibre
695 discontinuities in the matrix. So, the hygroscopic character of the filler was a drawback
696 to the improvement of the film properties. The reduction of the filler particle size is
697 necessary to minimize the negative effect of large particles.
698
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704
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878

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. Mean values and (standard deviation).

| | MC d.b (%) | | WVP ($g.mm.kPa^{-1}h^{-1}m^{-2}$) | | OP($10^{-14}cm^3m^{-1}s^{-1}kPa^{-1}$) | |
|---|---|---|---|---|---|---|
| Film | 1week | 5week | 1week | 5week | 1week | 5week |
| PE | $11.4(0.4)^{a1}$ | $8.7\ (0.4)^{a2}$ | $6.0(0.3)^{a1}$ | $6.7(0.7)^{a1}$ | $3.8(0.3)^{a1}$ | $2.7(0.2)^{ab2}$ |
| 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 | $9.9(0.9)^{b1}$ | $8.2\ (0.3)^{a2}$ | $5.4(0.4)^{a1}$ | $6.8(0.5)^{a2}$ | $4.2(0.4)^{b1}$ | $2.45(0.12)^{b2}$ |
| WITH BRAN | | | | | | |
| 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 | $12.9(1.2)^{b1}$ | $8.1(0.3)^{a2}$ | $7.3(0.9)^{a1}$ | $8.3(0.2)^{b2}$ | | |

a, b, c. Different superscripts within a column indicate significant differences between starch matrix and fine or coarse rice bran in the same matrix. ($p<0.05$).

1,2. Different superscripts within the same file indicate significant differences between storage times for the same formulation. ($p<0.05$).

888 Table 2: Elastic modulus (EM), tensile strength at break (TS) and percentage of
889 elongation at break (%) of pea (PE), potato (PO) and cassava (CAS) starch films at two
890 storage time (1 week and 5 weeks). Films with fine (F) and coarse (C) fractions
891 incorporated to starch films were also included. Mean values and (standard deviation).

| | EM(MPa) | | TS(MPa) | | %Ɛ | |
|---|---|---|---|---|---|---|
| Film | 1week | 5week | 1week | 5week | 1week | 5week |
| 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)$^{c2}$ |
| WITH BRAN | | | | | | |
| 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)$^{2a}$ |

892 a, b, c. Different superscripts within a column indicate significant differences between starch matrix and fine or coarse
893 rice bran in the same matrix. ($p<0.05$).
894 1,2. Different superscripts within the same file indicate significant differences between storage times for the same
895 formulation. ($p<0,05$).
896

897 Table 3: Gloss values at 60º and internal transmittance (Ti) of pea (PE), potato
898 (PO),and cassava (CAS) starch films at two storage times (1 week and 5 weeks). Films
899 with fine (F) and coarse (C) fractions incorporated to starch films were also included.
900 Mean values and (standard deviation).

| | 60º | | Ti (450nm) | |
|---|---|---|---|---|
| Film | 1week | 5week | 1week | 5week |
| 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)$^{b1}$ |
| WITH BRAN | | | | |
| 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)$^{a1}$ |

901 a, b, c. Different superscripts within a column indicate significant differences between starch matrix and fine or coarse
902 rice bran in the same matrix. ($p<0.05$).
903 1,2. Different superscripts within the same file indicate significant differences between storage times for the same
904 formulation. ($p<0,05$).
905

906 Table 4: Chemical composition of rice bran, % dry basis. Mean values (standard
907 deviation).

| | Moisture | Protein | Fat | Ashes | Starch | Fiber |
|---|---|---|---|---|---|---|
| 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 |

908

909

## FIGURE CAPTIONS

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

**Figure 2**: SEM micrographs of surface and cross section of pea, potato and cassava starch films.

**Figure 3**: Maps of Log DTM modulus obtained from AFM in surface of pea, potato and cassava starch films for samples stored for 1 and 5 weeks.

**Figure 4**: Typical particle size distributions of the different bran fractions. $D_{3,2}$ and $D_{4,3}$ , mean values and (standard deviation).

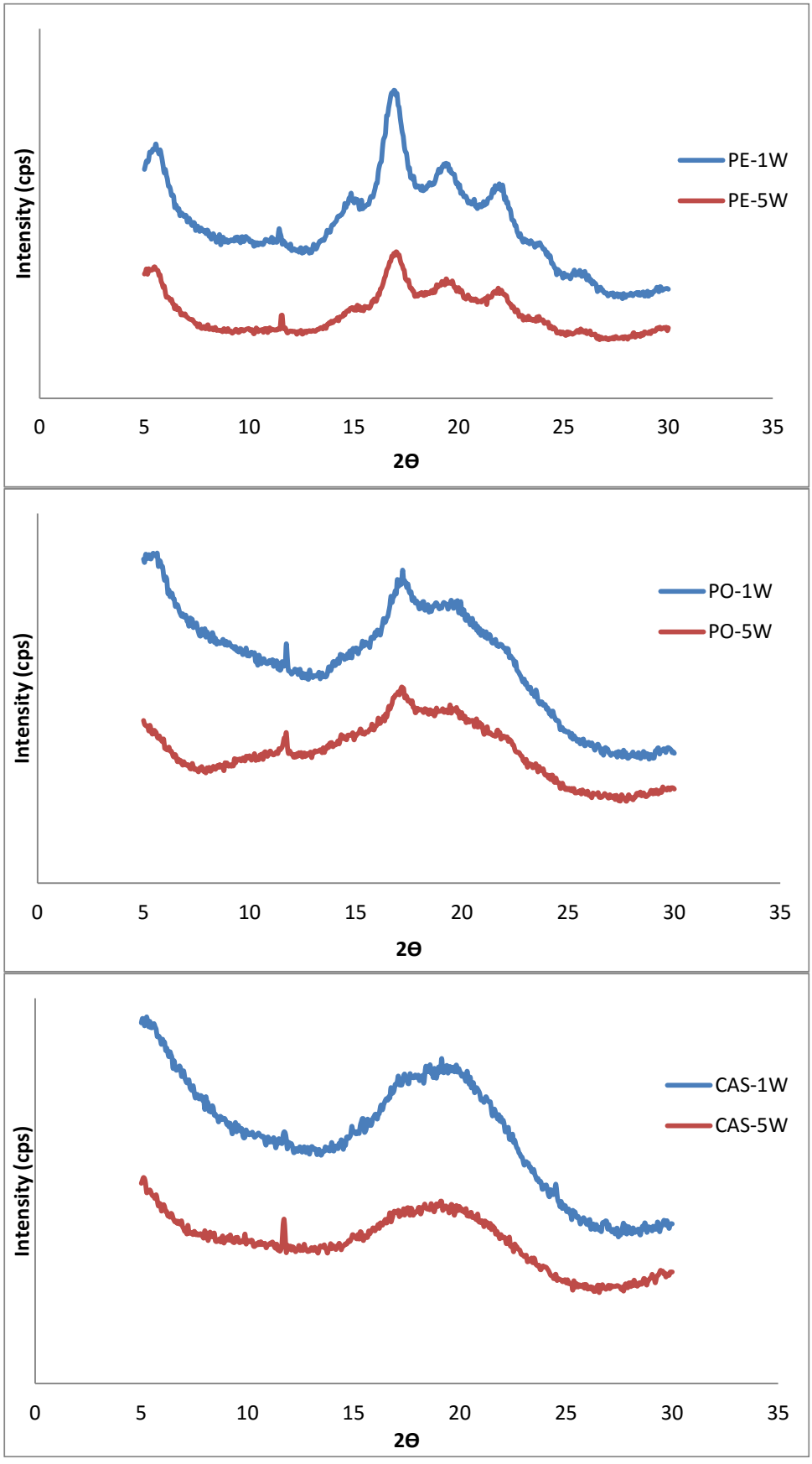
**Figure 5**: SEM micrographs of fine and coarse rice bran fractions.

**Figure 6**: SEM micrographs of surface and cross section of pea (PE), potato (PO) and cassava (CAS) starch films containing fine (F) rice bran.

**Figure 7**: SEM micrographs of surface and cross section of pea (PE), potato (PO) and cassava (CAS) starch films containing coarse (C) rice bran.

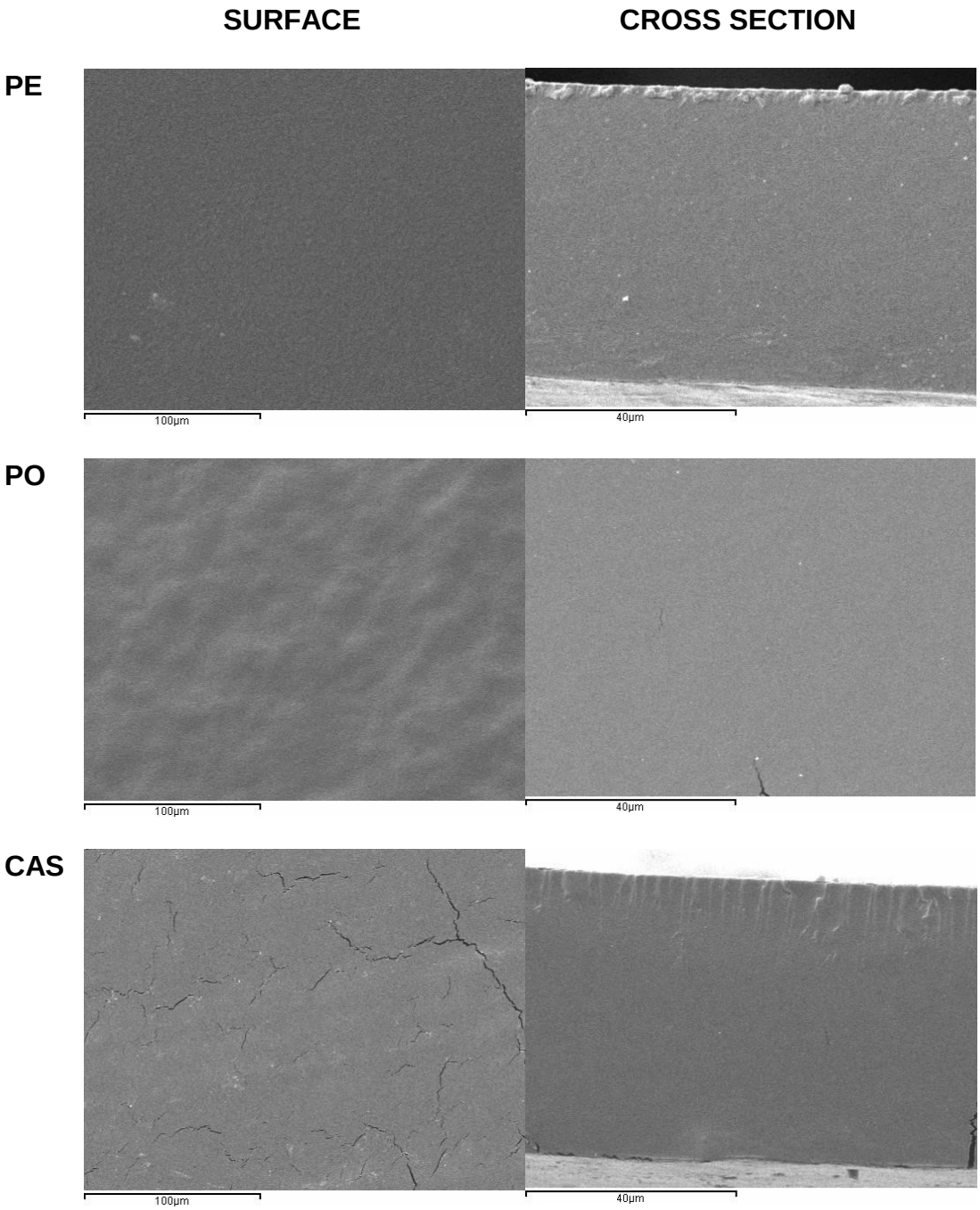
**Figure 8**: Maps of Log DTM modulus obtained from AFM in surface of pea, potato and cassava starch films containing Fine (F) and coarse (C) rice bran.

928 **Figure 1**




929

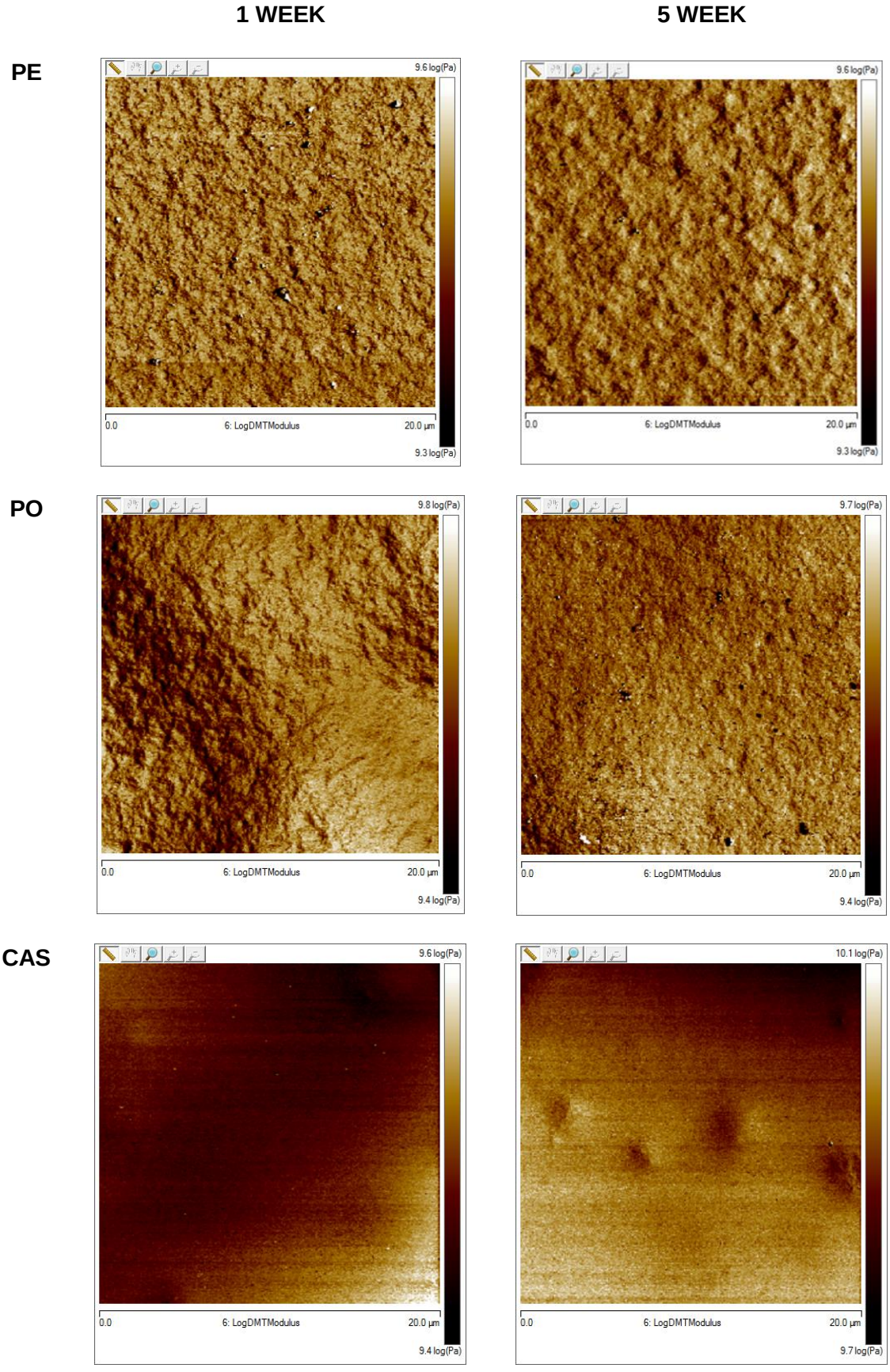
**Figure 2**



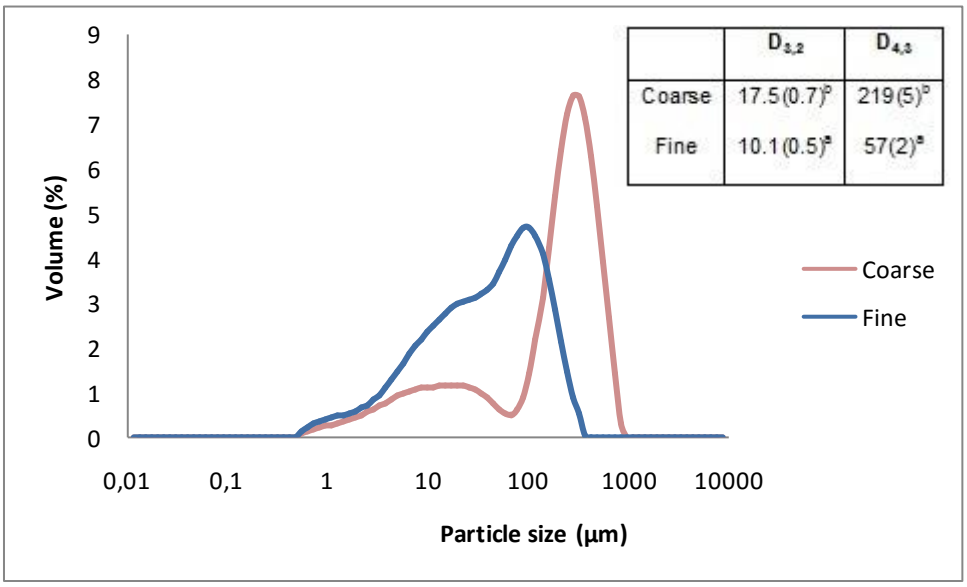

930

931

**Figure 3**


1 WEEK
5 WEEK
PE
PO
CAS
9.6 log(Pa)
0.0
6: LogDMTModulus
20.0 µm
9.3 log(Pa)
9.8 log(Pa)
9.4 log(Pa)
9.7 log(Pa)
10.1 log(Pa)

934 **Figure 4**

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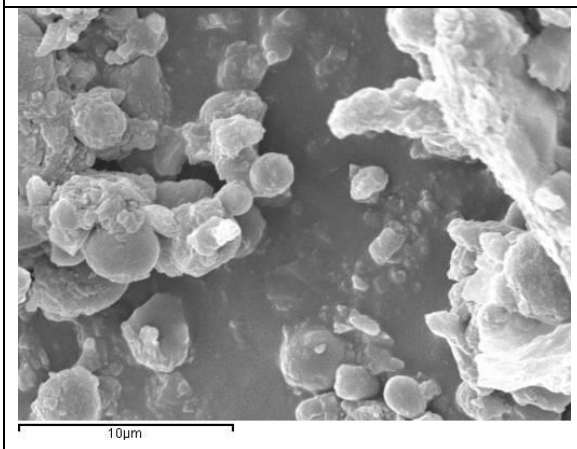
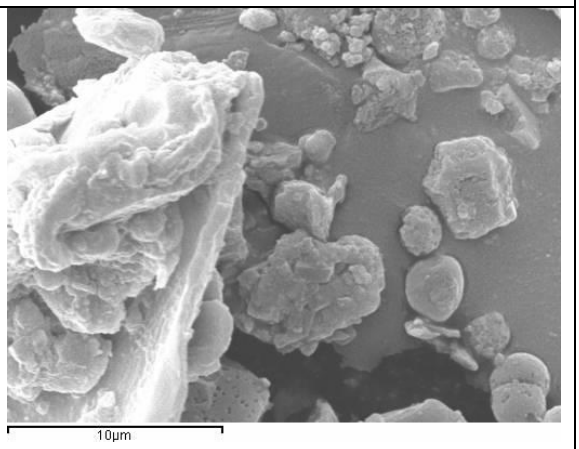
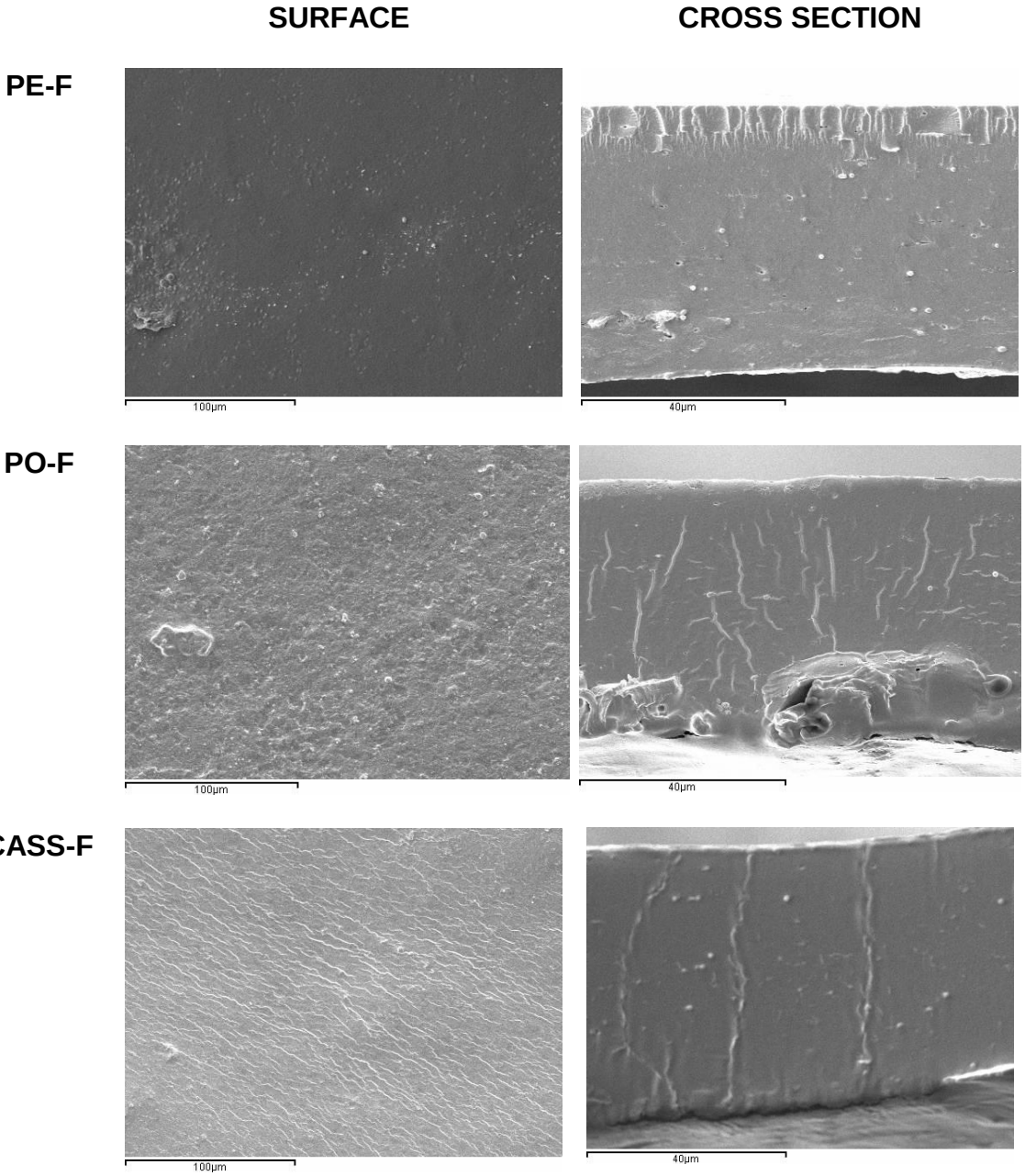
936

937 **Figure 5**

| FINE RICE BRAN | COARSE RICE BRAN |
| --- | --- |
|   |   |

938

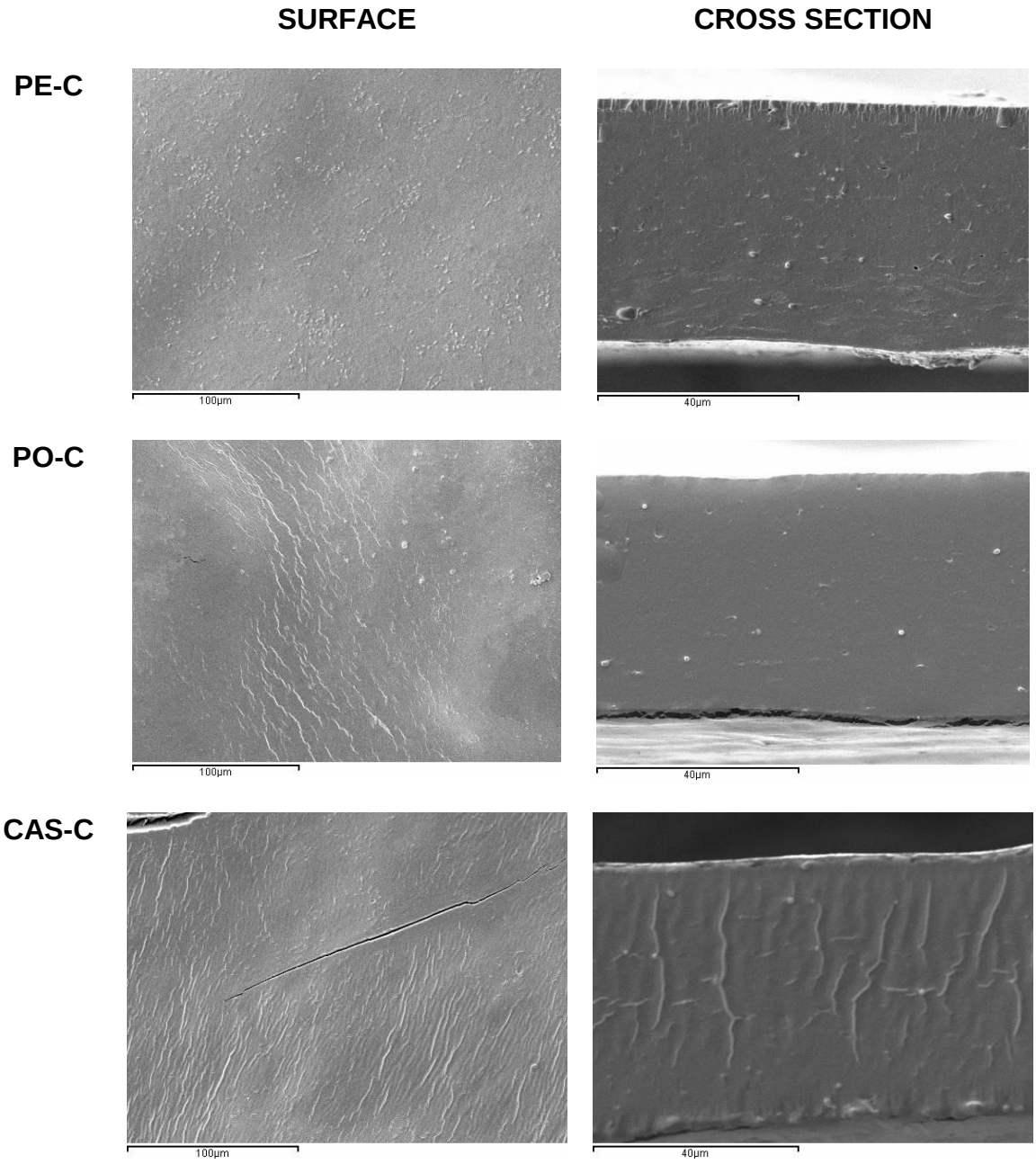
939 **Figure 6**




940

941

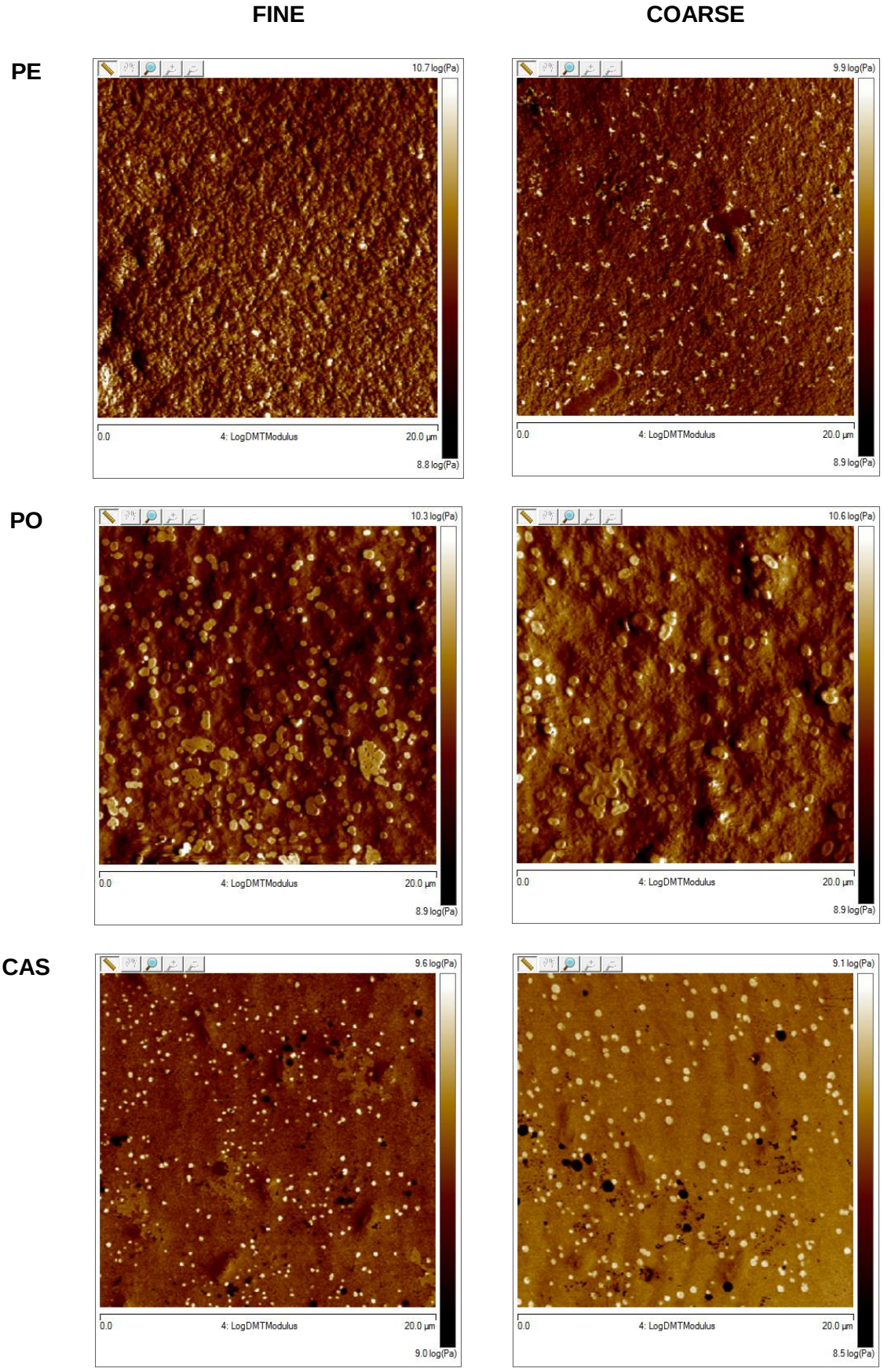
942 **Figure 7**




943

944

945 **Figure 8**


FINE
COARSE
PE
PO
CAS
10.7 log(Pa)
9.9 log(Pa)
10.3 log(Pa)
10.6 log(Pa)
9.6 log(Pa)
9.1 log(Pa)
0.0
4: LogDMTModulus
20.0 µm
8.8 log(Pa)
8.9 log(Pa)
9.0 log(Pa)
8.5 log(Pa)

946