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Extruded films of blended chitosan, low density polyethylene and ethylene acrylic acid

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ABSTRACT

The obtaining of chitosan extruded films was possible by using low density polyethylene (LDPE) as a matrix polymer and ethylene-acrylic acid copolymer as an adhesive, in order to ensure adhesion in the interphase of the immiscible polymers. The obtained blend films were resistant; however, a reduction in the mechanical resistance was observed as chitosan concentration increased. The thermal stability of the films showed a certain grade of interaction between polymers as seen in FTIR spectra. The antifungal activity of the extruded films was assessed against *Aspergillus niger* and high inhibition percentages were observed, which may be mainly attributed to barrier properties of the extruded films and the limited oxygen availability, resulting in the inability of the fungi to grow. A low adherence of fungal spores to the material surface was observed, mainly in areas with chitosan clumps, which can serve as starting points for material degradation.

1. Introduction

Versatility, low production cost, and raising demand of petroleum-derived plastics, has generated an increase of resulting waste, which is either non biodegradable or decompose after long periods of time producing toxic derivatives. The ecological problems related to the excessive use of these synthetic materials have led to the search of alternative materials, such as biomaterials, which are both biodegradables and non-toxic.

The direct combination of synthetic materials with biodegradable materials is one of the easier and economic ways for biomaterials production (Rogovina, Alexanyan & Prut, 2011). This allows the improvement of the functional properties of the materials and the expansion of their potential applications. Natural polymers such as collagen, chitosan, starch, and elastin are being studied as possible packing materials primarily due to their similarity with biomolecules, their biocompatibility, immunological properties, biodegradability, among other characteristics.

Chitosan, the linear and partly acetylated (1–4)-2-amino-2-deoxy-<beta>-D-glucan, is easily obtained from chitin (Goycoolea, Agulló, & Mato, 2004; Muzzarelli, 2012; Muzzarelli et al., 2012; Pastor & Higuera, 2004; Rabea, El Badawy, Stevens, Smagghe, & Steurbaut, 2003). Chitosan is biodegradable, non toxic, biocompatible, and displays antimicrobial properties against a wide range of bacteria, yeasts, and fungi. It exhibits filmogenic properties, which allows the production of films or membranes semipermeable to gases such as CO_2 and O_2 (Khan, Peh, & Ch'ng, 2000). This makes chitosan a promising material for the development of food packages.

Chitosan films or edible coatings have shown antimicrobial activity and ability to reduce the ethylene and carbon dioxide production, which can extend the shelf life of food such as fruits, vegetables, meat, eggs, and dairy products (Bhale et al., 2003; Jeon, Kamil, & Shahidi, 2002; Shahidi, Arachchi, & Jeon, 1999). Also, chitosan films are biodegradable, biocompatibles, thin, flexible, strong, resistant, and difficult to break, with mechanical properties that are comparable with those of commercial polymers of medium force such as cellulose (Agulló, Albertengo, Pastor, Rodríguez, & Valenzuela, 2004; Jeon et al., 2002). However, their high sensibility to humidity limits their application as food package (Yu, Dean, & Li, 2006).

Extrusion of chitosan with thermoplastic polymers represents an alternative for the obtaining of humidity resistant materials, partially or totally biodegradable, because of their high yield, lesser

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need of space, and greater control of the final material's characteristics, in comparison with the solvent evaporation process. Besides, this process is currently being used at an industrial level to produce plastic materials (Pelissari, Yamashita, & Grossmann, 2011). The production of chitosan and LDPE films by extrusion and press molding techniques has been reported, proving that the addition of chitosan in both methods results in antimicrobial activity confirmed on different bacterial strains (Park, Marsh, & Dawson, 2010; Zhang, He, Liu, & Qiao, 2009). However, the limited miscibility or immiscibility of both polymers complicates the film formation process. In this work, ethylene/acrylic acid (EAA) copolymer (Primacor 1430*, Dow) was used to improve the interphase interactions between chitosan and LDPE, evaluating the thermal, mechanical, structural and fungistatic properties against *Aspergillus niger* of the resulting blend extruded films.

2. Materials and methods

2.1. Materials

Low viscosity commercial grade chitosan (Fluka, Biochemika, Japan) (<200 mPa s) and chitosan obtained by chemical treated crab waste, were used. Low density polyethylene (LDPE), LBA 253 (FDA regulation 21CFR 177.1520 (c) 2.1+2.2, Muehlstein International-Latin America, Norwalk, CT, USA), was used, specially designed for production of clarity Blown films for general purpose packaging applications. Ethylene acrylic acid copolymer, Primacor 1430* (FDA regulation 21CFR 177.1310 (b), Copyright© The Dow Chemical Co.), was used for providing sealing capability, and hot tack and adhesion in the material interface; it was also used to improve toughness, clarity, and tear resistance.

2.2. Physicochemical characterization of chitosan

Molecular weight (MW) was determined by a viscometric method, using chitosan solutions at concentrations ranging from 0.25 to 6.0 g/L (w/v). The intrinsic viscosity of chitosan, in 0.3 M acetic acid/0.2 M sodium acetate solution, was measured in triplicate by using an Ubbelohde capillary viscometer in a constant temperature (at 25 °C) water bath (Hwang et al., 2002; Mathew, Brahmakumar, & Emilia Abraham, 2006). The MW values were determined using the Mark–Houwink–Sakurada equation (Eq. (1)):

$$[\eta] = \kappa M v^{a} \tag{1}$$

where $[\eta]$ is the intrinsic viscosity, Mv is the average viscometric molecular weight, and both k and a are empirical constants that depend of the polymer nature, solvent, and temperature. Values for k and a were 3.04×10^{-5} and 1.26, respectively (Rinaudo, Milas, & Le Dang, 1993).

The chitosan deacetylation degree (%DD) was determined by Fourier transform infrared spectroscopy analysis, FT-IR, (Perkin Elmer FT-IR Spectrum GX). Chitosan pellets prepared with potassium bromide, KBr, (Aldrich, spectroscopic grade) were analyzed performing 16 scans, at 4000–400 cm^{-1}. The analyses of the spectra were done according to Khan, Peh, and Ch'ng (2002) and the deacetylation degree was determined using Eqs. (2) and (3):

$$\mathrm{DD}\,(\%) = 100 - \left[\left(\frac{A_{1655}}{A_{3450}}\right) \times \frac{100}{1.33}\right] \tag{2}$$

$$\mathrm{DD}\,(\%) = 100 - \left[\left(\frac{A_{1655}}{A_{3450}}\right) \times 115\right] \tag{3}$$

where A_{1655} is the absorbance at 1655 cm^{-1} of the amide-I band, A_{3450} is the absorbance at 3450 cm^{-1} of the hydroxyl band, and 1.33 is the value for the A_{1655}/A_{3450} proportion for a completely acetylated chitosan.

Table 1
Formulations of the composite blends of chitosan, Primacor 1430* adhesive and low density polyethylene (LDPE).

Chitosan (%)	Chitosan:Primacor 1430:LDPE (%)[a]		
0	0:0:100	0:1:99	0:10:90
5	5:0:95	5:1:94	5:10:85

[a] 100 g of composite blend were prepared.

2.3. Films elaboration

Composite blends formulations (Table 1) were prepared by mixing the low density polyethylene as thermoplastic polymer and the chitosan powder. In order to achieve the adhesion of LDPE and chitosan in the film interface, Primacor 1430 (ethylene acrylic acid copolymer, EAA) was added to the mixture.

The composite blends formulations were first blown extruded (pelletized) with a pilot mono-spindle extrusion machine (Beutelspacher, Mexico, D.F.). The temperature for the three heating sections of the extruder barrel and the die were set at 145, 145, 150, and 150 °C, respectively. The total residence time was 2 min and the screw speed was 45 rpm.

To obtain the chitosan films, the obtained pellets were extruded in a LME Laboratory Mixing Extruder (ATLAS Polymer Evaluation Products, USA) (Castillo-Ortega et al., 2003) (Fig. 1). The barrel temperature was maintained at 150 °C in the feeding section and at 160 °C in the next two zones and the die. The screw speed was 120 rpm and the extruded films were maintained at 25 °C.

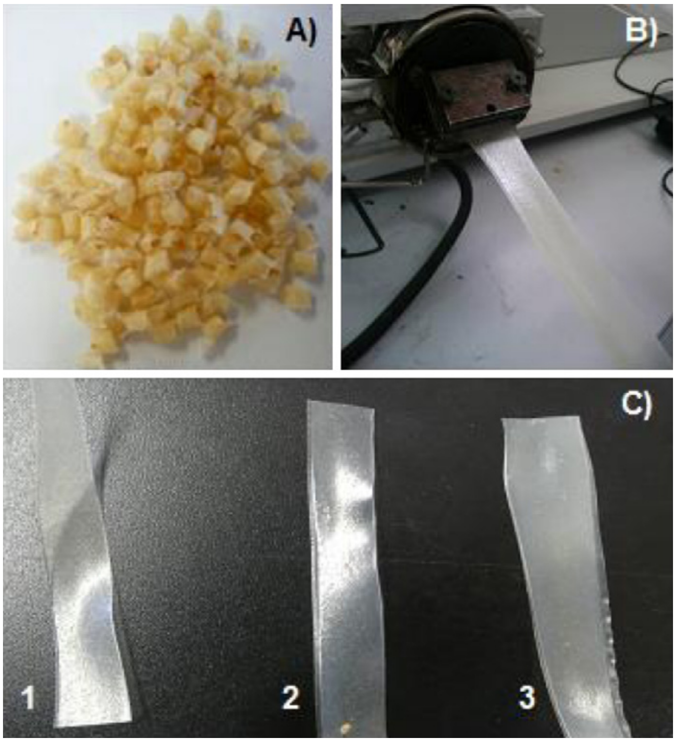

Fig. 1. Films elaboration by extrusion of blends containing chitosan, Primacor 1430* (Dow) and low density polyethylene (LDPE): (A) pellets of chitosan/LDPE/adhesive composite blend; (B) laminated film by extrusion of chitosan composite blend pellets; (C) extruded films containing chitosan: 1: 95% LDPE + 5% chitosan; 2: 94% LDPE + 5% chitosan + 1% adhesive; 3: 85% LDPE + 5% chitosan + 10% adhesive.

2.4. Physicochemical characterization of chitosan films

The thickness of the chitosan films was determined by using a Mitutoyo micrometer (PB-1 JIS.B.7502, USA), reporting the average value after 10 measurements randomly taken at different points.

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were carried out on a SDT 2960 simultaneous DSC–TGA TA instruments equipment. Samples of approximately 6 mg were taken and warmed up to 650 °C at a heating and cooling rate of 10 °C/min under 23 mL/min air flow (Martínez-Camacho et al., 2010).

The strength and elongation of films were evaluated by using an United (SSTM 5KN model, USA) equipment according to the conditions set by the Standard Test Method for Tensile Properties of Plastics by Use of Microtensile Specimens, ASTM D-1708-96. Average values from at least three measurements were reported.

Chitosan films were analyzed directly using Fourier transform infrared (FT-IR) spectrophotometry equipment (Perkin Elmer FT-IR Spectrum GX), within a spectral range from 4000 to 400 cm^{-1} (Martínez-Camacho et al., 2010).

Chitosan films surfaces were coated with gold before observation in a scanning electron microscope (Jeol JSM-6360LV, Japan) (Echlin, 2009; Plascencia-Jatomea, Viniegra, Olayo, Castillo-Ortega, & Shirai, 2003).

2.5. Fungistatic activity of chitosan films

Before being inoculated, the extruded films were cut into 6 mm-diameter pieces and sterilized by exposure to ultraviolet light for 5 min on each side. Radial growth and diameter of both, spores and hyphae of *A. niger*, were determined.

To evaluate the effect of the extruded films on the radial growth of *A. niger*, the sterile film pieces were placed on the agar, and the fungi inoculums were placed over the film surface. This inoculation system was called "agar/film/fungi" (above the film) (Martínez-Camacho et al., 2010). An inoculum (1×10^3 spores/mL) was deposited and incubated at 25 °C.

Daily, the diameter of the colonies was manually measured until the control reached the edge of the plate. Fungistatic inhibition was obtained by Eq. (4):

$$\text{Fungistatics inhibition}\,(\%) = 1 - \left[\frac{R_i}{R_c}\right] \times 100 \qquad (4)$$

where R_c was the mean value of the colony radius of control and R_i was the colony radius of the chitosan extruded film (Guo et al., 2006; Martínez-Camacho et al., 2010). All the measurements were carried out in triplicate.

The diameter of the fungi spores and hyphae was determined by image analysis using Image-Pro Plus version 6.3 software (2008 Media Cybernetics Inc., USA), using an optical microscope (Olympus CX31, Japan) connected to an Infinity 1 camera (Media Cybernetics, USA), and using a 40× objective.

2.6. Statistical analysis

Statistics on a completely randomized design were determined using the one-way analysis of variance (ANOVA) procedure in the JMP software (JMP version 5.0, SAS Institute Inc., USA), at a level of significance set at $P = 0.050$. Means for groups in homogeneous subsets were carried out using the Tukey multiple comparisons test (Tukey's post hoc test), at 95% confidence interval. All data were presented as mean value with their standard error indicated (mean ± SE). Differences were accepted as significant when ($P \leq 0.05$).

3. Results and discussion

3.1. Physicochemical characterization of chitosan

According to the physicochemical analysis (AOAC, 2005), chitosan showed a residual ash content of 0.103 ± 0.064%, 56.6% deacetylation, and an intrinsic viscosity of 8.11 ± 0.275 dL/g. The estimated molecular weight was 400 kDa, which corresponds to a medium molecular weight according to usually recognized values (150–700 kDa) (Goy, de Britto, & Assis, 2009; Jon, Lee, Ju, & Lee, 2007; Wong, 2009).

3.2. Physicochemical characterization of chitosan films

3.2.1. Thickness

The extruded films made of chitosan/LDPE/EAA blends were slightly transparent and shiny (Fig. 1). Although little agglomerates were detected along the film, separated phases were not observed. The addition of EAA increased ($P \leq 0.05$) the films thickness, where the higher thickness values were recorded in the 10% EAA and 90% LDPE blends extruded films (Table 2). This suggests the presence of electrostatic interactions between the LDPE and the EAA molecules throughout the material's interphase, resulting in different thickness. Thickness of the obtained material also depends on its molding conditions, as observed with the press and heat molding (Rogovina, Aleksanyan, Novikov, Prut, & Rebrov, 2009; Zhang et al., 2009).

It is well know that the morphology of the extruded material depends on the viscoelastic characteristics of the components of the blend. For immiscible polymers blends, there are domain structures due to the interfacial layer between them, which depends on the composition, preparation methods, and mixing conditions of the blends. According to Liang (2002), during the extrusion and flow of the melted polymer, the domain structures can be deformed, broken, and aggregated, making the viscoelastic behavior of the blend more complicated, resulting in differences in the characteristics of the extruded material, such as different volume, thickness or presence of aggregates, and separation of the blend components. In this work, polymers were first blown extruded at 145–150 °C and then extruded at 150–160 °C. It is possible that the EAA molecules strongly interacted with the LDPE and chitosan chains during heating, probably following a Newtonian movement along the fluid, until their structural rearrangement were achieved at room temperature.

3.2.2. Thermal studies

The thermal stability of the extruded films was evaluated by thermogravimetric analysis (TGA), and the glass transition temperature (T_g) was measured by differential scanning calorimetry (DSC). Figs. 2 and 3 show TGA–DSC diagrams of the extruded films, with and without the addition of chitosan, respectively. T_g values of the extruded films are shown in Table 2.

The onset temperature of thermo-oxidative degradation of extruded LDPE films with and without chitosan was observed at 240 °C, accompanied with a massive weight loss up to a 100% around 500 °C. Several exothermic peaks were observed during DSC analyses, indicating oxidative degradation. The extruded films obtained in the present research work had a higher thermal stability than chitosan films previously prepared by the casting process in our laboratory, where the onset temperature of thermo-oxidative degradation of chitosan films was observed at 135 °C (Martínez-Camacho et al., 2010).

Figs. 2 and 3 show that the addition of the EAA copolymer and chitosan does not affect the thermal stability of extruded films based in LDPE.

Table 2
Physicochemical properties of the chitosan films prepared by extrusion technique.

Blend film	Thickness (mm)	Elongation to break (%)	Tensile strength (MPa)	T_g (°C)
AX	0.17 ± 0.02^{c}	314.68 ± 78.01^{ab}	13.26 ± 1.84^{b}	167
AY	0.24 ± 0.05^{bc}	359.54 ± 125.76^{ab}	14.93 ± 0.83^{b}	161
AZ	$0.40 \pm 0.06^{\underline{a}}$	$428.45 \pm 31.08^{\underline{a}}$	14.32 ± 1.46^{b}	160
CX	0.34 ± 0.04^{ab}	85.36 ± 35.06^{c}	12.72 ± 0.24^{b}	166
CY	0.19 ± 0.01^{bc}	107.94 ± 38.41^{c}	10.53 ± 0.18^{b}	164
CZ	0.22 ± 0.05^{c}	183.07 ± 16.17^{cb}	11.51 ± 0.09^{b}	158

AX: low density polyethylene, LDPE. AY: 99% LDPE + 1% Primacor 1430 (adhesive). AZ: 90% LDPE + 10% adhesive. CX: 95% LDPE + 5% chitosan. CY: 94% LDPE + 5% chitosan + 1% adhesive. CZ: 85% LDPE + 5% chitosan + 10% adhesive.
Data, followed by their standard errors, are means of at least three experiments. Treatment means were separated using the Tukey test ($P \geq 0.05$).

The addition of EAA decreased the T_g value and increased the elongation percentage of the extruded films (Table 2), suggesting that the EAA copolymer could be working as a plasticizer. All extruded films showed only one T_g value. The addition of either chitosan or EAA, did not lead to the formation of endothermal peaks, indicating some degree of miscibility among chitosan, LDPE, and EAA molecules, possibly due to the interaction of chitosan amino groups with the other two molecules of the composite material.

The position of the endothermal peak in the DCS graphs indicates the polymer fusion temperature (T_m), and the width of this peak indicates the content of the other polymer in the blend (Rogovina et al., 2009). In the obtained films, T_m was around 100 °C, which corresponds to a previously reported LDPE fusion point (109–110 °C) (Ali, Youssef, Said, & Saleh, 2005; Pedroso & Rosa, 2005; Rogovina et al., 2009). This T_m could be also influenced by the EAA copolymer's T_m (96 °C) and its reducing effect on the crystallinity of the polymers present in the blend. T_m was displaced to 108 °C in the 5% chitosan blend; however, a lower T_m was achieved with the addition of the copolymer in all of the chitosan films.

Nevertheless, there was not a change in the peak area, which can possibly be attributed to the absence of a fusion point of chitosan (Honma, Senda, & Inoue, 2003; Jeung & Mishra, 2011), the low proportion of added chitosan in the LDPE film compared to the used amount in other studies, or to the low interaction between the $-NH_2$ groups of chitosan and the $-OH$ groups of the other polymers in the blend (Correlo et al., 2005; Honma et al., 2003; Pedroso & Rosa, 2005; Rogovina et al., 2009).

3.2.3. *Stress–strain mechanical properties*

The tensile properties of polymers, as well as the morphology of their blends, are influenced by the extrusion conditions (mixing camera specifications, type and number of screws, rotor temperature and speed, and residence time) and the processing parameters during molding, injection, or blowing (injection rate, packing pressure, and rotor/exit temperature), among other factors (Correlo et al., 2005). In this study, processing conditions were selected depending on the production of the extruded films and visual approval. Further studies on the effects of these parameters on the morphology and other properties of obtained materials are necessary, since extrusion variables strongly influence films properties (Pelissari et al., 2011).

Films showed typical strain-strength behavior (Fig. 4). Regardless the EAA copolymer concentration, the addition of chitosan did not significantly ($P > 0.05$) increase the tensile strength of the films compared to LDPE control (Table 2).

Chitosan decreased ($P \leq 0.05$) the elongation of the extruded films with respect to the LDPE control (Table 2 and Fig. 4). Same tendencies have been reported (Correlo et al., 2005; Rogovina et al., 2009; Zhang et al., 2009) when much higher proportions of chitosan, LDPE, and polyesters have been used in blends than those used in the present study. This decrease may be caused by the poor miscibility between LDPE and the biopolymer, or by the rupture of LDPE's crystalline structure (Park et al., 2010; Zhang et al., 2009), decreasing its mobility.

It has been reported that without a compatibilizer, the rheological properties of the material are unstable, causing swellings and separations that affect the final morphology (Matzinos, Bikiaris, Kokkou, & Panayiotou, 2001). In this work, the addition and increment of the EAA copolymer concentration increased the elongation percentage of the extruded films, even when chitosan was present (5%). This suggests the presence of possible electrostatic interactions, mainly hydrogen bonds, in the interphase between molecules of EAA copolymer and LDPE chains.

The tensile strength of these materials decreased when chitosan was added to the blend, even though a significant difference was not found between extruded films with and without chitosan (Table 2). When starch, chitosan, and glycerol are used, chitosan addition increased tensile strength of extruded films, due to the intermolecular hydrogen bonds formed between NH_2 and OH groups of starch exposed in the extrusion process (Pelissari et al., 2011). Based on the above, intermolecular hydrogen bonds possibly may not be formed between chitosan and LDPE, even when the copolymer was present.

According to Correlo et al. (2005), when having materials with chitosan blends, loss of adhesion between the interphase components causes the formation of pores due to the unfolding of matrix fibers in the material, when stress is applied, and when chitosan acts a particulate filler, due to its immiscibility with LDPE. Thus, mechanical performance of a filled polymer depends on the strength and the filler module, which explains the lower tensile strength showed by the extruded films in the present research work. The little or no interfacial interaction between chitosan and LDPE, caused by their thermodynamic immiscibility and inherent incompatibility, may lead to the decrease of tensile strength registered in the films obtained in this work, which in turn can cause rupture of the material in the interphase (Pedroso & Rosa, 2005).

A greater amount of copolymer seems to raise the tensile strength of the material, even if chitosan is present; however, no significant differences were observed. It is feasible that higher proportions of copolymer improve the tensile strength or equals the one of the original material. The use of a polyethylene-g-maleic anhydride copolymer, which proved to be more effective tan EAA copolymer in LDPE and starch blends (due to the fine dispersion of starch in the LDPE matrix), could be a better option than EAA copolymer (Matzinos et al., 2001).

3.2.4. *Fourier transform infrared spectroscopy (FT-IR) analysis*

FT-IR analysis is useful in elucidating the existence and type of interactions that might occur between molecules from chitosan, thermoplastic, and copolymer. In the present study, the FT-IR spectra of films with EAA (Fig. 5A and B) showed a peak at approximately 1700 cm^{-1} that, may belong to the carboxyl group (which peak is located at 1700–1725 cm^{-1}) and increased as the copolymer concentration increased (Coates, 2000). The characteristic bands of chitosan at 1655 and 1560 cm^{-1}, corresponding to the amide I and amide II bend respectively, diminished as the copolymer

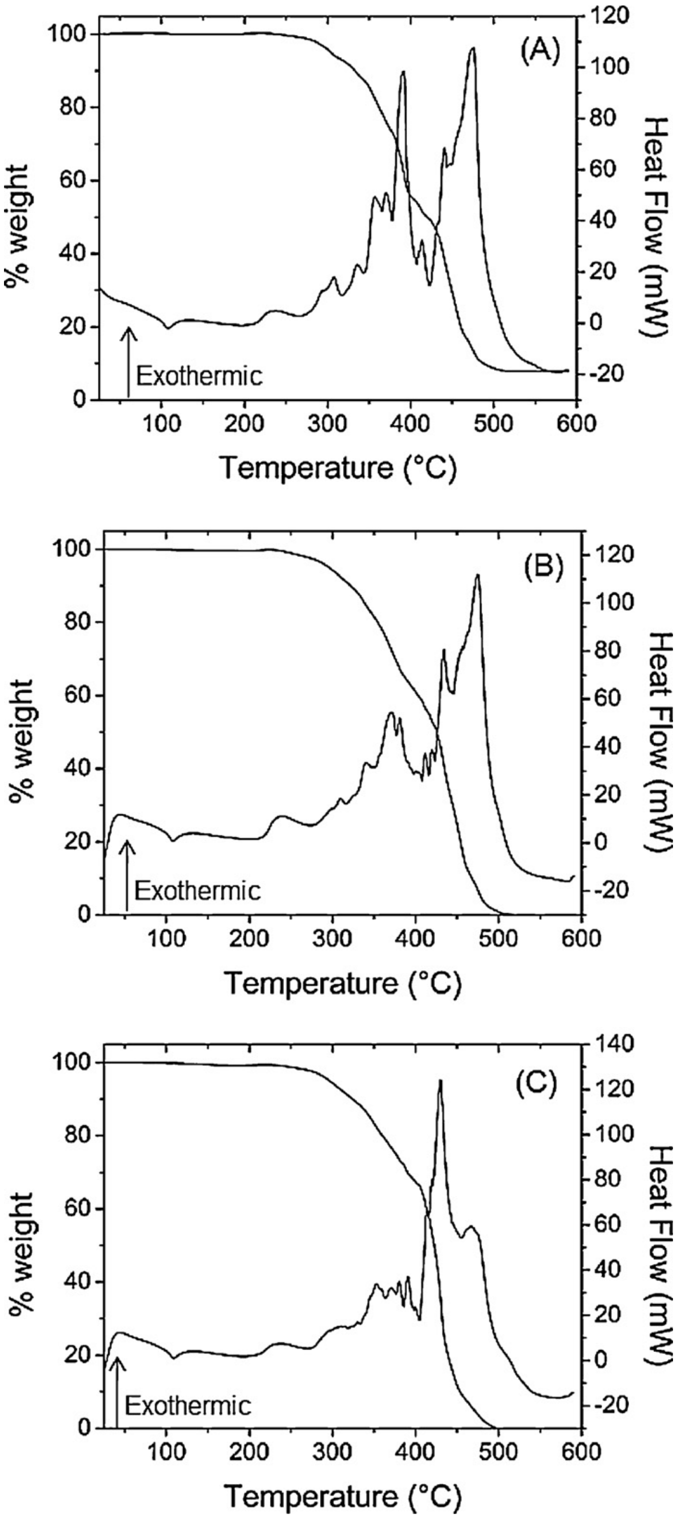

Fig. 2. Thermogravimetric analysis, TGA–DSC, of extruded composite films with chitosan: (A) 95% LDPE + 5% chitosan, CX, (B) 94% LDPE + 5% chitosan + 1% adhesive, CY, and (C) 85% LDPE + 5% chitosan + 10% adhesive, CZ.

concentration increased. This possibly could be due to the existence of interactions between the amino group and molecules of the copolymer, or to the scarce presence of the biopolymer coupled with the EAA, which can mask the characteristic peak of absorption of chitosan's amide group.

The widest peak observed in the spectra at 3200–3570 cm^{-1}, is usually attributed to the stretching of O—H bonds (Coates, 2000)

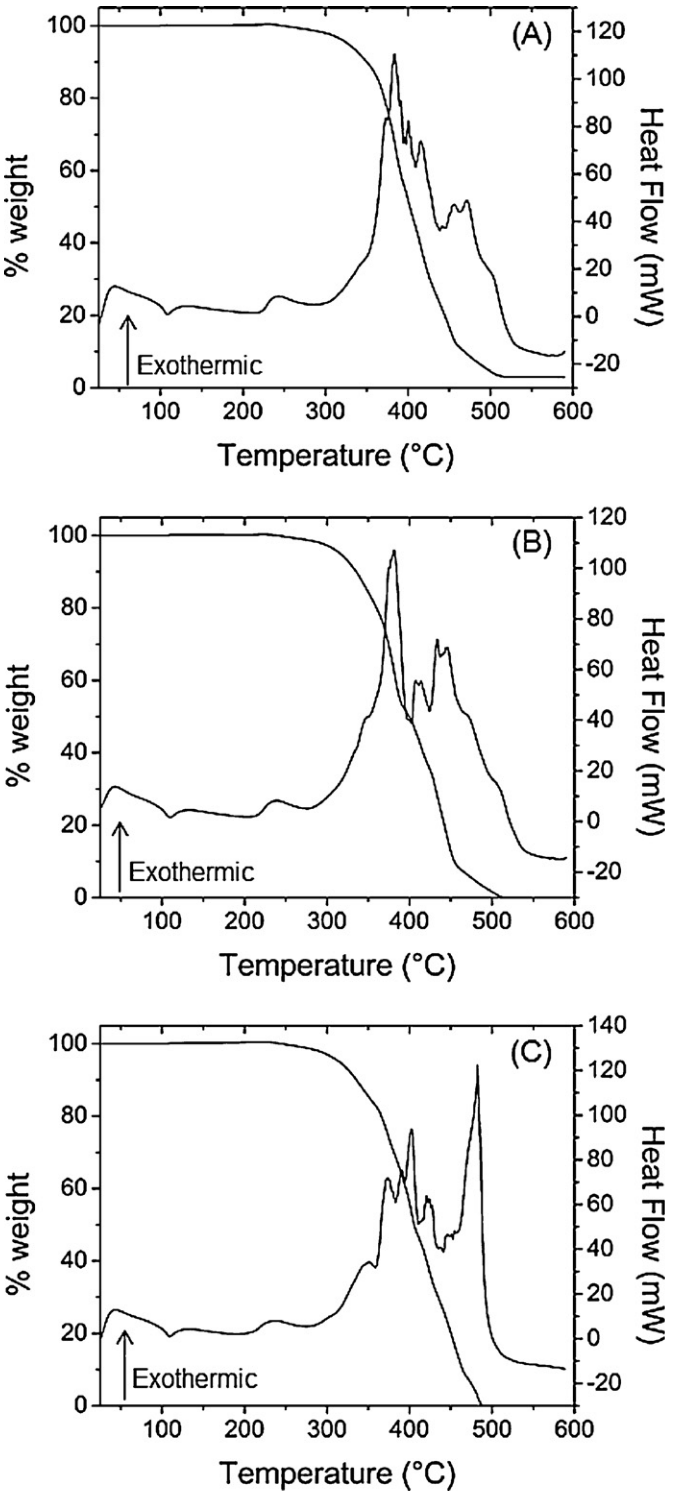

Fig. 3. Thermogravimetric analysis, TGA–DSC, of extruded composite films without chitosan: (A) LDPE, AX, (B) 99% LDPE + 1% Primacor 1430* (adhesive), AY, and (C) 90% LDPE + 10% adhesive, AZ.

which may be formed by chitosan's OH groups and helped by the presence of humidity in the films, which causes the widening of the peak. Rueda, Secall, and Bayer (1999) reported a widened peak around 3400 cm^{-1} for chitosan films made by casting and subjected to a drying process; this peak indicates strong interactions among hydroxyl groups. The FT-IR analysis makes evident the larger widening of this peak in films that were made by solvent evaporation (Martínez-Camacho et al., 2010) compared with films

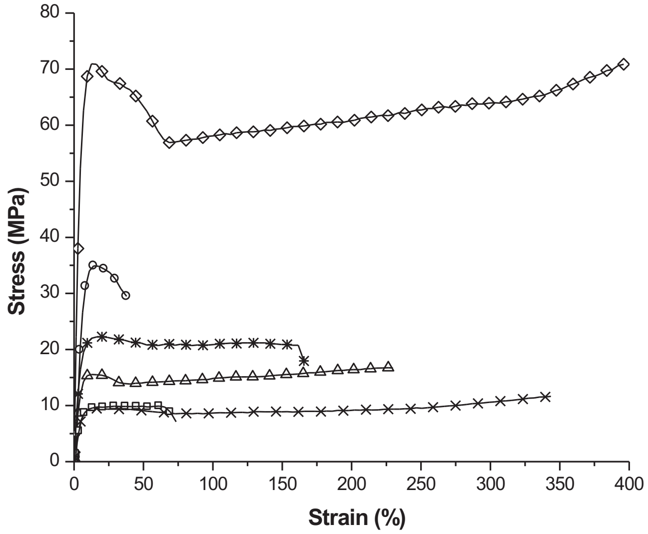

Fig. 4. Stress–strain graph of the composite blend films. (×) Low density polyethylene, LDPE; (△) 99% LDPE + 1% Primacor 1430 (adhesive); (◊) 90% LDPE + 10% adhesive; (○) 95% LDPE + 5% chitosan; (□) 94% LDPE + 5% chitosan + 1% adhesive; (*) 85% LDPE + 5% chitosan + 10% adhesive.

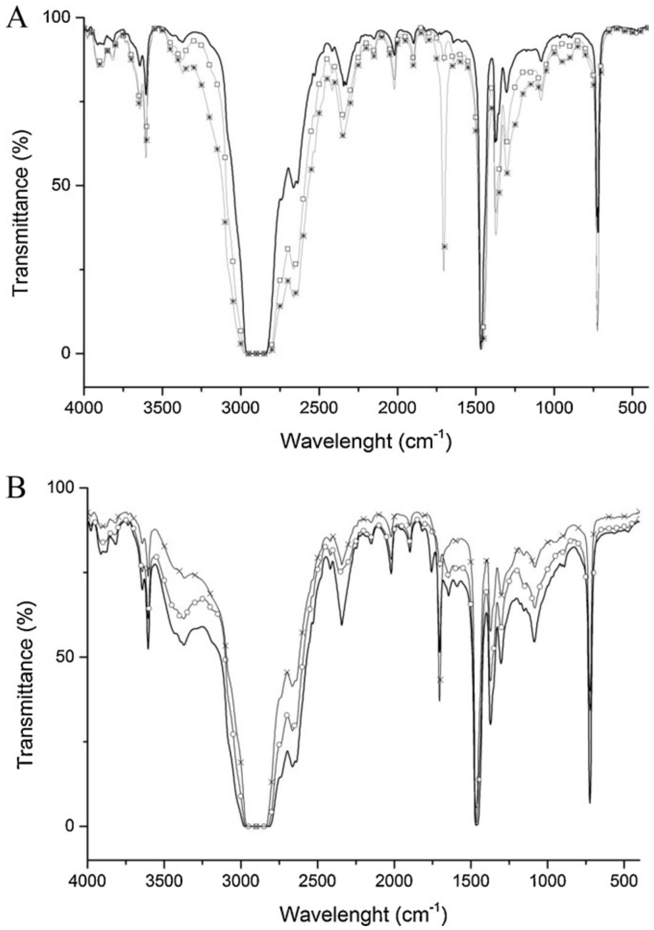

Fig. 5. FT-IR spectra of the composite blend films. (A) Controls, without chitosan: (—) low density polyethylene, LDPE; (□) 99% LDPE + 1% Primacor 1430 (adhesive); (*) 90% LDPE + 10% adhesive. (B) Composite blends with chitosan: (—) 95% LDPE + 5% chitosan; (○) 94% LDPE + 5% chitosan + 1% adhesive; (×) 85% LDPE + 5% chitosan + 10% adhesive.

elaborated by extrusion with polyolefins. This may be caused by the hydrophobic nature of the thermoplastic (LDPE) used as a base polymer for the production of the extruded materials. However, the extruded films with chitosan showed a slight displacement of the peak, from 3200 to 3500 cm^{-1} to larger wavelengths, and showing both, higher intensity and widening (Fig. 5B). This suggests the presence of weak hydrogen bonds (Cesteros, 2004), since those interactions would be established, mainly, between the amine and hydroxyl groups from chitosan and the carboxyl group of the adhesive compound, both present in little quantities in the blend.

3.2.5. *Scanning electron microscopy (SEM)*

As expected, chitosan films did not fuse completely and the morphology of the extruded films with chitosan was slightly heterogenic. Cavities were observed along and in the transversal section of the material, as well as polymeric aggregates located mainly under the film surface (Fig. 6), which were reduced and dispersed when 10% adhesive concentration was used. The function of the EAA used in this research work was aid the adhesion of chitosan and LDPE in the interphase through physicochemical interactions since both polymers are immiscible, helping to reduce size and quantity of the disperse phase (chitosan) in the continuous phase (LDPE) (Matzinos, Tserki, Gianikouris, Pavlidou, & Panayiotou, 2002). Similar observations have been reported in the surface and transversal section of films made from aliphatic polyester blends, suggesting that the chitosan domain is generally elliptic, oblong or circular, with dimensions of 40–300 μm length and 15–30 μm thick (Correlo et al., 2005; Jeung & Mishra, 2011). Also, hollows or cavities have been observed due to the lack of adhesion of the polymers in the interphase of the material (Jeung & Mishra, 2011). It is also possible the processing conditions were not the most suitable to achieved proper blending of chitosan particles (Correlo et al., 2005).

The polymer agglomerates in the continuous phase of the blend may affect the mechanical performance of the films, since the poor interaction or adhesion of the components in the blend causes an interphase zone less resistant to rupture (Correlo et al., 2005; Pedroso & Rosa, 2005). A larger amount of copolymer might be necessary to improve dispersion of chitosan in the continuous phase, as well as for a greater reduction in the particle size in order to favor the proportion of individual fibers of polymer, which would diminish the tendency to form agglomerates (Jeung & Mishra, 2011).

In addition to the characteristics and proportion of polymers, the presence of copolymers in the blend, and the method used to produce films, the conditions of the followed process, such as the screw rate, temperature, residence time, among others, may affect the morphology and rheological properties of the resulting extruded material (Pelissari et al., 2011). For example, using an extrusion process and blown molding, materials of different characteristics to the ones obtained in this study would be produced (Matzinos et al., 2001).

3.3. *Fungistatic activity of chitosan films*

3.3.1. *Radial growth*

A radial growth inhibition percentage for *A. niger* over 60% was observed in all of the analyzed films, even at chitosan absence; therefore, this inhibition might be due to the inoculation method used under the film, which may have mainly caused the inhibition of the fungus by low oxygen availability. However, the effect on the radial growth of the fungus could not be proved on the films inoculated with *A. niger* at their surface, because of the low adhesion of the spores to the films' surface, since they were suspended in an a aqueous solution and dragged away of the film's surface in the drop. It is also possible that, the release of the biopolymer from the material matrix was enough to achieve inhibition of microbial

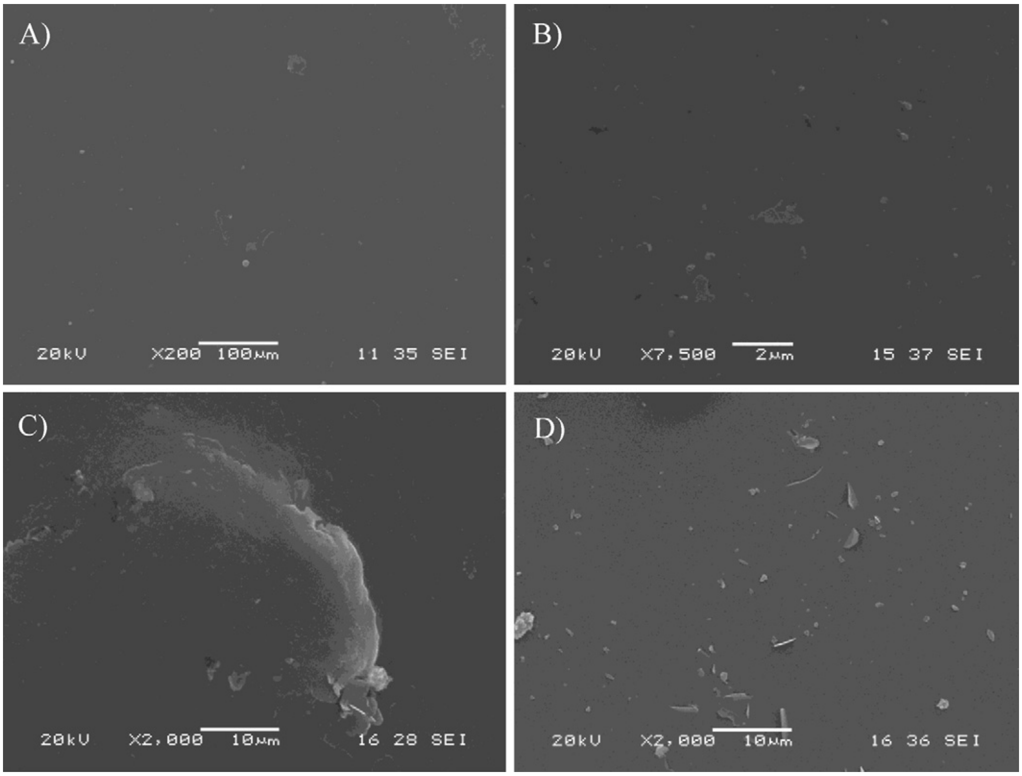

Fig. 6. Scanning electron micrography (SEM) analysis of extruded LDPE-chitosan films. (A) LDPE film at 200×, (B) LDPE + 10% adhesive at 7500×, (C) LDPE + 5% chitosan film at 2000×, (D) LDPE + 5% chitosan + 10% adhesive at 2000×.

growth, as reported in *E. coli*, *L. monocytogenes*, and *S. enteritidis* using LDPE and chitosan films obtained by press molding (Park et al., 2010). In materials made from LDPE and chitosan (5%), the growth of *E. coli* was inhibited in 73%; nevertheless, results suggest that the determinant factor in antimicrobial activity of the material was the materials structure caused by different modifications in the production process and not the chitosan proportion in the blend (Zhang et al., 2009).

Recently, it has been found that chitosan films obtained by evaporation showed no antibacterial activity on *E. coli*, *S. aureus*, and *S. epidermidis* (Foster & Butt, 2011). This is contrary to a number of previous reports; however, these studies assumed that the inhibition observed might have been caused by a barrier effect, as it is the oxygen permeability. Kurek, Scetar, Voilley, Galic, and Debeaufort (2012) observed that a coating of chitosan on LDPE films decreased the oxygen and carbon dioxide permeability at low relative humidity. Also, the solvent and plasticizer type can influence the permeability of chitosan films by modifying the molecular arrangement in the films. Molecular conformation in the blend may significantly affect the antimicrobial activity of the film since, according the proposed mechanisms for antimicrobial activity for chitosan, a direct interaction between the positively charged groups of chitosan and the negative charges of microbial membranes is necessary (Foster & Butt, 2011; Martínez-Camacho et al., 2010, 2011). However, the arrangement of the chitosan chains in the film may limit the amount of free amine groups necessary for the antimicrobial effect of the polymer. This is why the conformation that chitosan may acquire in the extruded films has to be taken to account.

3.3.2. Morphometric parameters

No differences ($P > 0.05$) in the average diameter of *A. niger* spores were observed (Table 3), this is probably due to low adhesion of spores to the surface of the films, making difficult their observation.

The average diameter of fungus hyphae exposed to extruded films showed significant differences ($P \leq 0.05$), being chitosan extruded films the more active against the fungus. Regardless the copolymer amount, chitosan in the extruded materials caused a thickening of hyphae.

Plascencia-Jatomea et al. (2003) reported a drastic increase of hyphae diameter as the concentration of a chitosan solution added to growth media increased, probably due to cell wall damage caused by changes in cellular pressure or tension. Due to the inoculation system followed in this study (film above the inoculum), a lower permeability to oxygen and water (also due to the presence of LDPE) may have resulted in a diminished hyphae diameter. The same analysis, but inoculating the fungus on the film surface, is suggested to rule out the limiting factor of permeability to oxygen and water. These observations agree with the previous reports for *A. niger* inoculated in chitosan films prepared by casting technique (Martínez-Camacho et al., 2010).

No significant differences were observed on the spore diameter with respect to the type of extruded films analyzed, even when EAA copolymer was added. However, the diameter of spores in the LDPE control film was the smallest, suggesting that chitosan increases the average diameter of the spore. This observation was reported by Plascencia-Jatomea et al. (2003) on *A. niger* spores treated with chitosan solutions added in the growth media. Rapid decrease in the number of *A. niger* spore germination, and damage and slimming of their cell membrane were found using low molecular weight (50 kDa) chitosan solution, assuming that damage was caused by the entrance of chitosan into the cell (Li, Feng, Yang, Wang, & Su, 2008). On the other hand, high molecular weight chitosan (over 800 kDa) did not enter into the cell, thus the growth of fungus was not inhibited. Antifungal activity of chitosan on *A. niger* seems to depend on both, concentration and molecular weight of the

Table 3
Fungistatic activity of the extruded blend films with and without chitosan, on the radial growth and morphometric parameters of *Aspergillus niger*, at 25 °C.

Extruded blend film	Fungistatic index (%) at 72 h	Hyphae diameter (μm) at 48 h	Spore's diameter (μm) at 10 h
LDPE	61.37 ± 1.48a	2.59 ± 0.83 cd	4.23 ± 1.41b
99% LDPE + 1% A	60.40 ± 3.92a	3.84 ± 0.36a	4.39 ± 1.89ab
90% LDPE + 10% A	64.28 ± 0.56a	3.06 ± 0.61b	5.75 ± 0.97ab
95% LDPE + 5% chitosan	61.69 ± 4.78a	1.92 ± 0.35e	2.54 ± 0.00b
94% LDPE + 5% chitosan + 1% A	62.66 ± 2.02a	2.94 ± 0.84bc	9.03 ± 0.00a
85% LDPE + 5% chitosan + 10% A	62.34 ± 3.67a	2.35 ± 0.58de	5.56 ± 0.72ab

LDPE: low density polyethylene; A: Primacor 1430* adhesive.
Data, followed by their standard errors, are means of three experiments. Treatment means were separated using the Tukey test ($P \geq 0.05$).

polymer (Li et al., 2008), as well as on the molecular conformation, hence further studies are needed for full understanding.

A more precise study is necessary in order to observe changes on membrane and cell wall caused by chitosan. Germination inhibition and diminished hyphae development of fungus treated with LDPE and 50% chitosan films was observed by Rogovina et al. (2009); however, fungi used and analysis conditions were not specified. It is possible that the effect of LDPE-chitosan films differs depending on the type of microorganism, as seen with chitosan solutions (Gómez-Rivas et al., 2004; Guo et al., 2006; Muzzarelli et al., 1990).

The proposed mechanism of antimicrobial activity of chitosan/LDPE/EAA extruded films is based on the releasing of amine groups from the material matrix, and the interaction of their positive charges with the negatives charges of the cell membranes of microorganisms (Rabea et al., 2003). However, recent studies have shown that antimicrobial activity of chitosan may not only perform in that way, but also through an effect of none charged hydroxyl groups in the carbonated skeleton of chitosan and influenced by the conformation of the chitosan molecule in solution (Pavinatto, Pavinatto, Barros-Timmons, & Oliveira, 2010). Since the formation of generally spherical or ellipsoidal chitosan agglomerates in extruded films has been proved, the conformation of the compound in the mentioned materials may affect their antimicrobial activity when evaluated on microorganism directly in contact with the material, and not as a compound partial or totally dissolved in a solution as it has been used in other studies (Torres-Giner, Ocio, & Lagaron, 2008).

More studies such as cellular viability, differential scanning microscopy or confocal microscopy among others, are necessary to clarify the effect of chitosan materials on microorganisms.

4. Conclusions

Chitosan can be mixed with low-density polyethylene and EAA copolymer Primacor (Dow) to obtain composite extruded films with acceptable mechanical properties and good thermal stability. The addition of Primacor as copolymer ensures adhesion in the interphase of both polymers, increasing elongation at rupture of chitosan/LDPE extruded films. Also allows size reduction of the chitosan conglomerates or aggregates located mainly under the film's surface, which may serve as initial points for the material biodegradation. Even when the total chitosan content in the film was 5%, the extruded films inhibited the radial growth of *A. niger*. Morphological changes and increased diameter of fungi hyphae were induced, however this effect can be mainly due to the low oxygen permeability of the extruded films. More studies are needed to clarify the antifungal properties reported in these composite films.

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