



Effect of replacement of corn starch by whey protein isolate in biodegradable film blends obtained by extrusion

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ABSTRACT

The aim of this study was to evaluate the effect of replacing corn starch by whey protein isolated (WPI) in biodegradable polymer blends developed by extrusion. X-ray diffraction showed the presence of a Vh-type crystalline arrangement. The films were homogeneous, indicating strong interfacial adhesion between the protein and the thermoplastic starch matrix (TPS) as observed in scanning electron microscopy. The addition of WPI on TPS matrix promoted an increase in the thermal stability of the materials. It was observed 58.5% decrease in the water vapor permeability. The effect of corn starch substitution by WPI on mechanical properties resulted in a more resistant and less flexible film when compared the TPS film. The addition of WPI caused greenish yellow color and less transparent films. The substitution of corn starch by WPI made it possible to obtain polymer blends with improved properties and represents an innovation for application as a packaging material.

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1. Introduction

Serious environmental pollution caused by synthetic plastics has led to increased research directed towards biodegradable plastics. The integral use of renewable resources and hydrocarbon reduction has aroused interest in developing alternatives to replace, totally or partially, the synthetic polymers by biodegradable polymers, mainly for food products (Ma, Chang, Yu & Stumborg, 2009; Ortega-Toro, Jiménez, Talens & Chiralt, 2014; Yan, Hou, Guo & Dong, 2012). Thus, films and coatings formed from biodegradable polymers, such as polysaccharides, proteins and lipids, have been studied due to their environmental benefits and sustainability (Hong & Krochta, 2003; Ramos, Fernandes, Silva, Pintado & Malcata, 2012; Redl, Morel, Bonicel, Guilbert & Vergnes, 1999; Sharma & Luzinov, 2013).

In biodegradable plastics development, starch has been considered one of the most promising materials (Ma, Chang, Yu & Stumborg, 2009), among natural biopolymers, due to its low cost, non-toxicity, biodegradability and easy availability (Aydn & Ilberg,

2016; Fajardo, Martins, Fuciños, Pastrana, Teixeira & Vicente, 2010; Šimkovic, 2013; Gross & Kalra, 2002; Wang, Chang & Zhang, 2010). It is composed of amylose and amylopectin in amounts that vary according to plant source, for example, corn starch has about 28% amylose while tapioca starch has 17%. In general, an increasing amount of amylose improves the mechanical, barrier and film formation properties, as well as the processing conditions (Forsell, Lahtinen, Lahelin & Myllärinen, 2002; Mendes et al., 2016; Raquez, Nabar, Srinivasan, Shin, Narayan & Dubois, 2008). In addition, starch has the ability to form a continuous matrix and has thermoplastic characteristics, which allows it to be easily processed (Bertuzzi, Vidaurre, Armada & Gottifredi, 2007; Ortega-Toro, Jiménez, Talens & Chiralt, 2014; Shah, Bandopadhyay & Bellare, 1995; Yan, Hou, Guo & Dong, 2012).

The thermoplastic starch (TPS) is one of the materials studied in the development of biodegradable materials (Chen, Zhang, Zhang, Fan & Wu, 2012; Curvelo, De Carvalho & Agnelli, 2001; López, Zaritzky, Grossmann & García, 2013; Ma, Chang, Yu & Stumborg, 2009; Teixeira, Da Roz, Carvalho & Curvelo, 2007). Starch becomes thermoplastic, when processed with plasticizers under high temperature and mechanical forces, with rupture of the granules and subsequent fusion (de Graaf, Karman & Janssen, 2003; Herrera Brandelero, Victoria Eiras Grossmann & Yamashita, 2011). It can

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easily be utilized in synthetic polymer processes, such as extrusion, blowing, injection and compression molding (Averous & Boquillon, 2004; Teixeira et al., 2007). Currently, there are several products obtained from TPS (López et al., 2013; Ma, Chang, Yu & Stumborg, 2009; Shamekh, Myllärinen, Poutanen & Forssell, 2002), however, the development of TPS films is still limited due to its fragility, low water resistance and dependence on the mechanical properties and environmental moisture (Averous & Boquillon, 2004; Bastos, Santos, da Silva & Simão, 2009; López et al., 2013; Zullo & Iannace, 2009). Thus, in order to compensate for these disadvantages, the starch may be blended with other polymers (Aydin & Ilberg, 2016; Shi et al., 2008).

If compared to other common proteins like gluten, soy or other biopolymers, whey protein has been studied for packaging applications due to its abundant availability (50 million tons of unprocessed whey per year) (Schmid et al., 2012), transparency, flexibility and possessing good aroma and oxygen and lipid barrier properties. However, these films have a low moisture barrier, thermal stability and traction properties (McHugh & John, 1994; Prommakool, Sajjaanantakul, Janjarasskul & Krochta, 2011).

In general, films made from a single polymer have shown good properties in some aspects and bad in others. Therefore, the use of biopolymer mixtures has become an alternative to improve biodegradable film performance properties (Yan, Hou, Guo & Dong, 2012). As such, films obtained by the combination of polysaccharides (starches, alginates, cellulose and chitosan) with protein (milk protein, soy protein, collagen and gelatin) have been developed (Cheng et al., 2003; Ferreira, Nunes, Delgadoillo & Lopes-da-Silva, 2009; García, Cova, Sandoval, Müller & Carrasquel, 2012; Haider, Park & Lee, 2008; Leuangskurk, Phupoksakul, Tananuwig, Borompichaichartkul & Janjarasskul, 2014; Mariani, Allgauer, Oliveira, Cardoso & Innocentini-Mei, 2009; Pereda, Aranguren & Marcovich, 2008; Sun, Sun & Xiong, 2013) and showed better properties when compared those obtained from the pure polymers.

Research has been conducted on proteins and polysaccharides blends, but nothing has been reported about the combination of corn starch and whey protein isolate. As a novel alternative to the food industry for increase the potential use of a by-product of the dairy industry for application as a packaging material, or other plastic stuffs with suitable performance properties, the objective of this study was to evaluate the effect of the replacement of corn starch by whey protein isolate in films produced by extrusion on structural, thermal, morphological, mechanical, optical and barrier properties.

2. Experimental

2.1. Material

Corn starch (Amidex 3001), with 70% amylose and 30% amylopectin, was obtained from Corn Products Brazil (Balsa Nova, PR, Brazil). Whey protein isolate (WPI 9410) with 90% protein was obtained from Hilmar Ingredients (Hilmar, CA, USA). Glycerol, stearic acid and granulated anhydrous citric acid were from Cargill (Uberlândia, MG, Brazil).

2.2. Preparation of TPS/WPI blends films

Corn starch and whey protein isolate (TPS/WPI) blends were obtained from the mixture of the following components: solid phase: 60% (TPS/WPI) and liquid phase: 24% glycerol and 16% water. To the final mixture, 1 wt% stearic acid and 1 wt% citric acid was also added, which act as anticaking agent and antioxidant, respectively. The blends were developed by replacing corn starch (TPS) by whey protein isolate (WPI) in the solid phase (60%) as shown in Table 1.

Table 1
Symbols of TPS/WPI of blend films developed.

Code	Solid phase (60%)	
	TPS (%)	WPI (%)
TPS	100	0
TPS90/WPI10	90	10
TPS80/WPI 20	80	20
TPS70/WPI 30	70	30
TPS60/WPI 40	60	40
TPS50/WPI 50	50	50

The mixture was processed in a Coperion extruder, model ZSK 18, with L/D = 40, co-rotating twin screw, 18 mm diameter screws with mixing and conducting elements, with 7 heating zones and 2 degassing zones. The screw speed was 300 rpm and the temperature profile was 80, 80, 90, 90, 100, 110 and 110 °C. Once the pellets of these materials were obtained, they continued for processing in a single-screw AX Plastics extruder to obtain the sheets of film. The screw speed was 35 rpm and the temperature profile was 85, 90 and 95 °C in the three heating zones. Then the sheets were pressed at 115 °C ± 5 °C for 3 min in a hydraulic press (15 ton capacity). The pressing conditions were: 3 ton/30s, 10 s without pressing; 6 ton for 3 min. After pressing, the film sheets, 12 × 12 cm, were cooled to room temperature. Table 1 shows the composition of each film and each component amount (starch and whey protein isolate).

2.3. Film conditioning and thickness

The films were conditioned under controlled temperature, at 23 ± 2 °C and 50 ± 5% relative humidity (RH) for 48 h prior to testing, according to ASTM D618-00 (ASTM, 2000a). The film average thickness was measured by readings at ten different points, randomly taken in each test body, using a digital micrometer Mitutoyo (0.01 mm precision, Mitutoyo Sul-Americana, Suzano, SP, Brazil).

2.4. X-ray diffraction (XRD)

The corn starch, whey protein isolate and film X-ray diffraction patterns were obtained on a Shimadzu XRD-6000 machine (Shimadzu, Tokyo, Japan) equipped with Cu-K_{α1} (λ = 1.5406 Å) filter radiation. Samples were mounted on aluminum stubs and analyzed with a step of 0.02° s⁻¹, 2θ from 4°–40°, speed of 1° min⁻¹ and 30 kV, 30 mA. The interlayer spacing (d) was calculated by the Bragg equation, (Equation (1)):

$$\sin\theta = n\lambda/2d \quad (1)$$

Where: λ corresponds to the wavelength used (λ = 1.5406 Å) and θ (theta) is the angle where the peak is detected on the diffractogram.

2.5. Scanning electron microscopy (SEM)

The morphology of powdered components (corn starch and WPI) and film was investigated in a JSM 6510 JEOL (Peabody, Massachusetts, USA) scanning electron microscope with an accelerating voltage of 10 kV. Surface images were obtained from powdered components and from the cryogenic fracture of the extruded material. Fractured films were mounted on stubs with carbon tape, coated in gold under vacuum for 90 s (metallization), attached to the microscope and photomicrographed.

2.6. Thermogravimetric analysis (TG)

The thermal stability of the corn starch and WPI powder and of the films was evaluated by thermogravimetric analysis on a TGA

Q500 equipment (TA Instruments, New Castle, USA). The analyzes were performed under a synthetic air atmosphere at a 40 mL min⁻¹ flow rate, heating from 25 °C to 600 °C at a rate of 10 °C min⁻¹.

2.7. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) was performed on a TA 60 DSC calorimeter (Shimadzu Corporation, Kyoto, Japan). Sample weight ranged between 2 and 5 mg and an empty capsule was used as a reference. The heating and cooling ramps for the powdered WPI were set at 10 °C min⁻¹ and varied between -50 °C and 250 °C. For powdered starch, ramps were set at 2 °C min⁻¹ and varied between -80 °C and 50 °C. For the film evaluations, the heating and cooling ramps were set at 10 °C min⁻¹, the and sequence being: heating from 25 to 190 °C/10min, isothermic, to eliminate the thermal history; cooling from 190 to -70 °C; and a second heating until 190 °C (ASTM, 2003a). From the DSC curves, the glass transition temperature (T_g) was measured in the second heating.

2.8. Mechanical properties

The film tensile properties of the films, tensile strength (σ_{max}), elongation at break (ϵ) and elastic modulus (EM), were measured according to ASTM D882-02 standard method (ASTM, 2002), in a EMIC DL3000 Universal Testing Machine (EMIC, São Paulo, Brazil) with 500 N load cell (50 Kgf). Samples of films were cut into strips (10 mm × 100 mm) and the initial separation of the grips was 70 mm with a 50 mm.min⁻¹ speed. The puncture of the films was carried out in a texture analyzer (Stable Microsystems, TATX2i model, England). The films were cut into 9 cm² squares and fixed on a support with a central opening (2.1 cm diameter). A 5.0 mm diameter spherical probe (P/5S probe) was perpendicularly displaced towards the film surface at a constant speed of 0.8 mm.s⁻¹ until the probe passed through the film (Azevedo, Dias, et al., 2015; Chen & Lai, 2008). The puncture strength/thickness (N/mm) was calculated by dividing strength at break by the film thickness to eliminate the effect of the thickness variation. Film deformation (mm) was determined at the breaking point (Azevedo, Dias, et al., 2015; Park & Zhao, 2004). For the tensile and puncture tests, five specimens for each film in three repetitions were used.

2.9. Water vapor permeability (WVP)

The water vapor permeability (WVP) the films was determined according to ASTM E96 method (ASTM, 2000b). The film was fixed in a polymethylmethacrylate (Plexiglas) capsule with four screws, symmetrically located around the circumference of the capsule with silicone grease, to ensure that the permeation takes place only through the film. Silica was added to the interior of the capsule. The permeation capsule was then placed in a chamber with controlled humidity and temperature at 25 ± 2 °C and maintained at 75% relative humidity (RH). The samples were weighed until constant weight was reached and the weight gain values were plotted as a function of time. The film WVP (g/(m.s.Pa)) was calculated according to Equation (2):

$$WVP = (WVTR \cdot \delta) / \Delta P \quad (2)$$

where δ is the film thickness, WVTR is the water vapor transmission rate and ΔP represents the difference in vapor pressure between the two faces of the film: $\Delta P = S \cdot (R_1 - R_2)$; S is the saturated vapor pressure at the test temperature (2809 kPa), R_1 is the RH outside the capsule (75%) and R_2 is the RH inside the capsule that contained silica (0%).

2.10. Optical properties

The film color was measured in a Konica Minolta CR-400 (Osaka, Japan) machine, using the CIE (L^* , a^* , b^*) system with a D65 light source, 10° observer angle and reflectance mode. The analyzed parameters were: (L^* , a^* , b^*), chroma saturation index and hue angle. The results were expressed as the mean of ten readings on three replicates. The transparency of the films was determined by measuring the transmittance percentage (%T) at 600 nm using UV/VIS 1601PC spectrophotometer (Shimadzu, Tokyo, Japan) according to the D1746-03 method (ASTM, 2003b). Transparency (T_{600}) was calculated by Equation (3), where δ is the film thickness.

$$T_{600} = (\text{Log}\%T) / \delta \quad (3)$$

2.11. Statistical analysis

The results of water vapor permeability (WVP) and mechanical and optical properties were evaluated using analysis of variance (ANOVA) with a 5% level of significance, using STATISTICA SOFTWARE (VER. 8, STAT SOFT INC., TULSA, USA). X-ray diffraction (XRD), scanning electron microscopy (SEM), thermal gravimetric analysis (TG) and differential scanning calorimetry (DSC) were submitted to descriptive analysis.

3. Results and discussion

3.1. X-ray diffraction (XRD)

The X-ray diffraction patterns of the starch and powdered WPI are shown in Fig. 1. Fig. 1a presents peaks around $2\theta = 14.2^\circ$; $d = 17.97 \text{ \AA}$; $2\theta = 17.02^\circ$; $d = 5.21 \text{ \AA}$; $2\theta = 18.2^\circ$; $d = 4.88 \text{ \AA}$; $2\theta = 22.6^\circ$; $d = 3.94 \text{ \AA}$, for the corn starch powder characteristic of an A-type crystalline arrangement. Previous studies have reported that waxy maize starch granules have an A-type crystalline arrangement, which has two weak diffraction peaks at 10.1° and 11.5° ; a peak of strong intensity at 15.3° ; a double peak at 17.1° and 18.2° and a final peak of high intensity at 23.5° (Angellier, Molina-Boisseau, Dole & Dufresne, 2006; Kim, Han, Kweon, Park & Lim, 2013). The diffractogram of the powdered WPI showed a possible amorphous structure which indicates the absence of lactose crystals in the WPI (Fig. 1b). The lactose present in the liquid whey during the drying process transforms into an amorphous state (Azevedo, Dias, et al., 2015; Nijdam, Ibach, Eichhorn & Kind, 2007). Thus, lactose removal and subsequent drying of the liquid whey during the concentration process to WPI or to whey protein concentrate (WPC) makes the products amorphous.

Fig. 1c shows the X-ray diffraction patterns of the TPS/WPI films in the region of $2\theta = 4-34^\circ$. The X-ray diffraction pattern of the TPS film showed peaks around $2\theta = 12.6^\circ$; $d = 7.02 \text{ \AA}$; $2\theta = 19.56^\circ$; $d = 4.54 \text{ \AA}$; $2\theta = 22.08^\circ$; $d = 4.03 \text{ \AA}$ (Fig. 1c). The peak around $2\theta = 19.56^\circ$ is attributed to the recrystallization induction process in a simple helical amylose molecule during cooling after processing and corresponds to the Vh-type crystalline arrangement. Starch amylose and lipids are also responsible for the Vh-type crystallite complex formations at high melting temperatures, being also resistant to enzyme attack (Magalhães & Andrade, 2009; de Campos et al., 2013). The Vh-type crystalline arrangement consists of an amylose recrystallization induced by lysophospholipids and complexes that form agents such as isopropanol and glycerol (Teixeira et al., 2011).

Peaks around $2\theta = 16.08^\circ$ are characteristic of amylopectin recrystallization (B-type crystallization) due to the presence of granules after extrusion (Teixeira et al., 2011). Thus, the films devel-

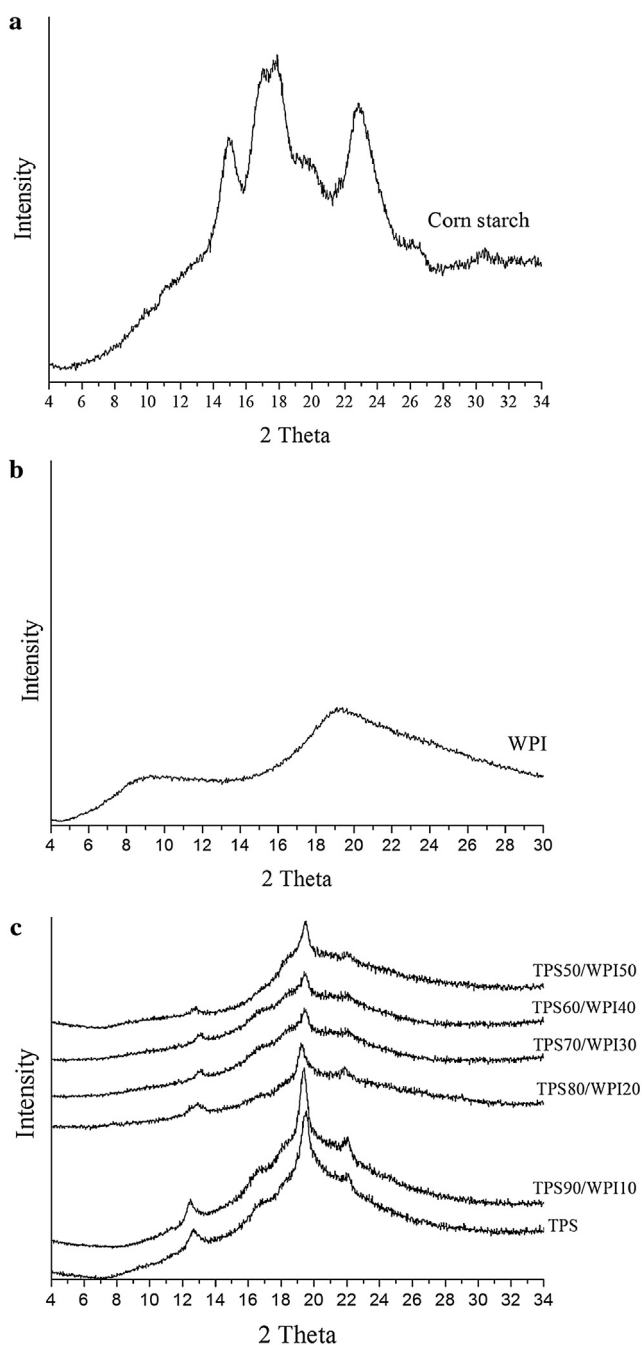


Fig. 1. X-ray diffraction patterns of: a) Powdered corn starch b) powdered WPI; c) corn starch and whey protein isolate films produced by extrusion.

oped in this present study showed no peaks around $2\theta = 16.08^\circ$ indicating the absence of amylopectin residual crystallinity.

The diffraction patterns of the TPS/WPI blends are similar to those of the TPS matrix. All films showed peaks around $2\theta = 12.9^\circ$ and spacing $d = 6.87 \text{ \AA}$. This peak can be attributed to the presence of glycerol complexed with amylose. Furthermore, only the TPS; TPS90/WPI10 and TPS80/WPI20 films showed a diffraction peak at about $2\theta = 22.04^\circ$ and spacing $d = 4.04 \text{ \AA}$ that could also be related to the presence of glycerol. However, it can be seen that with the increase of the WPI concentration and decrease of TPS in TPS70/30; TPS60/40 and TPS50/IPS50 films, the Vh-type crystallinity peaks ($2\theta = 19.56^\circ$) become wider. This fact is the result of the different ratio of starch and whey protein, and the possible decreased for-

mation of amylose-glycerol complex. (Mendes et al., 2016). Peaks at 13.7° , 19.6° and 21.1° are attributed to the Vh-type amylose crystals complexed with lipids and other substances such as glycerol formed during cooling after the process (Carmona, de Campos, Marconcini & Mattoso, 2014; Mendes et al., 2016; Teixeira et al., 2011), whereas peaks at 16.8° , 17.7° and 29.9° belong to the B-type crystals that may have been formed during storage due to amylopectin recrystallization (Dang & Yoksan, 2015). Lavorgna, Piscitelli, Mangiacapra & Buonocore, (2010) evaluated the effect of the presence of the plasticizer glycerol in chitosan films, finding a diffraction peak around $2\theta = 20^\circ$.

Fig. 1c shows the absence of A-type crystals, characteristic of cereal starch granules, for all films. This indicates that the native corn starch structure was completely destructured during extrusion (Shi et al., 2007), but some granules might be buried inside (Li et al., 2015) as shows the surface/fractured surface morphology (SEM) of the films, Fig. 2h.

3.2. Scanning electron microscopy (SEM)

X-ray studies are always supplemented by microscopy techniques. Fig. 2(a,b) shows the SEM micrographs of the powdered corn starch and powdered WPI granules. The starch granules (Fig. 2a) had shapes ranging from round to polygonal and their complete disintegration can be observed by the TPS/WPI blend films micrographs (Fig. 2c–h). The WPI particles showed to be spherical with rough and smooth surfaces. The morphology of the protein particles is attributed to the protein composition and their chemical structures. The principal WPI components are α -lactalbumin, β -lactoglobulin and bovine serum albumin (BSA), among others. These proteins are often agglomerated due to hydrophobic intermolecular interactions, disulfide bonds, hydrogen bonds and/or electrostatic interactions depending on the applied conditions, such as temperature, pressure, concentration and pH. Usually, WPI morphology is attributed to the presence of the principal compound, β -lactoglobulin (Ghosh, Ali, Selvanesan & Dias, 2010; Havea, Singh & Creamer, 2001).

The SEM micrographs of the fracture surface of the extruded films are shown in Fig. 2. The TPS film showed a smooth and homogeneous surface (Fig. 2c). This indicates the absence and complete destructuring of native corn starch granules after the extrusion process. This result explains the absence of A-type crystal for TPS film in the XRD graph (Fig. 2c). The TPS90/WPI10 film presented a rough surface and presence of agglomerates (Fig. 2d). The TPS80/WPI20 film showed to be more homogeneous and with few agglomerates (Fig. 2e). The TPS70/WPI30 film (Fig. 2f) presented as homogeneous, however with more evident cracks that might have been formed during the compression molding process. The TPS60/WPI40 and TPS50/WPI50 films (Fig. 2g and 2h) showed a homogeneous surface without cracks and with small agglomerated particles. This may indicate a strong interfacial adhesion between the protein and TPS matrix. During the extrusion process, certain surface irregularities may be formed in the structure of the films (Mendes et al., 2016; Tadmor & Gogos, 2013).

3.3. Thermogravimetric analysis (TG)

TG curve and its first derivative (DTG) for corn starch and WPI powdered, and TPS/WPI films are shown in Fig. 3 and were determined to evaluate the films thermal stability. Three degradation stages were observed (Fig. 3). The first stage occurs between 25 and 200°C and is not influenced by the mixture of polymers. The mass loss below 200°C is attributed to the presence of residual water and low molecular weight volatiles, since the start of protein degradation begins around 225°C (Azevedo, Dias, et al., 2015; Ma, Yu & Ma, 2005; Martínez-Camacho et al., 2010; Sharma & Luzinov,

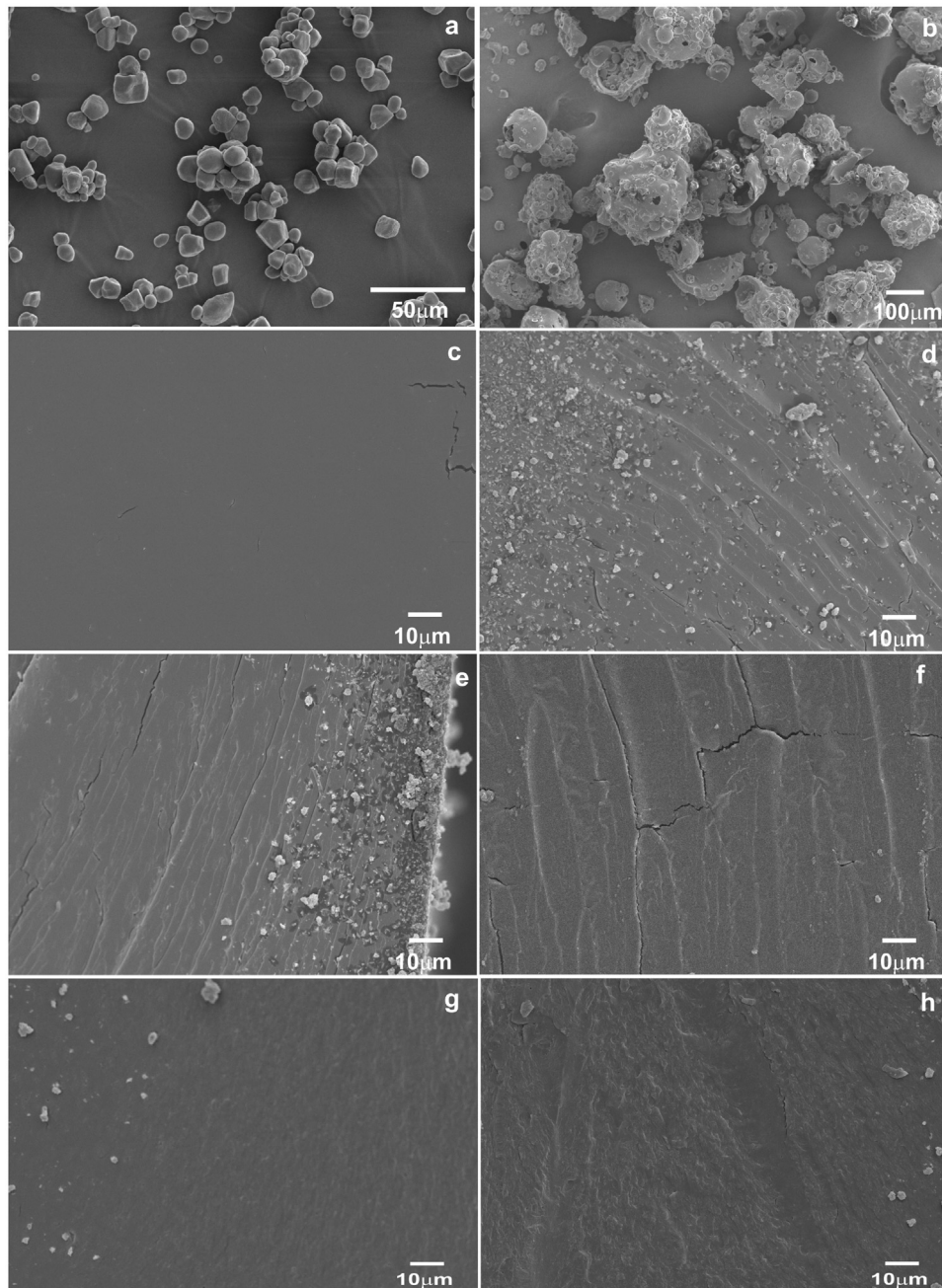


Fig. 2. SEM – Surface micrographs. a) powdered corn starch; b) powdered WPI. Fracture Micrographs of TPS/WPI. c) TPS; d) TPS90/WPI10; e) TPS80/WPI20; f) TPS70/WPI30; g) TPS60/WPI40; h) TPS50/WPI50.

2013). The second stage was observed from 200 °C to about 350 °C, corresponding to the thermal decomposition of the components present in the films. The third stage occurs above 350 °C and corresponds to the oxidative degradation of carbonaceous residues formed during the second stage, in which under air atmosphere, the complete oxidation of the materials occurs (Azevedo, Dias, et al., 2015; Canevarolo Jr, 2004; Pelissari, Grossmann, Yamashita & Pineda, 2009).

The powdered components (WPI and corn starch) and TPS/WPI films thermal stability parameters are shown in Table 2. The initial of thermal degradation (T_i) for the TPS decreased compared to powdered corn starch (Table 2). This may be attributable to the extruded material plasticization, which in the presence of water and glycerol decreases the material T_i . Mendes et al. (2016), in research with TPS and chitosan film blends, reported that the addition of chitosan

did not significantly alter the thermal stability of mixtures compared to pure TPS. These authors reported an initial degradation temperature for the pure TPS around 277 °C.

For the DTG_{max} curves (Fig. 3d), the temperature at which maximum degradation speed occurs, two thermal events are also presented. Table 2 shows an increase of the temperature range for the second DTG_{max} peak, possibly due to increased WPI concentration. The first peak (between 294.9 and 310.8 °C) is attributed to TPS degradation while the second peak (between 463.8 °C and 543.21 °C) is related to WPI degradation according to DTG_{max} temperatures of the WPI and powdered corn starch (Table 2).

When there is substitution of corn starch by WPI, an increase in the film T_i is noteworthy (Table 2), indicating an improvement in the film thermal stability. This can be expected since the mixture of polymers is an alternative in order to improve the performance

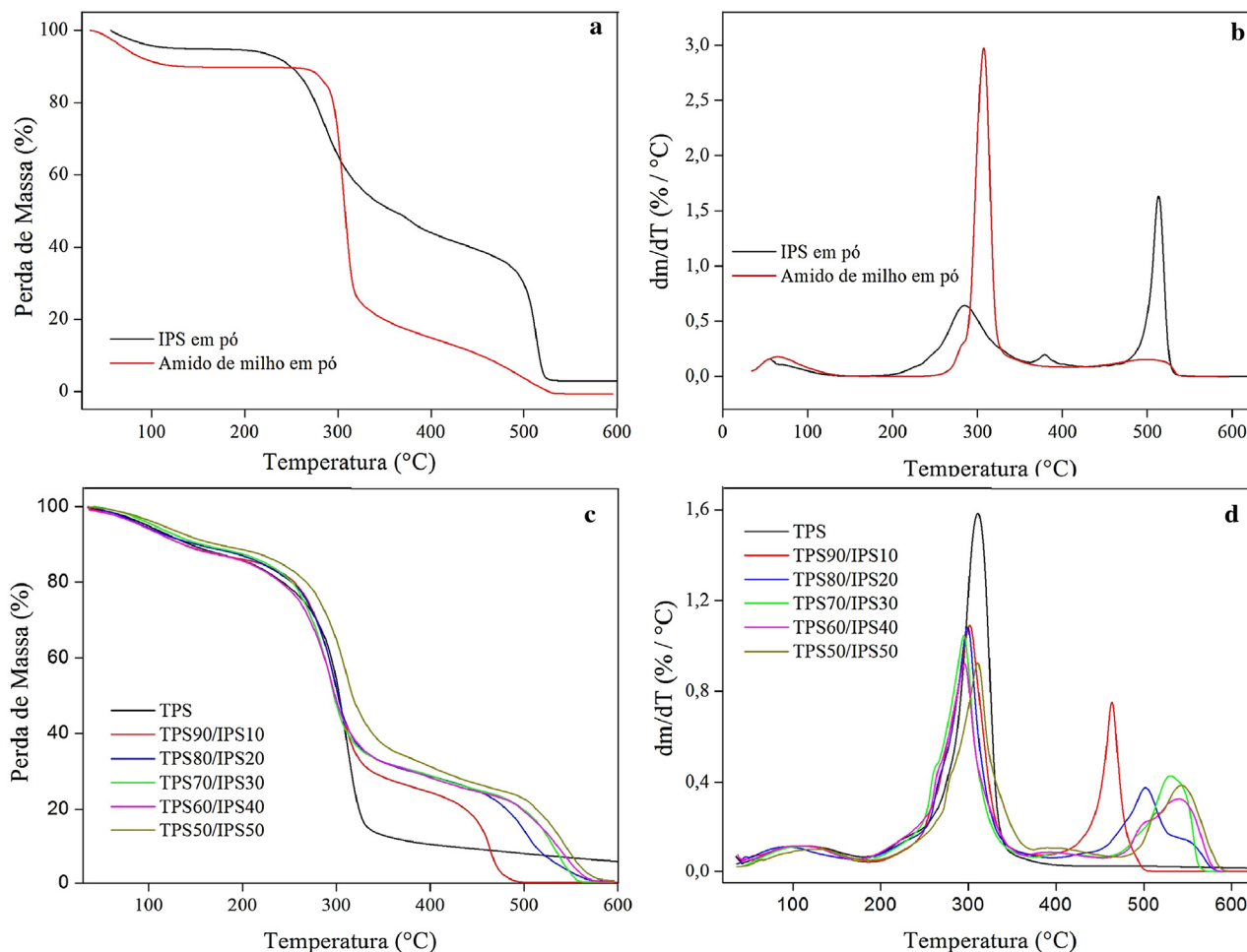


Fig. 3. TGA (a, c) and DTG curves (b, d) of powdered corn starch, powdered WPI and TPS/WPI blend films in synthetic air atmosphere.

Table 2
Thermal stability of corn starch and WPI powder and TPS/WPI blend films obtained by TGA and DSC.

	T_i^* (°C)	DTG_{max} (°C)	T_f (°C)	(%) ML ($T_i - T_f$)	Res _{600 °C} (%)	T_m (°C) ^a	T_g (°C)
Corn starch powder	285.2	307.3	541.9	84.6	0.12	109.7	-28.9
WPI powder	250.6/401.2	285.1/512.8	360.7/540.6	89.7	1.8	-	186.1
TPS	230.1	309.2	369.7	84.2	3.2	144.5	56.9
TPS90/WPI10	229.2/395.6	301.8/463.7	365.1/505.7	81.7	0.3	189.2	102.6
TPS80/WPI20	232.7/431.9	298.9/502.1	366.5/587.7	82.7	0.6	189.1	104.1
TPS70/WPI30	234.53/459.72	294.9/530.4	371.3/573.1	83.2	0.4	189.4	106.7
TPS60/WPI40	248.8/462.8	295.7/539.8	365.1/586.9	81.5	0.5	188.7	101.5
TPS50/WPI50	252.5/475.8	310.8/543.2	372.7/585.3	83.7	0.8	189.1	102.1

^a T_i (initial decomposition temperature); DTG_{max} (maximum decomposition temperature); T_f (final decomposition temperature); M_{Loss} (mass loss percentage in the decomposition range); Res_{600 °C} (percentage of residue at 600 °C); T_m (crystalline melting temperature); T_g (glass transition temperature).

properties in films. Moreover, with the addition of WPI to the TPS matrix, it can be noted that the second thermal decomposition stage takes place in two stages (two temperature peaks), unlike for the TPS film, probably due to the presence of other components present in WPI which interferes with the degradation mechanism. However, the substitution of corn starch by WPI did not alter the film degradation profile compared to the first T_f peak (365.1 °C to 372.7 °C) compared to the TPS film. Nevertheless, there is a T_f increase in the second temperature peak (505.7 °C to 587.7 °C) indicating that the presence of WPI is responsible for increasing the thermal stability of the blends. Teixeira et al. (2011) reported on work conducted on TPS and cellulose nanofiber that thermal degradation of the TPS is between 300 °C and 350 °C. Mariani, Allganer, Oliveira, Cardoso & Innocentini-Mei, (2009) studied the effect of

increasing concentrations of soy protein isolate (SPI) on corn starch and polycaprolactone (PCL) blends processed by extrusion. These authors reported that the corn starch and SPI addition was responsible for the reduction of the thermal properties of the materials compared to pure PCL. However, with the increased amount of SPI and reduced starch incorporated in the sample, the thermal properties tended to increase.

With the increasing concentration of WPI in the blends, there is a reduction in the mass loss (%ML) percentage compared to the corn starch and the powdered WPI. This indicates that the TPS/WPI blends became more stable to thermal decomposition. Authors report that material stability to thermal decomposition is related to a decrease in the mass loss (% ML) percentage range (Azevedo, Dias, et al. 2015; Canevarolo Jr, 2004).

Table 3
Tensility properties (σ_{\max} , EM, ϵ); puncture (PS and PD) of TPS/WPI films.

Film	Tensile strength (σ_{\max}) (MPa)	Elastic Modulus (EM) (MPa)	Elongation at break (ϵ) (%)	Puncture strength/thickness (PS) (N/mm)	Puncture Deformation (PD) (mm)
TPS	3.85 ± 0.62 a	143.71 ± 4.6 a	32.76 ± 3.2 a	92.40 ± 5.3 a	4.96 ± 0.36 a
TPS90/WPI10	4.04 ± 0.07 a	151.40 ± 22.8 a	21.60 ± 1.5 b	70.50 ± 9.7 b	3.67 ± 0.18 b
TPS80/WPI20	3.84 ± 0.38 a	152.53 ± 11.9 a	11.70 ± 3.4 c	56.74 ± 3.3 c	3.01 ± 0.27 c
TPS70/WPI30	5.73 ± 0.38 b	198.90 ± 11.0 b	10.65 ± 1.3 c	75.40 ± 5.8 b	3.38 ± 0.32 d
TPS60/WPI40	4.24 ± 0.34 a	230.35 ± 22.1 b	2.83 ± 1.1 d	19.57 ± 9.6 d	1.78 ± 0.18 e
TPS50/WPI50	3.07 ± 0.49 a	150.74 ± 16.7 a	3.84 ± 1.3 d	11.45 ± 3.1 d	1.86 ± 0.45 e

^aMeans observed in the column with the same letter are not statistically different ($p < 0.05$).

In studies with WPI and whey protein concentrate blends at different concentrations of glycerol, Ramos et al. (2012), obtained high mass loss at temperatures between 280 °C and 500 °C. These authors reported that this loss is associated with protein degradation and with the glycerol plasticizer, incorporated in the film matrix. Table 2 shows the absence of residual substances (Res_{600 °C}) after burning the films, since there is complete oxidation of the material under air atmosphere. Thus, the TPS/WPI blends showed increased thermal stability compared to the TPS film.

3.4. Differential scanning calorimetry (DSC)

The DSC analysis was performed for a better understanding of the interactions between TPS and WPI and transition temperatures. All films showed matrix crystalline phase melting temperature (T_m) (Table 2). The occurrence of a single crystalline melting temperature is related to homogeneity and miscibility among the film components (Lopez, Garcia, Villar, Gentili, Rodriguez & Albertengo, 2014).

T_m increased with the WPI addition compared to the TPS film (Table 2). This increase can be attributed to the interaction between starch molecules and proteins due to intermolecular forces with stronger bonds. Lopez, Garcia, Villar, Gentili, Rodriguez & Albertengo, (2014) reported that in the crystalline regions of the TPS matrix, the interactions among polymer chains are strong because of their chemical compatibility. These authors reported $T_m = 155$ °C for TPS films and T_m from 147.2 °C to 154.8 °C in TPS/chitosan blends. Sharma and Luzinov, (2013) reported the absence of crystallinity for WPI due to whey protein globule denaturation, indicating a plasticization effect of water. These authors suggest that water molecules were linked to proteic macromolecules via hydrogen bonds. This information was corroborated by the absence of crystallinity in the X-ray diffractograms of this study for WPI.

It was observed that the TPS film T_g was 56.9 °C (Table 2). The addition WPI in the TPS matrix increased the T_g , thereby decreasing the mobility of the polymer chains. This indicates an increase in thermal stability of the films. Sharma and Luzinov, (2013) found a $T_g = 120$ °C for in films with WPI and albumin blends. Azevedo, Dias, et al. (2015) reported $T_g = 143.46$ value for pure WPI films. Hernandez-Izquierdo, Reid, McHugh, Berrios & Krochta, (2008) have reported the existence of an endothermic peak at 148.3 °C for pure WPI films obtained by extrusion. T_g values for powdered WPI were reported by Zhou and Labuza (2007), who found 153 °C and Adhikari, Howes, Bhandari & Langrish, (2009) who observed values from around 75–119 °C containing 7 to 16% water. The T_g value for polyvinyl and corn starch film blends (WVP/starch 1:1) was higher (T_g 76.1 °C) when compared to pure WVP polymer (T_g 70.5 °C). This increase is attributed to the presence of hydrogen bond interactions between starch and WVP (Aydin & Ilberg, 2016).

3.5. Mechanical properties

The effect of replacing corn starch by WPI was significant ($p < 0.05$) for tensile strength (σ_{\max}), elastic modulus (EM), elongation (ϵ), puncture strength (PS), puncture deformation (PD) and are shown in Table 3. For σ_{\max} , there was a significant difference only for the TPS70/WPI30 film with an increase of 48.8% compared to the TPS film (Table 3). This indicates that a higher force was needed to break the film. For films with concentrations above 30% WPI, the σ_{\max} value was reduced. We observed an increase in the EM (60.3%) with up to 40% WPI for TPS/WPI film blends. This indicates less movement of the polymer chains, characterizing the films them as being highly rigid, i.e., less flexible. However, TPS70/30WPI and TPS60/WPI40 films showed no significant difference between each other (Table 3). The high rigidity can be attributed to a reduction of film plasticization during the extrusion process at high temperatures due to possible water and glycerol evaporation. The water also has a plasticizing effect on hydrophilic films, but in the presence of low humidity, the film becomes brittle due to reduced residual water in the film (Coupland, Shaw, Monahan, O'Riordan & O'Sullivan, 2000).

With the EM increase, there was an 88.3% reduction of the elongation (ϵ) for all films compared to the TPS film, indicating decreased plasticizer effect and less film stretching. In general, protein-based films have low tensile strength, i.e., high modulus of elasticity and low extensibility (Sharma & Luzinov, 2013). Yan, Hou, Guo & Dong, (2012) investigated the effects of extrusion for film formation and the glycerol concentration on the properties of starch films. With increasing glycerol content, the film becomes more flexible with a greater elongation at break and lower tensile strength. Plasticizers alter the film flexibility and extensibility, with a subsequent modulus of elasticity and tensile strength decrease due to the reduction of hydrogen bonds among polymer chains, (Alves, Mali, Beléia & Grossmann, 2007; Mali & Grossmann, 2003). The addition of plasticizers, providing flexibility, becomes necessary due to the fragility of WPI-based films (Shaw, Monahan, O'Riordan & O'Sullivan, 2002). Previous research has reported that for obtaining plastic with an acceptable performance, from protein, the use of plasticizers such as water or others with low molecular weight molecules is necessary to increase the protein processability and thermoplasticity/polymerization during molding (Matveev, Grinberg & Tolstoguzov, 2000; Rouilly, Orliac, Silvestre, & Rigal, 2001).

In addition to interfering with the WPI-based films properties, the absence of plasticizers also affects the development of starch films. According to Liu and Han (2005), the absence of plasticizers would form a film with a biphasic structure composed of amylose and amylopectin. The presence of plasticizers in starch films assists in the disruption of the double helix of amylose with amylopectin fragments formation, thus reducing the amylose/amylopectin interaction, forming a flexible film.

For the puncture test, WPI addition decreased the film PS and PD by 87.6% and 62.5% respectively (Table 3). This indicates less poly-

Table 4
Effect of corn starch substitution on WVP and color parameters (a^* , b^* , hue, and chroma) of TPS/WPI films.

Films	WVP $\times 10^{-12}$ ($\text{g m}^{-1} \text{s}^{-1} \text{Pa}$)	Transparency ($\log(\%T)/\text{mm}$)	a^*	b^*	chroma	hue
TPS	4.63 \pm 0.32 a	5.39 \pm 0.11 a	-0.76 \pm 0.12 a	4.07 \pm 0.32 a	3.48 \pm 1.15 a	-77.76 \pm 0.29 a
TPS90/WPI10	3.83 \pm 0.07 b	4.41 \pm 0.04 b	-0.91 \pm 0.07 a	4.13 \pm 0.45 a	4.17 \pm 0.32 a	-77.56 \pm 0.40 a
TPS80/WPI20	3.09 \pm 0.15 c	4.43 \pm 0.10 b	-0.88 \pm 0.30 a	3.40 \pm 1.10 a	4.23 \pm 0.48 a	-77.64 \pm 0.93 a
TPS70/WPI30	2.71 \pm 0.26 c	4.99 \pm 0.10 c	-0.50 \pm 0.08 b	5.08 \pm 0.52 b	5.10 \pm 0.52 b	-84.41 \pm 0.45 b
TPS60/WPI40	2.84 \pm 0.28 c	4.62 \pm 0.14 d	-1.25 \pm 0.19 c	6.76 \pm 0.93 c	6.89 \pm 0.84 c	-79.50 \pm 0.27 c
TPS50/WPI50	2.45 \pm 0.34 c	3.91 \pm 0.05 e	-1.47 \pm 0.11 d	11.93 \pm 1.36 d	12.02 \pm 1.38 d	-82.93 \pm 0.30 d

* Means observed in the column with the same letter are not statistically different ($p < 0.05$).

mer chain mobility, less film deformation and perforation strength and, consequently, films that are weaker and more fragile.

3.6. Water vapor permeability (WVP)

The corn starch substitution by WPI was significant ($p < 0.05$) for WVP (Table 4). In barrier properties studies, thickness control becomes a very important variable because with increased thickness, WVP decreases (McHugh & John, 1994). In this work, the thickness of the films showed no significant difference ($p < 0.05$) and ranged from 330 to 390 μm .

According to Table 4, the TPS film had the highest WVP. This increase can be attributed to the high affinity of glycerol for starch which promotes the diffusion of these molecules. Because it is a hydrophilic molecule, glycerol can easily lodge between the starch molecules and form hydrogen bonds with amylose and amylopectin hydroxyl groups (Laohakunjit & Noomhorm, 2004). Yan, Hou, Guo & Dong, (2012) reported that the extrusion process can also cause a certain degree of depolymerization and hydrogen bond breakage in starch, with consequent water absorption facility.

The WVP of TPS/WPI blend films decreased with increasing substitution of corn starch by WPI (Table 4). However, the film did not differ significantly above 20% corn starch replacement by WPI. WPI is a hydrophilic polymer which has hydroxyl groups, thus, the water could easily permeate the film (Wang, Zhang, Han & Bai, 2009). However, possibly due to the crystalline phase formed in the polymer matrix, as shown by the X-ray diffraction, the water vapor passage becomes limited. The water vapor and gas permeation occurs in the polymer amorphous regions in empty spaces while in the crystalline phase, a reduction in the water vapor passage occurs (Sarantopoulos et al., 2002). Moreover, WVP is related to factors such as film structure, amount of hydrophilic/hydrophobic material, polymer chain mobility due to the presence of plasticizers and the ratio between amorphous and crystalline regions (Garcia, Martino & Zaritzky, 2000). Another factor responsible for the WVP reduction may be associated with protein denaturation above 50 °C. Since the whey protein denatures, it becomes insoluble and aggregated (Onwulata, Konstance, Cooke & Farrell, 2003). Changes in the globular structure of proteins occur above 50 and 80 °C for α -lactalbumin and β -lactoglobulin, respectively (Hong & Creamer, 2002).

Apart from possible crystallinity formed in the TPS matrix and formation of insoluble WPI aggregates, as shown in the X-ray diffraction, resistance to water vapor of the TPS/WPI blends increased, possibly due to the presence of citric acid in the film formulation. Azevedo, Silva, Pereira, da Costa & Borges (2015) studied the effect of citric acid on the physical properties of WPI film and reported a decrease in WVP with the addition of 5% citric acid. These authors reported that in the presence of citric acid, the hydrogen bond formed with a hydroxyl group and a carboxyl group enhances the inter/intramolecular interaction among additives present in the film. This result corroborates with the SEM micrographs, which indicated a strong interfacial adhesion between the protein and TPS matrix. Lopez, Zaritzky, Grossmann & García, (2013) reported a

62% reduction of WVP in thermoplastic corn starch with acetylated starch and glycerol blends. These authors attributed this result to the hydrophobic character of the acetylated starch.

3.7. Optical properties

Color attributes are important because they are directly related to consumer acceptance. The optical transparency properties, a^* , b^* , hue and chroma presented significant difference ($p < 0.05$) among the films with substitution of corn starch by whey protein isolate (Table 4). The luminosity (L^*), which ranges from zero (black) to 100 (white), did not present significant difference ($p < 0.05$) among treatments, ranging from 85.17 to 88.75, indicating lighter films approaching the color white.

The films presented significant differences ($p < 0.05$) with a transparency reduction compared to the TPS film (Table 4). Transparency is related to the film internal structure (Sanchez-Gonzalez, Chafer, Chiralt & Gonzalez-Martinez, 2010a). The film crystallinity, as shown by X-ray diffraction, possibly decreased light transmission through the film thereby reducing the transparency. Brindle and Krochta (2008), reported that WPI films with glycerol were transparent whereas WPI/hydroxypropylmethylcellulose (HPMC) blends and glycerol became translucent, indicating some degree of immiscibility between WPI and HPMC.

The ($-a^*$) parameter, that corresponds to a tendency towards green, showed significant difference ($p < 0.05$) and increased with WPI addition only for the TPS60/WPI40 and TPS50/WPI50 films. The hue, chroma and b^* parameters had a significant effect ($p < 0.05$) with the substitution of starch by whey protein isolate. The ($+b^*$) parameter, related to yellow color, increased with WPI addition in the TPS70/WPI30; TPS60/WPI40 and TPS50/WPI50 films. Chroma is a measure of color saturation (Zhong, Song & Li, 2011). An increase in chroma values was observed, characterizing films with high color intensity. The Hue parameter is a value in degrees and refers to the color tone in the three-dimensional color diagram: chroma $+a^*$ 0° (red), $+b^*$ 90° (yellow), $-a^*$ 180° (green) and $-b^*$ 270° (blue). Although yellow and red are two totally different tonalities, mixing yellow with red results in orange color (yellow-red), mixing yellow and green results in yellow-green, blue mixed with green results in blue-green and so on (Sensing, 2007).

There was a hue increase only for TPS70/WPI30; TPS60/WPI40 and TPS50/WPI50 films. Thus, the position of the TPS/WPI film blends can be estimate in a greenish-yellow tonality within Cielab color solid. Silva, Pereira, Carvalho & Ferrua, (2007) reported that the film color can be influenced by the starch source. Cassava starch films presented higher color difference when compared to corn and potato starch films. During the starch gelatinization process, changes in the coloration occur due to the loss of structure and crystallinity of the starch granules, the gel thus becoming a structure with a certain opacity (Guerrero, Beatty, Kerry & De La Caba, 2012). Films based on thermoplastic corn starch (TPS) and chitosan/chitin were obtained by thermo-compression and showed a yellowish color. These authors reported that the processing temperature promoted chemical reactions such as the Maillard reaction related to

non-enzymatic browning (Lopez, Garcia, Villar, Gentili, Rodriguez & Albertengo, 2014).

4. Conclusion

Based on the X-Ray diffractograms and scanning electron microscopy, native starch granules were completely unstructured during extrusion, resulting in homogeneous films and strong adhesion between the TPS matrix and whey proteins. All films presented reduced WVP, not being significant at concentrations above 20% WPI compared to the TPS film. The TPS70/WPI30 film presented as more resistant, less flexible and with low elongation compared to the TPS film. The addition of WPI in the TPS matrix made the films more stable to thermal decomposition and caused alterations in the optical properties, with a greenish-yellow tonality and reduction of transparency compared to the TPS film. Thus, extrusion of biodegradable polymer blends is an important step for the development of films for use in packaging, the TPS70/WPI30 being the best film for continued future work.

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