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Development and characterization of multilayer films based on polyhydroxyalkanoates and hydrocolloids

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ABSTRACT: New biodegradable polymeric materials have been developed in order to minimize the environmental impact caused by the traditional packaging found in the market. Polyhydroxyalkanoates (PHAs) are biopolymers with important features such as biodegradability and biocompatibility; however, the costs associated with PHAs production and the limited mechanical properties reduce their application. Whey is a residual product from dairy industry and gelatin is a biopolymer with good processing characteristics. In this context, a filmogenic solution based on these two biopolymers was incorporated into pure PHA films to improve their optical, mechanical, and structural properties. The filmogenic solution was prepared from gelatin, cheese whey and PHAs, using glycerol as plasticizer agent. The multilayers films (gelatin concentrations at 3 and 5%, w/v) showed higher values for all properties when compared with the PHA standard film. In this sense, the addition of this solution was responsible for the improvement of the film properties. The 5% gelatin multilayers films added of cheese whey and PHAs showed better results when compared with the multilayers films with gelatin at 3%. The film composed of gelatin 5% and cheese whey showed a water vapor permeability ranging from 0.45 g mm/m^2/d/kPa, elongation of 2.18%, and opacity of 14.5%. However, the results of the morphological analysis showed that both films presented a homogeneous surface without cracks. Moreover, the results of the thermal analysis of both films indicate polymeric miscibility. Thus, the choice of the best film will depend on its applicability. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 44458.

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INTRODUCTION

The use of plastic materials is increasing due to their mechanical properties, durability and low cost. Among the various types of polymers utilized by society, synthetic plastics have been the most used ones. However, their inadequate discard represents a huge environmental impact.

Polyhydroxyalkanoates (PHA) are biodegradable polyesters accumulated by bacteria, in the form of intracellular granules, as carbon, and energy storage. PHAs may be produced from carbon source obtained from low cost substrates.[1–3] Their properties are similar to that found in various synthetic thermoplastics, for example, polyethylene and polypropylene, having a wide range of applications.[4]

Beyond the PHAs, there are other types of biodegradable biopolymers. The most used ones are obtained from proteins (gelatin, casein, egg albumin, wheat gluten, zein, and myofibrillar proteins), polysaccharides (starch and its derivatives, pectin, cellulose and its derivatives, alginate, and carrageenan), and lipids (acetylated monoglycerides, stearic acid, waxes, and fatty acid esters) or thereof combination.[5]

These biopolymers can be edible and/or biodegradable, which will guide their applicability. The use of gelatin as a biopolymer is extremely interesting by the fact that it is produced in abundance in Brazil, and has low commercial cost, with appropriate functional properties for the production of biofilms.[6]

Gelatin is obtained by thermal denaturation or physicochemical degradation of collagen, and may be used together with other components.[7] The plasticizer and solvent utilized for forming the films must ensure the compatibility with the polymer.[8]

Cheese whey is a polluting residue of the cheese industry and it cannot be discarded in rivers, due to the high biological oxygen demand needed for its biodegradation. Because of its low price,

cheese whey can be an excellent carbon source for bacteria. Production of PHAs with different composition using cheese whey as the main carbon source will allow their use in several applications.[9]

In this sense, the production of films by combining gelatin, cheese whey, and PHAs can be a viable alternative to reduce the environmental impact and production costs by the utilization of residues and low cost raw materials. Thus, the aim of this work was to develop multilayer films composed of gelatin, cheese whey and PHAs, and to characterize them in function of their properties, for packaging food products.

EXPERIMENTAL

PHA Obtaining

Microorganism and Preservation. *Burkholderia sacchari* LMF 101 was obtained lyophilized. Cells were reactivated by cultivation in 125 mL-Erlenmeyer flasks shaken with 50 mL of nutrient broth (24 h, 30 °C, 200 rpm) and cryopreserved by the addition of 20% glycerol solution in the same proportion of the medium. The final solution (glycerol, 10% v/v) containing the cells was distributed into 2 mL sterile microtubes, which were kept at domestic freezer (−18 °C, 40 min) before stored at −80 °C in ultrafreezer.

Pre-cultures were prepared by transferring a loop from the frozen stock vial to a Petri plate containing nutrient broth agar (48 h, 30 °C). A loop from the plate was transferred to a 500 mL-Erlenmeyer flask containing 250 mL of mineral medium (as described below) added of carbon source. After 16–18 h of growth in an orbital shaker (250 rpm) at 35 °C, an aliquot (calculated $OD_{600} = 0.1$) was utilized as inoculum for the main cultivation in the same synthetic medium, as described below.

Culture Medium. Cultivations were performed in defined mineral medium, containing (per liter of distilled H_2O): KH_2PO_4, 6.67 g; $(NH_4)_2HPO_4$, 4 g; $MgSO_4 \cdot 7H_2O$, 0.8 g; $C_6H_8O_7 \cdot H_2O$, 0.8 g; thiamine, 0.01 g, and trace elements solution, 0.5 mL. The composition of the trace elements solution was (per liter of 5 *M* HCl): $FeSO_4 \cdot 7H_2O$, 10 g; $CaCl_2$, 2 g; $ZnSO_4 \cdot 7H_2O$, 2.2 g; $MnSO_4 \cdot 4H_2O$, 0.5 g; $CuSO_4 \cdot 5H_2O$, 1 g; $(NH_4)_6Mo \cdot 7O_{24} \cdot 4H_2O$, 0.1 g; $Na_2B_4O_7 \cdot 10H_2O$, 0.02 g.[10] All medium components, except the carbon source, and thiamine were sterilized by autoclaving at 121 °C for 15 min. The solution containing the carbon source (glucose, 10 g/L) was autoclaved separately and thiamine sterilized by filtration. Components were mixed after reached room temperature.

Cultivations. Cultivations were performed in 500 mL Erlenmeyer flasks with 250 mL working volume and started by adding a certain volume of the pre-culture, so that the initial cell concentration in the flask was 0.1 absorbance units at 600 nm (Abs_{600}). Cultivation conditions were 35 °C, initial pH of 6.0 in an orbital shaker at 250 rpm.

Polymer Extraction. Upon reaching the stationary phase, cultures were stopped, centrifuged (1100 rpm, 40 min), and the biomass pellet was dried in an oven (40 °C) until constant weight. Extraction was performed by using a Soxhlet extractor with chloroform as solvent, during a 12 h flow. Then the solution was concentrated and the polymer precipitated with a 95% ethanol solution under stirring (150 rpm). The precipitated polymer was dried at 30 °C for 24 h.

Preparation of Pure PHA Films

After the extraction process, the PHA solution was obtained by mixing 8 g of PHA in 60 mL of $CHCl_3$. The solution was then stirred (150 rpm, 2 h) at 25 °C. The PHA films were obtained through the technique of solvent evaporation, using a solution of PHA dissolved in chloroform, poured into glass Petri dishes of 10 cm diameter containing 15 mL per plate. Plates were kept on a leveled bench where the solution was allowed to dry at 25 °C for 48 h.

Preparation of Multilayer Films

For the preparation of multilayer films, it was used the dip coating technique, where the pure PHA film was immersed for 1 min. in a filmogenic solution prepared with two macromolecules: gelatin (GEL) and cheese whey (CW) in nature, glycerol as plasticizer agent. After the immersion, were then dried at 24 °C for 24 h. The preparation of the filmogenic solution of gelatin and cheese whey was obtained by modification of the method described elsewhere.[11] Films containing only PHAs and gelatin at 3 and 5% (w/v) were utilized as control samples. After drying, films were conditioned at 25 °C and 52% relative humidity for 48 h before analysis, forming a total of three layers (pure PHA wrapped by two other layers, one in each side).

Physical Properties. ***Visual aspect.*** Visual and tactile analyses were performed with the objective of selecting the bioplastics that were: homogeneous, presenting uniform color, with no insoluble gelatin particles, flexible on handling, and with no cracks or zones prone to breakage.

Film thickness. The film thickness was measured using a Mitutoyo Digimatic Co. Proof. The final value represented the average of five random measurements taken at different parts of the film.

Physicochemical Properties. ***Solubility in water.*** The solubility of the bioplastics in water was determined according to the method proposed elsewhere.[12] Triplicate samples were prepared with 2 cm diameter circles extracted from the bioplastics. The initial dry matter of the samples was obtained by drying them for 24 h at 105 °C in an oven with air recirculation and renovation. After the first weighing, the samples were immersed in a recipient containing 50 mL distilled water and maintained under slow agitation for 24 h. The swollen samples were then removed and dried at 105 °C for an additional 24 h before determining the final dry matter.

Solubility in acid. The solubility in acid was determined by initially preparing the samples as described in the above item. However, after determining the initial dry matter, the samples were immersed in a recipient containing hydrochloric acid (1 *N*) for 24 h and then dried and weighed to determine the final dry matter.

Color and opacity. The color of the bioplastics was measured by the Hunterlab standards: L^*, ranges from 0 (black) to 100 (white); a^*, from green (−) to red (+); and b^*, from blue (−)

Figure 1. (A) Pure PHA (polyhydroxyalkanoates); (B) PHA/cheese whey/gelatin 3%; (C). PHA/cheese whey/gelatin 5% (PHA, polyhydroxyalkanoates). [Color figure can be viewed at wileyonlinelibrary.com]

to yellow (+).[13,14] The films were superposed on a surface of a standard white plate, and the patterns L^*, a^*, and b^* were measured in real time, resulting in the color difference (ΔE^*). The opacity of the bioplastics was determined using the colorimeter Hunterlab (Colorquest II, Fairfax). The measurements were made in triplicates after calibration of the colorimeter with a white and a black background. The values for opacity were calculated according to the eq. (1).[15]

$$Op = \left(\frac{Op_N}{Op_B}\right).100 \quad (1)$$

where, Op is the opacity of the bioplastic (%); Op_N is the opacity of the bioplastic against a black background; and Op_B is the opacity of the bioplastic against a white background.

Barrier Properties. ***Water vapor permeability evaluation.*** The tests, in triplicate, for the determination of water vapor permeability (WVP) were performed according to the modified standard method E-96.[16] The bioplastics were fixed in permeation aluminum cells containing calcium chloride and sealed with paraffin to ensure migration of moisture only through the exposed area of the bioplastic. The permeation cells were placed in desiccators kept at 25 °C and 75% relative humidity. The amount of water vapor migrating through the film was determined from the gain in mass of the calcium chloride, measured every 24 h. The effect of the air space between the region below the film and the surface of the calcium chloride of the test cells was not considered in the calculation.[17,18]

Mechanical Properties. The tensile strength and elongation at break were determined using a TAXT2 Texture Analyzer (SMS, Surrey), operated according to the standard method ASTM D 882-83, as modified elsewhere.[19]

Scanning electron microscopy (SEM). The surface morphology of the films was observed using a Scanning Electron Microscope (SEM), model Leo 440i, LEO brand operated at 10 kV and 50 mA. Before testing the samples were covered with a thin gold layer to the thermal conduction.

X-ray diffraction. The diffractograms were obtained using an X-ray diffractometer, model X'Pert Philips brand. The analysis conditions were: (i) Voltage and current: 40 kV and 40 mA, respectively; (ii) scanning range: 2θ from 5 to 30°; (iii) Step: 0.1°, and (iv) Speed 1°/min, provided with secondary beam monochromator graphite. The variation of the sizes of the crystals was determined using the PC-APD software Diffraction. The samples were stored at 25 °C ambient temperature and 50% RH, and analyzed in triplicates.

Thermal Properties. ***Differential scanning calorimetry (DSC).*** The determination of the glass transition temperatures and the melting enthalpy variations of films were made by analyzes of differential scanning calorimetry, using a calorimeter TA Instruments (The United States), TA 2010 model with cooling module by liquid nitrogen. Samples were prepared and preconditioned at 25 °C and controlled relative humidity of 50%. The measurements were performed in an inert atmosphere of ultra-dry nitrogen gas chromatographic grade, to the same feed flow rate and the drag of 50 cm^3/min. The tests were started at 30 °C and then samples were heated at a rate of 10 °C/min until attain 180 °C. The material reference for this analysis was the atmospheric air.

Statistical Analysis

The *Statistica® 5.5* (*Stasoft*, The United States) program was used to calculate the analysis of variance (ANOVA). The Tukey test was used to determine the differences between the biopolymer films properties in the range of 95% confidence interval.

RESULTS AND DISCUSSION

Properties of the Multilayer Films

Pure PHA, PHA/cheese whey/Gelatin 3% and PHA/cheese whey/Gelatin 5% films were developed [Figure 1(a–c), respectively]. The films were visually analyzed in order to select only the most uniform films. Among the three kinds of films produced, all of them were visually symmetrical, without breaks or blistering (Figure 1). It was noted in the multilayer films the presence of higher gloss compared with the standard sample of

Table I. Thickness and Water Vapor Permeability (WVP) of the Obtained Films

Film	Thickness (mm)*	Water vapor permeability (g mm/m^2/d/kPa)*
PHA	$0.0274^b \pm 0.004$	$0.697^a \pm 0.06$
PHA/CW/Gelatin 3%	$0.0392^a \pm 0.001$	$0.347^b \pm 0.06$
PHA/CW/Gelatin 5%	$0.0448^a \pm 0.005$	$0.457^{a,b} \pm 0.04$

*The analyses were performed in triplicate and the results presented as mean and standard deviation (PHA, polyhydroxyalkanoates; CW, cheese whey). Different letters within the same column indicate significant differences ($P < 0.05$).

Table II. Analysis of Solubility in Water and Acid of the Obtained Films

Film	Solubility in water (%)	Solubility in acid (%)
PHA	$0.69^{c} \pm 0.19$	$15.94^{b} \pm 0.74$
PHA/CW/Gelatin 3%	$9.87^{b} \pm 0.91$	$11.26^{a} \pm 1.18$
PHA/CW/Gelatin 5%	$17.76^{a} \pm 0.02$	$16.86^{a} \pm 1.28$

*The analyses were performed in triplicate and the results presented as mean and standard deviation (PHA, polyhydroxyalkanoates; CW, cheese whey). Different letters within the same column indicate significant differences ($P < 0.05$).

PHA film. It happened due to the macromolecules formed by gelatin and whey solution.

Five measurements were performed in different random positions from films. The thicknesses of the multilayer films varied from each other (Table I). The water vapor permeability (WVP) ranged from 0.45 to 0.69 g mm/m^2/d/kPa for the films (Table I).

It may be noted that when the filmogenic solutions consisting of cheese whey and gelatin were added to the films produced only from PHA, there was an increase in the thickness from 0.027 to 0.039 mm and 0.044 mm, respectively. In addition, the PHA/cheese whey/5% gelatin film showed a higher value due to the amount of gelatin inserted in the solution. This behavior was previously observed elsewhere.[20]

The analysis of water vapor permeability showed that PHA/cheese whey/gelatin 3% and PHA/cheese whey/gelatin 5% films presented lower values in relation to the PHA film (control). It occurred because the films formed by PHA and gelatin solution in the two indicated concentrations eliminated possible micropores in the polymeric matrix, forming a more cohesive matrix, which increased the water barrier and decreased the WPV value. However, the PHA/cheese whey/gelatin 5% film was not statistically different from the PHA film ($P < 0.05$). Thus, the diffusion depends on the size, shape, and polarity of the penetrating molecules, as well as the segmented movement of the polymeric chain in the film matrix.

However, permeability is the rate of water vapor transmitted per unit of area through a film of known thickness, induced by a pressure gradient between two specific areas, with determined relative humidity and temperature.[20] The droplets in the film increases the distance traveled by water molecules, which diffuse through the film, thereby reducing the permeability and water vapor.[21] It may be noted that the PHA/cheese whey/gelatin 5% film obtained a higher value than the PHA/cheese whey/gelatin 3% film. It happened because the thickness and the temperature exerted great influence on the permeability to water vapor of films with gelatin.[22]

Table III. Color Analysis of the Obtained Films

Film	Color difference according to Hunterlab*
PHA/CW/Gelatin 3%	$1.44^{b} \pm 0.11$
PHA/CW/Gelatin 5%	$2.56^{a} \pm 0.57$

*The analyses were performed in triplicate and the results presented as mean and standard deviation (PHA, polyhydroxyalkanoates; CW, cheese whey). Different letters within the same column indicate significant differences ($P < 0.05$).

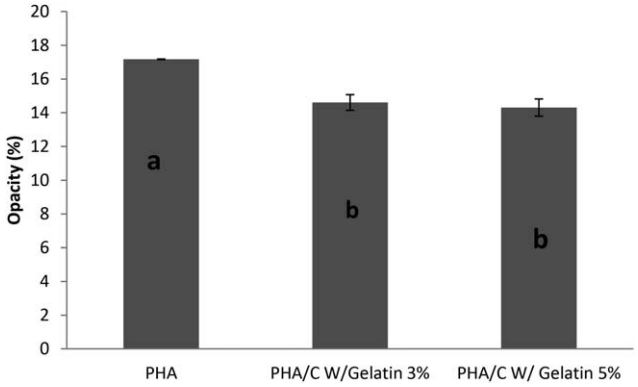

Figure 2. Analysis of variance of the opacity of the obtained films. *The analyses were performed in sextuplicate and the results presented as mean and standard deviation.(PHA, polyhydroxyalkanoates; CW, cheese whey). Different letters within the same column indicate significant differences ($P < 0.05$).

Films and compound or multilayer covers have being investigated in order to improve the characteristics of permeability, strength, and flexibility.[23] When adding a hydrophobic component to the forming suspension of the film, composite films are produced, in which the lipid component acts as a barrier to water vapor, and the protein or polysaccharide provides the oxygen barrier and the mechanical characteristics necessary for a good film.

It may be noted that the multilayer films constitute distinct properties from the presented data (Table II), which are in accordance with the results of solubility in water and acid.

Samples containing cheese whey, in gelatin concentrations of 3 and 5%, presented values of solubility in water greater than the PHA film. Higher water solubility was observed for the film containing 5% of gelatin in cheese whey. As higher is the content of hydrophilic components in the material, increased are the solubility values.[24]

Some applications of biofilms require water-insolubility characteristics to maintain the integrity of the product to be protected.[25] However, the solubility in acid of the samples showed that the values of the PHA and PHA/cheese whey/Gelatin 5% biofilms did not differ statistically ($P > 0.05$), whereas the sample containing 3% of gelatin in whey had a lower solubility in acid. It may have occurred due to the fact that there was greater interaction between polymeric matrices when the gelatin 3% solution and cheese whey were added. In this sense, the analyses of solubility in water and acid in films become advantageous from their applications, for both protection and food processing.[26]

Color and Opacity

The color of the films was measured in real time, resulting in the color difference (ΔE^*) of samples compared with the control PHA, PHA/cheese whey/gelatin 3%, PHA/cheese whey/gelatin 5% biofilms (Table III). The PHA/cheese whey/gelatin 5% film

Table IV. Mechanical Properties of the Obtained Films

Film	Tensile strength (MPa)	Elongation (%)
PHA	$9.98^{a} \pm 1.82$	$1.59^{b} \pm 0.22$
PHA/CW/Gelatin 3%	$33.80^{b} \pm 5.52$	$1.57^{b} \pm 0.44$
PHA/CW/Gelatin 5%	$26.90^{c} \pm 4.82$	$2.18^{a} \pm 0.75$

*The analyses were performed in sextuplicate and the results presented as mean and standard deviation (PHA, polyhydroxyalkanoates; CW, cheese whey). Different letters within the same column indicate significant differences ($P < 0.05$).

obtained a greater staining than the PHA/cheese whey/gelatin 3% film (Table III). The color and the opacity of the polymer are due to the morphology or the chemical structure related to the molecular weight of the material.[27]

The opacity results showed that the pure PHA film obtained a higher value in relation to the films containing gelatin solution and cheese whey. However there was no significant variation in the samples ($P > 0.05$) (Figure 2).

PHA/cheese whey/gelatin 3% and PHA/cheese whey/gelatin 5% films presented values similar to others published elsewhere.[28] These authors reported 14.5% opacity to a tapioca starch, gelatin, and glycerol based film. Films that have the addition of gelatin produce best color and higher brightness.[11]

Mechanical and Thermal Properties

The PHA/cheese whey/gelatin 3% and PHA/cheese whey/gelatin 5% films presented greater tensile strength than the PHA film ($P < 0.05$). The addition of gelatin and cheese whey to form multilayer films were essential to get a better result in relation to the standard PHA film. Therefore, there was an increase in its resistance to traction, demonstrating that their addition improved the film composition (Table IV). It may be considered that multilayer films generally possess superior mechanical properties than regular films.

The PHA/cheese whey/gelatin 5% film presented a greater elongation in relation to the other films ($P < 0.05$), which did not showed significant variation ($P > 0.05$).

In this sense, a high tensile strength is required, while the elongation value depends on the type of application of the film, once that to maintain the integrity and barrier properties, a film must bear the normal stresses found during its application, beyond shipping and handling.[12]

Figure 3(a) shows the results for differential scanning calorimetry (DSC) of PHA, PHA/gelatin 3% and PHA/cheese whey/gelatin 3% films. The results show that the glass transition temperature for all the samples is located at approximately 115 °C, except for the control sample (PHA film), which showed a slight displacement of this transition, being displayed at approximately 119 °C.

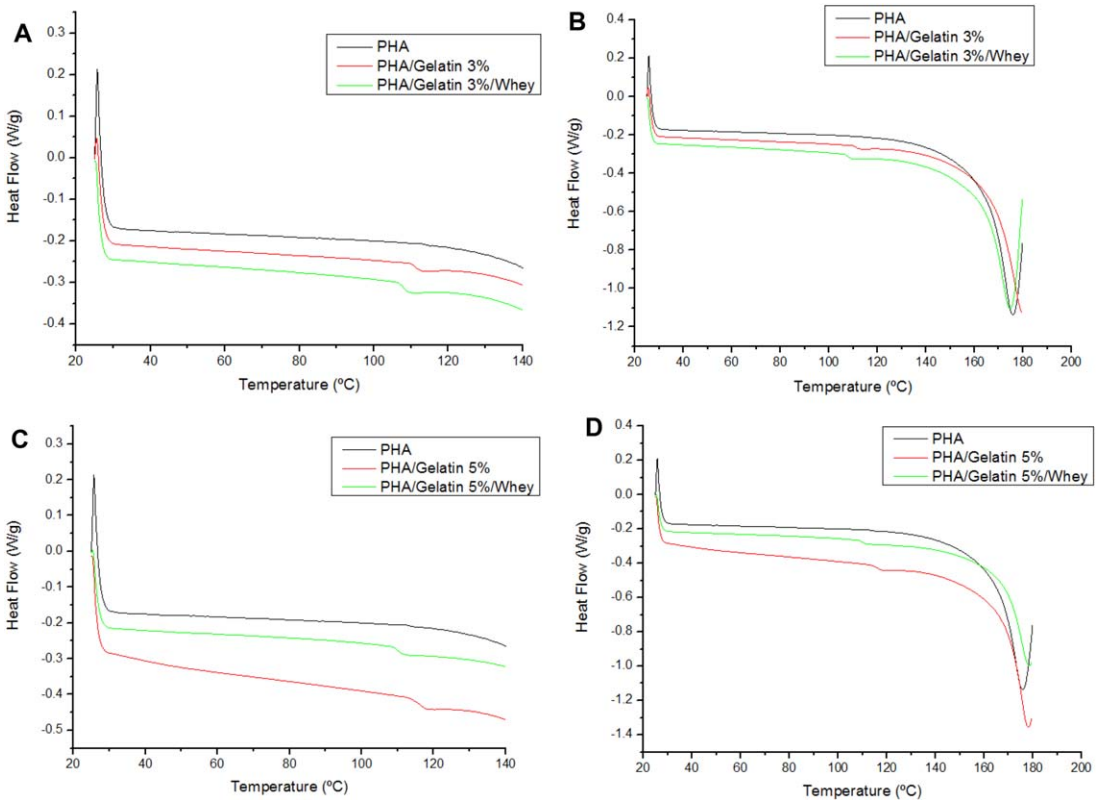

Figure 3. DSC glass transition in (A) PHA/cheese whey/gelatin 3% and endothermic transition film (B) PHA/cheese whey/gelatin 3% and DSC glass transition film (C) PHA/cheese whey/gelatin 5%; endothermic transition film in (D) PHA/cheese whey/gelatin 5%. (PHA, polyhydroxyalkanoates). [Color figure can be viewed at wileyonlinelibrary.com]

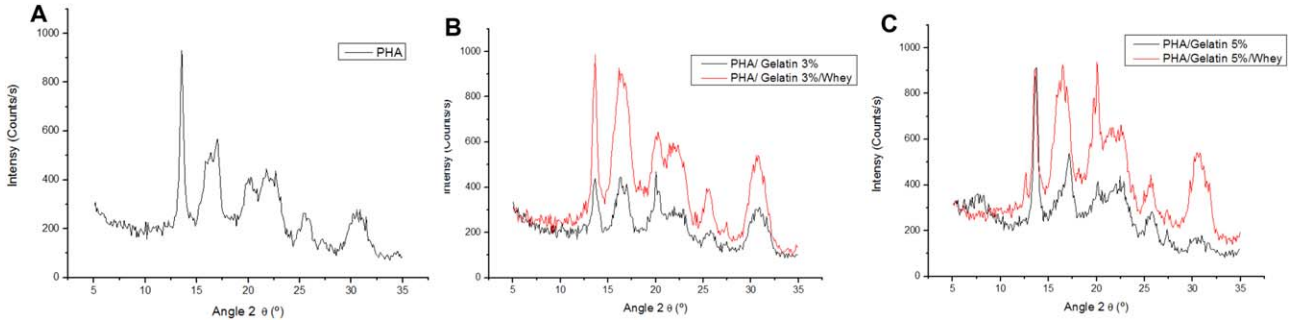

Figure 4. X-ray diffraction of the: (A) pure PHA film; (B) PHA/cheese whey/gelatin 3% film; and (C) PHA/cheese whey/gelatin 5% film (PHA, polyhydroxyalkanoates). [Color figure can be viewed at wileyonlinelibrary.com]

The glass transition temperature is taken as the average value of the temperature range that during the heating of a polymeric material, which is at a very low temperature, changes to higher values, allowing that the polymer chains that are in the amorphous phase acquire mobility, that is, the possibility of change in conformation. Below the T_g, the polymer has not enough

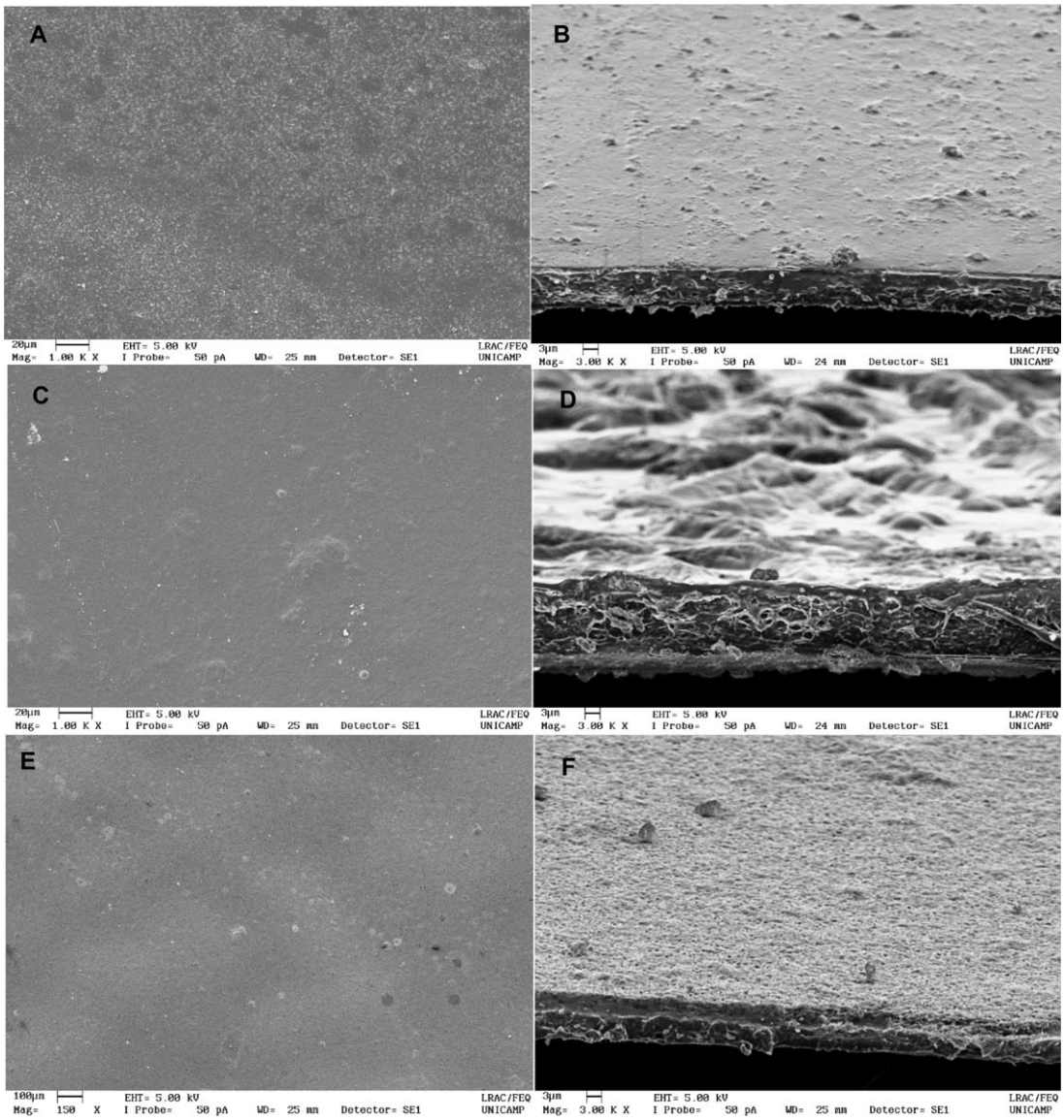

Figure 5. Electron microscopy images obtained from scan films of PHA/cheese whey/gelatin 3%, [surface (A) and break (B)]; PHA/cheese whey/gelatin 5% [surface (C) and break (D)]; pure PHA [surface (E) and break (F)]. (PHA, polyhydroxyalkanoates).

inner energy to allow the strand displacement in relation to the others, by conformational changes. It is in the glassy state characterized as hard and brittle like glass. To acquire mobility, that is, to become mobile, a molecule needs the ability to respond to a mechanical stress with enough time. On the other hand, the immobility is the incapacity to respond within the time interval available.[28] As can be seen the samples containing PHA/gelatin 3%, added or not of cheese whey, are all situated in the same transition zone together with PHA (control), presenting a miscibility between the polymer samples.

From the results of endothermic transitions of the samples containing 3% of gelatin in PHA films it can be observed similar transactions in the regions of approximately 174 °C [Figure 3(b)]. It must be underlined that the application and handling of this material may only be carried out up to 174 °C.

Figure 3(c) shows the glass transitions of the PHA, PHA/gelatin 5% and PHA/cheese whey/gelatin 5% films. It can be noticed that there were distinct glass transitions, where the PHA/gelatin 5% film is inserted at approximately 119 °C range, and that the PHA and PHA/cheese whey/gelatin 5% films are situated about 115 °C, also presenting a polymeric miscibility between the samples.

The endothermic transitions of the samples containing 5% of gelatin with or without addition of cheese whey in the PHA films are situated at approximately 174 °C. This value is similar to the endothermic transition of the PHA film [Figure 3(d)].

It is considered from these results that the melting temperature (T_m) of the PHA is consistent with values found in literature for polyhydroxyalkanoates produced from glucose (174–184 °C).[29–31] This parameter is important due to the limitations in applications of polymers that present thermal degradation temperature close to the melting temperature in molding processes and that require manipulation of PHA at high temperatures.[30]

The X-ray diffraction tests showed the crystalline regions in the films. They were clearly observed in the diffraction peaks in 2θ between 15 and 33 °C. Figure 4(a) shows that there are two crystalline peaks located in 2θ ranging from 12.5 to 32.5, which are common in PHA films. As observed, there are intense peaks when cheese whey is added to the film, differentiating from the sample PHA/gelatin 3% [Figure 4(b)]. This result is in accordance with the literature.[30–33]

In films containing a concentration of 5% gelatin more intense peaks also occur in samples containing cheese whey, except for the peak at 12.5 2θ [Figure 4(c)].

The results of the SEM obtained with PHA/cheese whey/gelatin 3% and PHA/cheese whey/gelatin 5% films showed a homogeneous surface without fissures. Figure 5 shows the images of the PHA/cheese whey/gelatin 3% film, where image (a) shows a homogeneous surface with no apparent fissures, and the image (b) shows the outline of the film thickness indicating the formation of the multilayer.

Figure 5(c,d) shows the SEM from the PHA/cheese whey/gelatin 5% film. It presents the same characteristics of the PHA/cheese whey/gelatin 3% film. It is remarkable in the sample the central layer of PHA wrapped by the two layers of gelatin solution with cheese whey at the concentrations of 3 and 5%. The microscopy of the pure PHA film also showed a smooth surface without fissures [Figure 5(e,f)].

The result of the good formation of the films presented by SEM is considered by the fact that biofilm developed with protein, as gelatin, are featured by their good mechanical properties.[34]

CONCLUSIONS

Cheese whey proved to be a great component for film production, mainly due to its composition rich in protein. The addition of gelatin to the multilayer films produced with PHA and cheese whey provided improved physical and mechanical properties. The chosen film will depend on its application, considering that the analysis of the properties indicate a positive value for each application. Actually, the films containing 3% gelatin presented higher tensile strength than the 5% gelatin films, making this film more attractive for industrial manufacture. However, PHA/cheese whey/gelatin 3% films possess important characteristics, mainly by the fact that in its preparation it is utilized a lower amount of material, thus reducing production costs.

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REFERENCES

1. Fonseca, G. G.; Antonio, R. V. *J. Microbiol.* **2006**, *1*, 234.
2. Fonseca, G. G.; Antonio, R. V. *Am. J. Food Technol.* **2007**, *2*, 12.
3. Fonseca, G. G.; Arruda-Caulkins, J. C.; Antonio, R. V. *Waste Manag. Res.* **2008**, *26*, 546.
4. Michel, P.; Loganayagi, R.; Nancy, D.; Ranandkumar, S. G.; Arulselvi, P. *Elixir Appl. Biol.* **2012**, *48*, 9424.
5. Fontes, L. C. B.; Ramos, K. K.; Sivi, T. C.; Queiroz, F. P. C. *Am. J. Food Technol.* **2011**, *6*, 555.
6. Thomazine, M.; Carvalho, R. A.; Sobral, P. J. A. *J. Food Sci.* **2005**, *70*, E172.
7. Gorgieva, S.; Kokol, V. Collagen- vs. Gelatine-Based Biomaterials and Their Biocompatibility: Review and Perspectives, Biomaterials Applications for Nanomedicine, (Prof. Rosario Pignatello), ISBN: 978-953-307-661-4 **2011**.
8. Fakhouri, F. M.; Costa, D.; Yamashita, F.; Martelli, S. M.; Jesus, R. C.; Alganer, K.; Collares-Queiroz, F. P.; Mei, L. H. I. *Carbohydr. Polym.* **2013**, *95*, 681.
9. Fonseca, G. G.; Da Silva, L. F.; Gomez, J. G. C. *Transworld Res. Netw.* **2008**, *5*, 1.
10. Lee, S. Y.; Choi, J. L. *Adv. Biochem. Eng. Biotechnol.* **2001**, *71*, 183.
11. Fakhouri, F. M.; Fontes, L. C. B.; Gonçalves, P. V. M.; Milanez, C. R.; Steel, C. J.; Queiroz, F. P. C. *Food Sci. Technol.* **2007**, *27*, 369.

12. Gontard, N.; Duchez, C.; Cuq, J. L.; Guilbert, S. *J. Food Sci. Technol.* **1994**, *29*, 39.
13. Gennadios, A.; Weller, C. L.; Hanna, M. A.; Froning, G. W. *J. Food Sci.* **1996**, *61*, 585.
14. Kunte, L. A.; Gennadios, A.; Cuppet, S. L.; Hanna, M. A.; Weller, C. L. *Cereal Chem.* **1997**, *74*, 115.
15. Hunterlab. The color management company. Universal software (version 3.2). Reston, **1997**.
16. American Society for Testing and Materials (ASTM) Standard test methods for tensile properties of thin plastic sheeting. In: Annual Book of ASTM Standards. Conshohocken, PA. Method D 882-83 **1980**.
17. McHugh, T. H.; Krochta, J. M. Permeability properties of edible films. In Edible Coatings and Films to Improve Food Quality; Krochta, J. M.; Baldwin, E. A.; Nisperos-Carriedo, M., Eds.; Technomic Publishing Co, Inc.: Lancaster, **1994**.
18. Gennadios, A.; McHugh, T. H.; Weller, C. L.; Krochta, J. M. Edible coatings and films based on proteins. In Edible Coatings and Films to Improve Food Quality; Krochta, J. M.; Baldwin, E. A.; Nisperos-Carriedo, M., Eds.; Technomic Publishing Co, Inc.: Lancaster, **1994**.
19. Tanada-Palmu, P. S.; Hyvonen, H. H. L. *Agric. Food Sci.* **2000**, *9*, 23.
20. American Society for Testing and Materials – ASTM. Designation e96-95: Standard Method for water Vapor Transmission of Materials. Philadelphia: ASTM, **1995**. (Annual book of ASTM standards).
21. Tanaka, M.; Ishizaki, S.; Suzuki, T.; Takai, R. *J. Tokyo Univ. Fish.* **2001**, *87*, 31.
22. Carvalho, R. A.; Grosso, C. R. F. *Braz. J. Chem. Eng.* **2006**, *23*, 45.
23. Davanço, T.; Tanada-Palmu, P.; Grosso, C. *Ciên. Tecnol. Alimen.* **2007**, *27*, 408.
24. Fakhouri, F. M.; Martelli, S. M.; Bertan, L. C.; Yamashita, F.; Mei, L. H. I.; Queiroz, F. P. C. *Food Sci. Technol.* **2012**, *49*, 149.
25. Perez-Gago, M. P.; Krochta, J. M. *J. Agric. Food Chem.* **2000**, *51*, 60.
26. Gontard, N.; Guibert, S.; Cuq, J. L. *J. Food Sci.* **1992**, *57*, 190.
27. Chen, H. *J. Dairy Sci.* **1995**, *78*, 2563.
28. Canevarolo, S. V. Jr. *Sci. Pol. (Artliber, São Paulo)* **2006**, *430*, 36.
29. Gomez, J. G. C.; Netto, C. L. *Rev. Bras. Eng. Quím.* **1997**, *17*, 24.
30. Figueiredo, T. V. B.; Campos, M. I.; Sousa, S. L.; Silva, J. R.; Druzian, J. I. *Quím. Nova* **2014**, *37*, 1111.
31. Campos, M. I.; Figueiredo, T. V. B.; Sousa, L. S.; Druzian, J. I. *Crops Prod.* **2014**, *52*, 338.
32. Vogelsanger, N.; Formolo, M. C.; Pezzin, A. P. T.; Schneider, A. L. S.; Furlan, S. A.; Bernardo, H. P.; Pezzin, S. H.; Pires, A. T. N.; de Duek, E. A. R. *Mater. Res.* **2003**, *6*, 359.
33. Catoni, S. L. M.; Trindade, K. N.; Gomes, C. A. T.; Pezzin, A. P. T.; Soldi, V. *Polymers* **2013**, *23*, 320.
34. Tapia-Blácido, D.; Sobral, P. J.; Menegalli, F. C. *J. Food Eng.* **2005**, *67*, 215.