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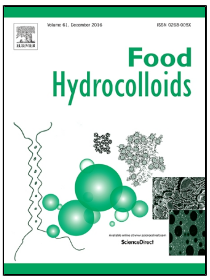

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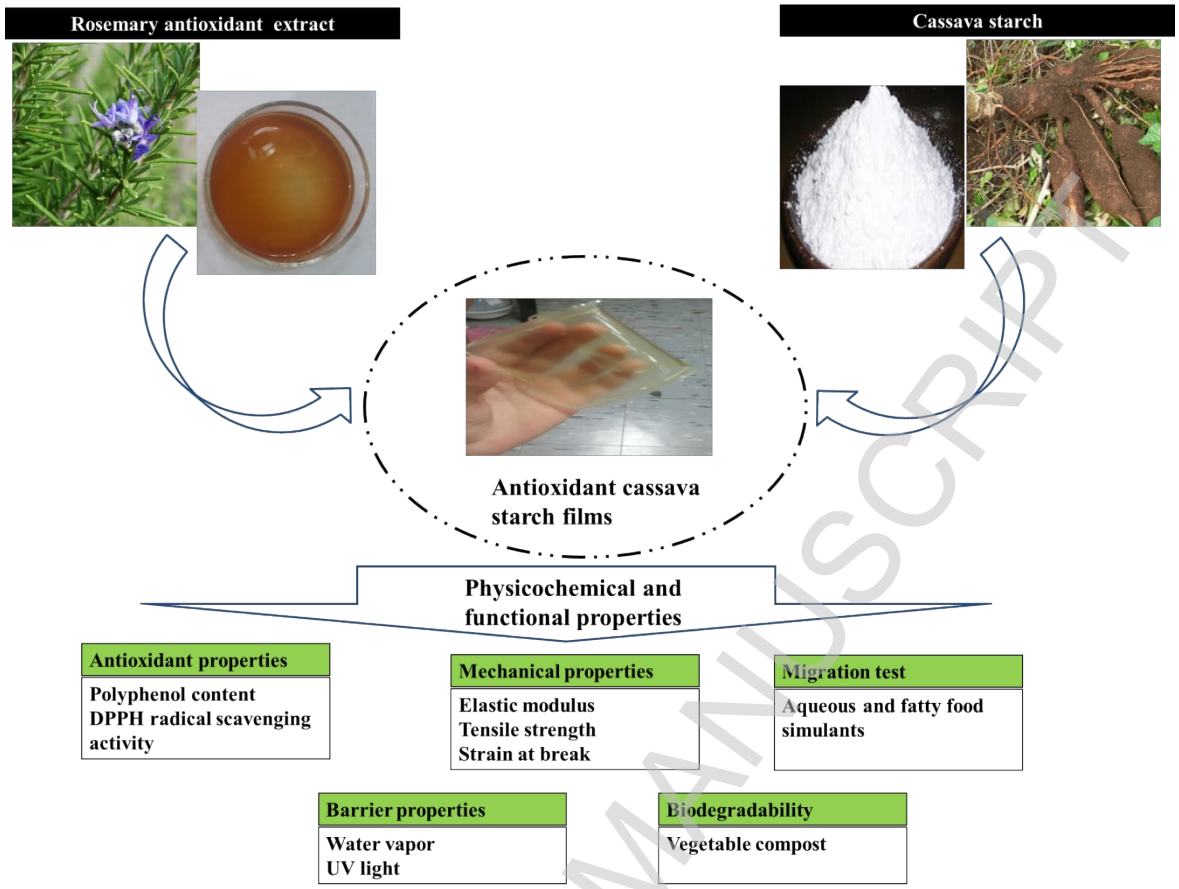

- Rosemary extracts (RE) were successfully incorporated within cassava starch films
- Active films showed a significant antioxidant activity
- UV-properties of the films were enhanced due to the RE presence
- RE incorporation inhibited the bonding between glycerol and starch molecules
- Films containing RE showed a high biodegradation extent after 14-days of composting

Edible cassava starch films carrying rosemary antioxidant extracts for potential use as active food packaging

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Abstract

Polyphenols-rich rosemary extracts (RE) were successfully incorporated within cassava starch films in order to produce active food packaging with antioxidant properties. Films with similar thicknesses (about 200 μm) and water content (15-20%) were obtained ($p>0.05$).The polyphenols content of the active films ranged between 4.4 and 13.6 mg of gallic acid equivalents per gram. As the polyphenol content increased, the films showed an increase in their antioxidant activity. Moreover, the films containing the greater extract concentration showed better barrier properties against UV light. Surface hydrophobicity of the films was affected by extract presence; the active films showed about 40% higher contact angle values (about 51°) than the control ones (about 37 °). Fourier transform infrared spectroscopy and themogravimetric analysis suggested that RE presence inhibited the bonding between glycerol and starch molecules and as a result the water vapor permeability and mechanical properties of the active films were affected. Migration tests were carried out using water and ethanol 95% as food simulants for aqueous and fatty foods, respectively. After 7 days of film exposition, the total polyphenols content loaded in the films was migrated within the aqueous food simulant, while, only a negligible polyphenol amount was detected in the fatty food one. Finally, the bio-disintegration of the films was tested finding that as the RE content increased the integrity of the RE-containing films was better preserved along the composting.

Keywords

Edible films; Starch; Rosemary; Antioxidant activity; Biodegradability

1. Introduction

World production of plastics materials has demonstrated a continuous growth for more than 50 years. It is currently estimated that the production of these materials rose to 300 million tons (Plastics Europe, 2015). Packaging applications represent around 39.6% of the total plastics demand and is considered the largest market for the plastic industry (Plastics Europe, 2015). However, the polymers mainly used in this field are from non-renewable sources and therefore are associated with environmental pollution issues.

Natural polymers constitute an actual alternative for diminishing the use of non-degradable and non-renewable materials in the packaging industry. Among them, starch has been considered as one of the most promising candidates for future materials because of its low price, abundance, and thermoplastic behavior (Jiménez, Fabra, Talens & Chiralt, 2012). Moreover, several researchers have reported the good film-forming properties of starches from a variety of botanical sources such as corn, wheat, cassava, rice, potato, yam, among others (Bonilla, Atarés, Vargas & Chiralt, 2013; Chang-Bravo, López-Córdoba & Martino, 2014; Dias, Müller, Larotonda & Laurindo, 2010; García, Martino & Zaritzky, 2000; García, Famá, D'Accorso & Goyanes, 2015; López, Lecot, Zaritzky & García, 2011; Mali, Grossmann, García, Martino & Zaritzky, 2006; Sessini, Arrieta, Kenny & Peponi, 2016). It has been stated that the physical properties and chemical structure of the starch films vary greatly depending upon the starch botanical origin, the content and type of the plasticizer and the processing conditions (García, Martino & Zaritzky, 2000; Jiménez, Fabra, Talens & Chiralt, 2012; Mali, Grossmann, Garcia, Martino & Zaritzky, 2002). Cassava starch is appreciated for its paste clarity, low gelatinization temperature and good gel stability (Mali, Grossmann, García, Martino & Zaritzky, 2006). In addition, cassava starch films have been described as odorless, tasteless, colorless, non-toxic and biodegradable (Famá, Flores, Gerschenson & Goyanes, 2006; García, Famá, Dufresne, Aranguren & Goyanes, 2009; López & García, 2012; Mali, Sakanaka, Yamashita & Grossmann, 2005; Medina Jaramillo, Gutiérrez, Goyanes, Bernal & Famá, 2016; Morales, Candal, Famá, Goyanes & Rubiolo, 2015; Seligra, Jaramillo, Famá & Goyanes, 2015; Souza, Benze, Ferrão, Ditchfield, Coelho & Tadini, 2012; Versino & García, 2014).

In order to obtain active food packaging, biodegradable films have been added of functional additives, like antioxidant and antimicrobial agents, which may be migrated from the packaging to the food product (or the surrounding headspace) so as to extend the shelf life of food and to improve its safety and quality properties (Bonilla, Talón, Atarés, Vargas & Chiralt, 2013; Chang-Bravo, López-Córdoba & Martino, 2014; Gonçalves, Tomé, Garcia, Brandão, Mendes & Marrucho, 2013; López-de-Dicastillo, Gómez-Estaca, Catalá, Gavara & Hernández-Muñoz, 2012; Medina Jaramillo, Gutiérrez, Goyanes, Bernal & Famá, 2016; Moreno, Atarés & Chiralt, 2015; Quilaqueo Gutiérrez, Echeverría, Ihl, Bifani & Mauri, 2012). In particular, antioxidant-containing films could be used to prevent the oxidation damage of fatty foods (Moreno, Atarés & Chiralt, 2015). For this purpose, the use of natural compounds instead of synthetic

additives is preferred due to the association of the last ones with adverse effects on human health (Kechichian, Ditchfield, Veiga-Santos & Tadini, 2010; Moure et al., 2001).

Polyphenols-rich extracts are considered potent films additives because help to prevent the lipid oxidation of foods and the microbial spoilage. Starch films have been added of herbal extracts from red cabbage (*Brassica oleraceae*), oregano (*Origanum vulgare*), Murta (*Ugni molinae Turcz*), yerba mate (*Ilex paraguariensis*), among others (Chang-Bravo, López-Córdoba & Martino, 2014; Medina Jaramillo, González Seligra, Goyanes, Bernal & Famá, 2015; Silva-Pereira, Teixeira, Pereira-Júnior & Stefani, 2015; Silva-Weiss, Bifani, Ihl, Sobral & Gómez-Guillén, 2013).

Rosemary (*Rosmarinus officinalis L.*) extracts from the Lamiaceae family, are a source of bioactive ingredients including phenolic acids, flavonoids, diterpenoids and triterpenes (Aguilar et al., 2008). Among their constituents, carnosic acid, carnosol and rosmarinic acid have attributed the higher antioxidant activity (Erkan, Ayranci & Ayranci, 2008). Rosemary extracts are widely used as additives in the food (e.g. flavorings, antioxidant and antimicrobial agent) and pharmaceutical (e.g. hepatoprotective, diuretic, hypocholesterolemic, antirheumatic and antithrombotic) industries (Aguilar et al., 2008; Erkan, Ayranci & Ayranci, 2008). Several studies have demonstrated that the addition of rosemary extract into food products, such as processed meat and processed fish and fishery products, slowing down or preventing the oxidation reactions (Aguilar et al., 2008). Nevertheless, only few works deals with the delivery of rosemary extract from active packaging materials to food products have been reported. Several authors have analyzed the effects of the addition of rosemary extract on films based on natural (e.g. gelatin, chitosan, whey protein, oxidized and acetylated corn starch–sodium alginate blends) and synthetic polymers (e.g. polyethylene) (Abdollahi, Rezaei & Farzi, 2012; Barbosa-Pereira, Aurrekoetxea, Angulo, Paseiro-Losada & Cruz, 2014; Bentayeb, Rubio, Batlle & Nerin, 2007; Gómez-Estaca, Montero, Fernández-Martín, Alemán & Gómez-Guillén, 2009; Musuc, Badea-Doni, Jecu, Rusu & Popa, 2013; Ouattara et al., 2002; Ponce, Roura, del Valle & Moreira, 2008; Seydim & Sarikus, 2006; Yan, Zhang, Dong, Hou & Guo, 2013). It has been found that the rosemary extract addition into biodegradable films led to materials with high antioxidant (as measured by the peroxide and TBARS indices) and antimicrobial (e.g. against *E. coli*) activities allowing to prevent food spoilage and contamination (Gómez-Estaca, Montero, Giménez & Gómez-Guillén, 2007; Yan, Zhang, Dong, Hou & Guo,

2013). However, to the best of our knowledge, studies dealing with cassava starch films carrying rosemary extracts not have been performed. The objective of this work was to investigate the effect of the incorporation of aqueous rosemary extracts on the physicochemical properties of edible and biodegradable cassava starch films. The systems were characterized in terms of their water content, surface hydrophobicity, tensile and barrier properties, thermal stability and biodegradability. Moreover, migration tests were carried out using water and ethanol 95% as food simulants for aqueous and fatty foods, respectively. The findings suggested that films based on plasticized cassava starch containing RE can be considered as interesting food packaging materials.

2. Materials and methods

2.1. Materials

Cassava starch (18 wt% amylose and 82 wt% amylopectin), provided by Industrias del Maíz S.A (Buenos Aires, Argentina), was used as the film-forming biopolymer. Analytical grade glycerol (Aldrich, USA) was used as plasticizer. Dried and milled rosemary leaves were purchased at a local market in Buenos Aires, Argentina.

2.2. Preparation of aqueous rosemary extracts

Extraction was performed according to optimized protocol reported by Rodríguez-Rojo, Visentin, Maestri and Cocero (2012). A blend containing 10 g of dried and milled rosemary leaves and 100 mL of distilled water was placed in a thermostatic bath at 50°C for 60 min. Once obtained, the extracts were cooled, filtered (pore size 0.45 μm) and stored at 4°C in dark flasks until used. The extraction yield, as determined gravimetrically at 80 °C until constant weigh, was 2.6 mg of dried extract/100 mL of sample.

2.3. Preparation of cassava starch films

Cassava starch films were produced by solvent casting process as reported in previous works (García, Famá, Dufresne, Aranguren & Goyanes, 2009; Medina

Jaramillo, González Seligra, Goyanes, Bernal & Famá, 2015; Seligra, Jaramillo, Famá & Goyanes, 2015). Control films (TPS) were prepared based on blends containing starch (5.0 g), glycerol (1.5 g) and distilled water (93.5 g). In order to prepare the active films, a water mass (5g, 10g or 20 g) from the formulations was replaced with the same amount of aqueous rosemary extract. Each blend was homogenized for 40 min and then heated until 96°C (heating rate = 3°C/min), under constant stirring. The formulations were degassed for 7 min with a mechanical vacuum pump, dispensed into polypropylene plates and dried at 50°C for 24 h. Films were conditioned at room temperature into desiccators containing supersaturated solution of sodium bromide (RH≈ 57%) for 48 h, prior to characterization studies. The active films added of 5g, 10g or 20g of aqueous rosemary extract (RE) per each 100 g of film-forming solution will be referred as "TPS-RE5%", "TPS-RE10%" and "TPS-RE20%", respectively.

2.4. Microscope observations

Micrographs of cross-sections of the films were obtained using a scanning electron microscope (FE-SEM) (SUPRA 40, Carl Zeiss NTS, Germany). The specimens were cryofractured by immersion in liquid nitrogen. The samples were mounted on stubs and sputtered with a thin layer of gold (thickness below 50 nm) prior to SEM observations. Images at different magnifications (from 2,000x up to 50,000x) were obtained using a voltage of about 3 kV and a spotsize of ~ 2 nm. The thickness of each film was measured from SEM images at six randomly selected points, using the ImageJ software.

2.5. Water content and surface hydrophobicity of the films

Water content (%) was measured gravimetrically by drying the film samples in an oven at 100°C until constant weight (AOAC, 2016).

The contact angle (θ) was used to estimate the surface hydrophobicity of the films. A 2 µL-droplet of ultrapure water was deposited on the film surface and the image of the drop was recorded with a digital microscope (MicroView, China). The contact angles were calculated using the ImageJ free software (Stalder, Kulik, Sage, Barbieri & Hoffmann, 2006).

180 2.6. Film barrier properties

181 Water vapor permeability (WVP) tests were carried out at room temperature
182 following the ASTM E96/ ASTM E96M-16 method. Film samples were sealed over a
183 circular opening of $4x10^{-4}$ m^2 in a permeation cell, containing calcium chloride. Then,
184 the cells were placed in desiccators conditioned with sodium chloride saturated solution
185 (75% RH). Changes in the weight of the cell were recorded to the nearest 0.0001 g and
186 plotted as a function of time and the slope of each line was calculated by linear
187 regression. WVP (g Pa^{-1} s^{-1} m^{-1}) was calculated as follows:
188
189 $$WVP = [WVTR/P.RH)]\, d \qquad \text{Eq. 1}$$
190
191 where WVTR is the water vapor transmission rate calculated as the ratio between the
192 slope of the straight line (g/s) and the cell area (m^2); P is the saturation vapor pressure of
193 water (Pa); *RH* is the relative humidity in the desiccator, and *d* is the film thickness (m).
194 Film transparency was measured by their ability to transmit light in the visible region
195 (Han & Floros, 1997). Films were cut into rectangles (50 mm × 10 mm) and placed on
196 the internal side of a quartz spectrophotometer cell. The percent transmittance (% T) of
197 light at 600 nm (T_{600}) was measured using a UV-visible spectrophotometer
198 (SHIMADZU UV-1800, Japan) and the transparency was calculated as the ratio
199 between $logT_{600}$ and the thickness (mm) of each film.
200
201 2.7. Uniaxial tensile tests

202 Uniaxial tensile tests were carried out using Instron dynamometer 5982 at a strain
203 rate of 5 mm/min, according to ASTM D882-12 standard. Probes with similar
204 dimensions were used (length: 50 mm, wide: 5 mm and thickness: 0.2 mm) and at least
205 3 replicates of each film type were tested. Nominal stress–strain curves were obtained
206 and Young's modulus, tensile strength and strain at break were calculated according to
207 ASTM D882-12 standard.
208

209 2.8. Fourier transform infrared spectroscopy (FTIR)

210 FTIR analysis was performed using Nicolet 380 equipment (Thermo Scientific,
211 USA) equipped with attenuated total reflectance (ATR) module. The samples were

placed on the ATR accessory and then were analyzed under transmission mode, taking 64 scans per experiment with a resolution of 4 cm^{-1}. FTIR spectra were normalized with the smallest absorbance set to 0 and the highest to +1.

2.9. Thermogravimetric analysis (TGA)

TGA was performed using SHIMADZU DTG-60 (Japan) equipment. Samples (3.0-5.0 mg) were placed in aluminum pans inside the thermogravimetric balance and then heated under dry nitrogen atmosphere (30 mL/min) in the range of 30 to 400 °C at a heating rate of 10°C min^{-1}.

2.10. Polyphenol content and antioxidant activity of the rosemary extract and the films

Rosemary extracts were diluted with distilled water in order to reach different polyphenols concentrations.

Film extract solutions were prepared by mixing 80 mg of the film samples (TPS-RE5%, TPS-RE10% or TPS-RE20%) with 5 mL of distilled water. The blends were placed in a shaker at 125 rpm and room temperature. After 24 h of assay, aliquots of the supernatant were removed and the polyphenols content and the antioxidant activity were determined as follows:

Total polyphenols content was determined by the Folin-Ciocalteu method (Singleton, Orthofer & Lamuela-Raventós, 1999). Briefly, 400 µL of each sample (RE or film extract solution) were mixed with 2 mL of Folin-Ciocalteu reagent (Anedra, Argentina, 1:10 diluted). Then, 1.6 mL of sodium carbonate (7g/100 mL) (Anedra, Argentina) were added to each sample. After 30 min, the absorbance was measured at 760 nm using spectrophotometer (SHIMADZU UV-1800, Japan). The calibration curve was performed using gallic acid (Sigma Aldrich, USA) as a standard. The results were expressed as gallic acid equivalents (GAE).

Antioxidant activity was tested as described Brand-Williams, Cuvelier and Berset (1995). A volume of 100 µL of each sample (RE or film extract solution) was mixed with 3.9 mL of 1,1-diphenyl-2-picrylhydrazyl (DPPH•) ethanol solution (25 mg DPPH•/ L). Absorbance was determined at 515 nm until the reaction reached a plateau. The DPPH• -scavenging activity of each sample was expressed as the inhibition percentage calculated with the following equation:

243 $$\text{DPPH}\bullet \text{ inhibition (\%)} = ((A_b - A_s)/A_b) \times 100 \quad \text{Eq. 2}$$

244 where A_b is the absorbance of the blank and A_s is the absorbance of the sample.

245

246 2.11. Migration tests of rosemary extract from active films to food simulants

247 Migration tests were performed as recently reported Busolo and Lagaron (2015).
248 Water and ethanol 95% were selected as food simulants for aqueous and fatty foods,
249 respectively (Baner, Bieber, Figge, Franz & Piringer, 1992). Samples (Film pieces of 2
250 cm^2) were immersed in 5 mL of food simulant and placed in a shaker at 25 °C and 125
251 rpm for 7 days. After completing the exposure time, the migration of rosemary
252 polyphenols to each simulant was tested by the Folin-ciocalteu method as above
253 described (Section 2.10). The results were expressed as mg of GAE per kilogram of
254 food simulant.

255

256 2.12. Biodegradation tests

257 Composting tests were carried out as recently reported Medina Jaramillo, Gutiérrez,
258 Goyanes, Bernal and Famá (2016). Film samples (20 mm x 20 mm) were weighed and
259 buried at 1-2 cm depth in plastic trays containing organic compost made of vegetable
260 wastes (relative humidity =85%), purchased at a local market (Buenos Aires,
261 Argentina). The plastic trays were incubated at room temperature under aerobic
262 conditions. Then, film samples were recovered at 7 and 14 days of the disintegration
263 test. The composting process was monitored by visual inspection of the buried films:
264 samples were collected and photographed periodically (Cerruti et al., 2011).

265

266 2.13. Statistical analysis

267 The statistical analysis was performed using the Systat Inc. software (Evanston,
268 USA). Assumptions of normality and variance-homogeneity were tested with the
269 Shapiro-Wilk and Levene tests, respectively. Analysis of variance (ANOVA) and Tukey
270 pairwise comparisons were carried out using a level of 95% confidence (α= 0.05). The
271 experiments were performed at least in duplicate and the data were reported as mean ±
272 standard deviation.

273

3. Results and discussion

3.1. Morphological analysis of the films

Films with thicknesses of around 200 μm were obtained. Fig. 1 shows SEM micrographs of cryofractured surfaces of control (TPS) and active films (TPS-RE5%, TPS-RE10% and TPS-RE20%). TPS films showed an ordered and homogeneous structure (*i.e.* without pores and cracks); while, in the active films some cracks were observed on the fractured surfaces (Fig. 1).

3.2. Water content, surface hydrophobicity and water vapor permeability of the films

RE presence did not affect the water content of the films, regardless of the used concentration ($p>0.05$). Water content values of 15.7±1.5 %, 19.8±2.1%, 19.4±1.6% and 19.6±1.5% were found for TPS, TPS-RE5%, TPS-RE10% and TPS-RE20%, respectively.

Surface hydrophobicity of the films was evaluated via measuring the contact angle of water (θ) upon the film surface by the sessile drop method (Table 1). In general, films with higher θ values exhibit a higher surface hydrophobicity; quantitative differentiation between "hydrophobic" and "hydrophilic" surfaces is indeed based on whether $\theta > 65$ or $\theta < 65$, respectively (Vogler, 1998). All samples exhibited values of contact angle lower 65°, which are indicative of hydrophilic surfaces. Control films (TPS) showed contact angles around 37°, while the films containing RE showed a statistically significant increase in the values of contact angle (θ about 51°). This behavior can be explained on the basis of hydrophobicity of the main bioactive compounds isolated in aqueous rosemary extracts (*i.e.* rosmaniric and carnosic acid) (Rodríguez-Rojo, Visentin, Maestri & Cocero, 2012). Rosmaniric acid have four –OH groups and a –COOH group in its chemical structure; while, carnosic acid is more hydrophobic with two –OH groups and a –COOH group. In this sense, the increase in the film surface hydrophobicity could be attributed to little amounts of carnosic acid, which can have been migrated to the film surface during the drying process.

Water vapor permeability (WVP) values for control and active films are shown in Table 1. TPS and TPS-RE5% films showed similar WVP values ($p>0.05$); while, TPS-RE10% and TPS-RE20% showed a statistical significant increase in the WVP values ($p<0.05$). This behavior was probably due to the cracks presence on these materials (Fig. 1). Several authors have reported that water vapor transmission through a material

is a balance of its hydrophilic/hydrophobic ratio of the film components, the film crystallinity, the tortuosity of the pathway, and the presence of surface or structural defects (Ludueña, Vázquez & Alvarez, 2012; Versino & García, 2014).

3.3. Infrared spectra of control and active films

Infrared spectra of powdered rosemary extract (RE), control (TPS) and active films (TPS-RE5%, TPS-RE10% and TPS-RE20%) are shown in Fig. 2.

RE showed typical bands at 3400 cm^{-1} (O–H stretching), 2927 cm^{-1} (C–H stretching), 1752 cm^{-1} (C=O stretching), 1717 cm^{-1} (C=O stretching), 1515 cm^{-1} (C=C stretching) and 1272 cm^{-1} (C–O stretching) (Musuc, Badea-Doni, Jecu, Rusu & Popa, 2013; Ribeiro, Caleja, Barros, Santos-Buelga, Barreiro & Ferreira, 2016)

IR spectra of all films showed the characteristic bands of starch at 3300 cm^{-1} (O–H stretching), 2947 cm^{-1} (C–H from alkyl groups) and 1149 cm^{-1} (C–O stretching) (Fig. 2). Besides, the films carrying RE showed signals corresponding to the stretching of the aromatic rings due to the presence of phenolic compounds from the extract at 1515 cm^{-1}. Other signals around at 1750 cm^{-1} and1714 cm^{-1} were also detected and attributed to the presence of C=O groups from carboxylic acids.

In order to compare the available amount of OH groups in the different systems, the ratios between the intensities of the peaks at 3300 cm^{-1} (I_{3300}) and at 1149 cm^{-1} (I_{1149}) were calculated. This last signal was chosen as the reference peak assuming that the total number of 'C–O' bonds in 'C–O–H' groups remained the same in the samples with and without RE (Seligra, Jaramillo, Famá & Goyanes, 2015; Shi et al., 2008). In general, the films containing RE showed lower I_{3300}/ I_{1149} ratio than TPS films (i.e. lower available amount of OH groups). Moreover, the samples TPS-RE10% and TPS-RE20% showed a greater reduction in the OH amount compared with TPS and TPS-RE5% ones. This behavior may be attributed to the RE presence, which interacted with the glycerol-starch matrix resulting in a change in the OH availability within the polymer network.

3.4. Thermogravimetric analysis

Fig. 3 shows the TGA curves of powdered rosemary extract, control films and active films. The rosemary extract exhibited a high thermal stability, maintaining their mass almost unaltered up to 150°C (weight retention> 95%). The mass loss events between

150-300°C could be attributed to the decomposition of bioactive constituents, possibly from phenolic diterpenes, such as carnosic acid and carnosol, as well as from the rosmarinic acid (Cordeiro et al., 2013).

The thermal degradation of control films (TPS) followed the pattern described in the literature typical of glycerol-plasticized starch-films (García, Famá, Dufresne, Aranguren & Goyanes, 2009). A small step of weight loss (c.a. 10 %) between 50 and 150°C was attributed to water loss. The mass loss event between 200 and 255 °C could be attributed to the decomposition of the glycerol-rich phase. At temperatures higher than 255°C occurs the oxidation of the partially decomposed starch. In the films carrying RE, the degradation temperatures showed a shift towards lower values, respect to the TPS films. Moreover, a higher shift was observed for the samples TPS-RE20%, *i.e.* with greater added extract amount. These results agree with FTIR studies showing that extract weaken the starch-glycerol interactions.

The chars amount at temperatures higher than 250°C increased in the following order: TPS-RE20%> TPS-RE10%> TPS-RE5%> TPS. This behavior was attributed to the high thermal stability of the extract (Fig. 3).

3.5. Tensile properties

Stress-strain curves for the control and active films are shown in Fig. 4.

Active films showed elastic modulus values (c.a. 1.5 MPa) about 3-fold higher than TPS films (c.a. 0.5 MPa). With respect to tensile strength, all samples showed values ranging between 0.5 and 0.8 MPa ($p>0.05$) (Fig. 4). For all formulations containing RE (TPS-RE5%, TPS-RE10% and TPS-RE20%), the strain at break showed a statistically significant decrease higher 60%, compared with TPS films ($p<0.05$) (Fig. 4). This behavior was possibly due to the development of a heterogeneous film structure and to the weakening of glycerol-starch interactions by effect of RE presence, as it was observed by SEM, FTIR and TGA studies. Similar results were found by Yan, Zhang, Dong, Hou and Guo (2013) when worked with starch–alginate composite films incorporated with rosemary extract, the values of tensile strength and elongation at break decreased with the increase of rosemary extract concentration.

3.6. Light barrier properties

All films were clear enough to be used as see-through packaging. TPS, TPS-RE5% and TPS-RE10% showed similar transparency (%) (c.a. 7.3%) ($p > 0.05$). While the TPS-RE20% showed a statistically significant decrease in the film transparency (%) (c.a. 5.7%). Furthermore, negligible UV light transmission (200-400 nm) for the active films was observed, compared with TPS films (data not shown). This behavior was attributed to the high content of aromatic compounds in the RE, which were available to act as an excellent UV barrier.

3.7. Polyphenol content and antioxidant activity of the rosemary extract and the active films

Rosemary extract showed an average polyphenol content of 5.04 ±2.3 mg GAE/mL. With respect to its antioxidant activity, the polyphenols concentration necessary to reduce the initial amount of DPPH radical to 50%, called efficient concentration (EC_{50}), was found around 0.14 mg GAE/mL. Rodríguez-Rojo, Visentin, Maestri and Cocero (2012), when worked on RE fresh leaves, reported a polyphenol content of 5.5 mg GAE/mL and EC_{50} of 0.07 mg/mL. Moreover, these authors tested the composition of the aqueous rosemary extract by high performance liquid chromatography (HPLC), finding that rosmaniric acid was the main bioactive compounds present in the aqueous extracts (concentrations around 0.014 mg/mL) followed by carnosic acid, which was found in very low concentrations (0.0035 mg/mL) (Rodríguez-Rojo, Visentin, Maestri & Cocero, 2012).

Table 2 shows the polyphenol content, the DPPH•-scavenging activity and the migration test results obtained for the active films. As expected, the increase in the added amount of RE to the formulations led to edible films with higher polyphenol content. Moreover, the DPPH-radical scavenging activities of the films increase progressively when increased the polyphenol content in the samples. This fact is interesting from the industrial point of view, because the antioxidant activity of the edible cassava starch films may be tailored and different applications could be found for each type of film.

The polyphenol concentrations migrated from the edible films to the food simulants after 7 days of film exposition are shown in Table 2. As expected, phenolic compounds migrated quickly to aqueous medium, releasing the total amount of polyphenols along the migration assay. In contrast, a very low amount of phenolic compounds was

detected when ethanol was used as a fatty food simulant. As it is well known, several factors may influence the release behavior of active compounds from biopolymer films, including the chemical composition of the active compounds, polymer–active compound interactions, matrix structure and surrounding medium conditions (Buonocore, Del Nobile, Panizza, Corbo & Nicolais, 2003; Pothakamury & Barbosa-Cánovas, 1995). In this sense, the high released amount of rosemary polyphenols into the aqueous medium could be attributed to the quick water penetration into to the cassava starch-glycerol matrix, allowing the diffusion of the active compounds to the simulant. Similar results were reported by Chang-Bravo, López-Córdoba and Martino (2014) when worked on carrageenan-starch films carrying aqueous *ilex paraguariensis* extract. On the contrary, starch matrices are less swellable in the ethanolic medium and therefore a low amount of the simulant reached the penetration of the film matrix. This film behavior could be useful for applications where longer release time of the antioxidants is required.

3.8. Biodegradation of the films

Table 3 shows the evolution of biodisintegration of edible films buried under the solid composting material. After 7 days of assay, all systems showed strong changes in their tonality and integrity, suggesting the beginning of degradation. It has been reported that the action of heat and moisture as well as the enzymatic activity of microorganisms shorten and weaken the starch polymer chains (Cerruti et al., 2011; Mohee, Unmar, Mudhoo & Khadoo, 2008).

The decomposition of the TPS films occurred almost entirely after 14 days of assay. For this time, the integrity of the RE-containing films (TPS-RE5%, TPS-RE10% and TPS-20%) was better preserved, indicating that the biodegradation was retarded by the presence of RE. Cerruti et al. (2011) studied the effect of a grape-based additive rich in polyphenols on the bio-disintegration rate of a starch-based polymer finding that the natural polyphenols affects the activity of the microorganisms present in the composting environment. Medina Jaramillo, Gutiérrez, Goyanes, Bernal and Famá (2016) reported that the addition of yerba mate extract decreased the biodegradation time of cassava starch films, observing the complete disintegration of the films before two weeks of composting.

4. Conclusions

Edible active films based on cassava starch, glycerol and natural polyphenols extracted from rosemary leaves were successfully developed. As the content of rosemary extract increased, the films showed an increase in both the polyphenols content and the antioxidant activity. The incorporation of rosemary extract in glycerol-plasticized cassava starch films enhanced the UV-blocking properties of the films. Migration tests showed that after 7 days of film exposition, their total polyphenols content was migrated within the aqueous food simulant; while; only a negligible polyphenol amount was detected in the fatty food simulant. The engineered active films showed a high biodegradation extent after 14-days of composting. The results of this work suggest that rosemary extract has a strong potential, as a natural antioxidant agent, in developing active and biodegradable cassava starch films.

Acknowledgements

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Figure captions are as follows:

Figure 1 Micrographs obtained by scanning electron microscopy of the cross-sections of the films. Magnifications: 2,000x (left column) and 10,000x (right column)

Figure 2 ATR-FTIR spectra of powdered rosemary extract, control and active films

Figure 3 TGA curves of powdered rosemary extract, control films and active films carrying rosemary extract.

Figure 4 Stress-strain curves for the control and active films

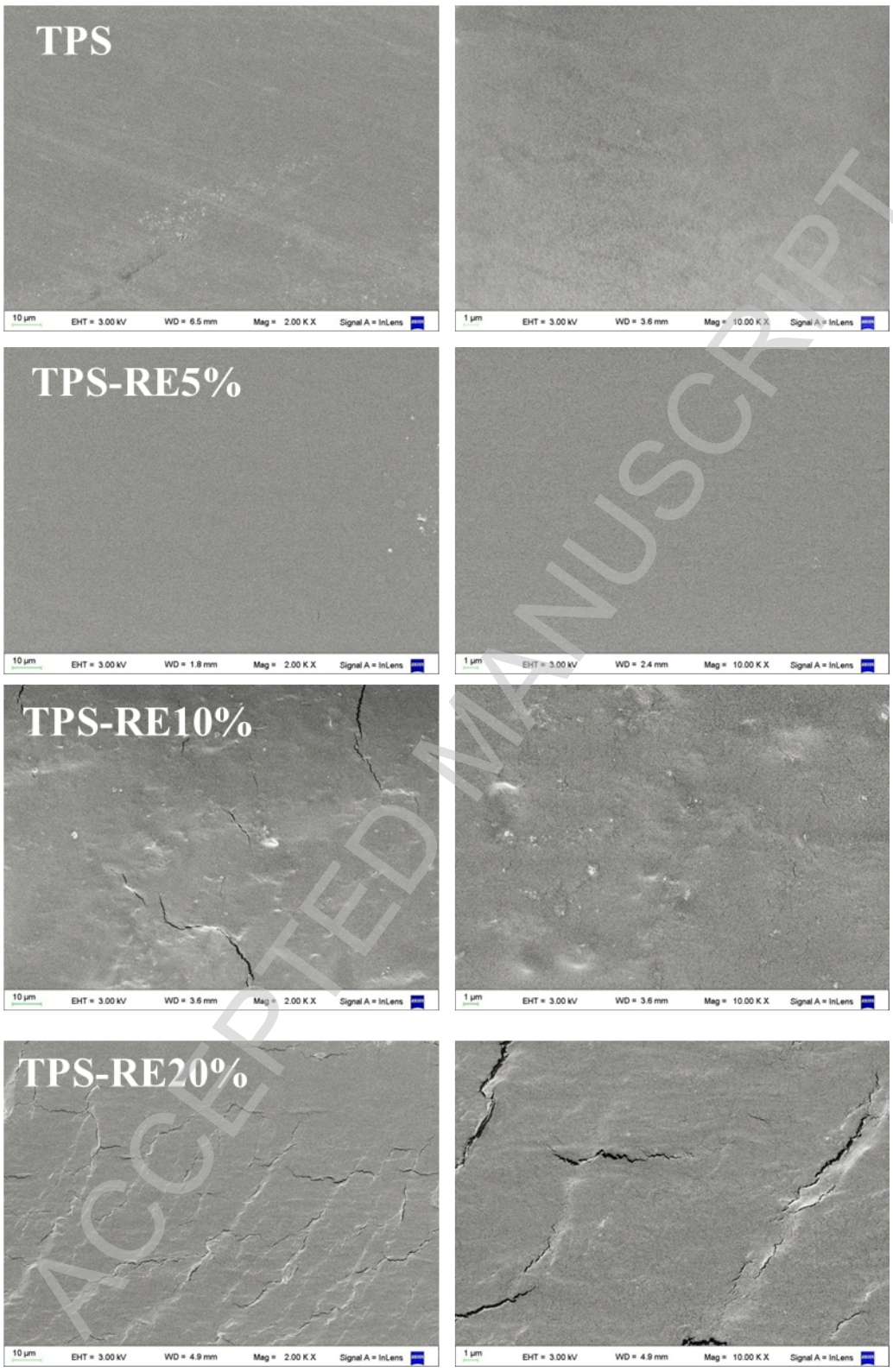

Figure 1

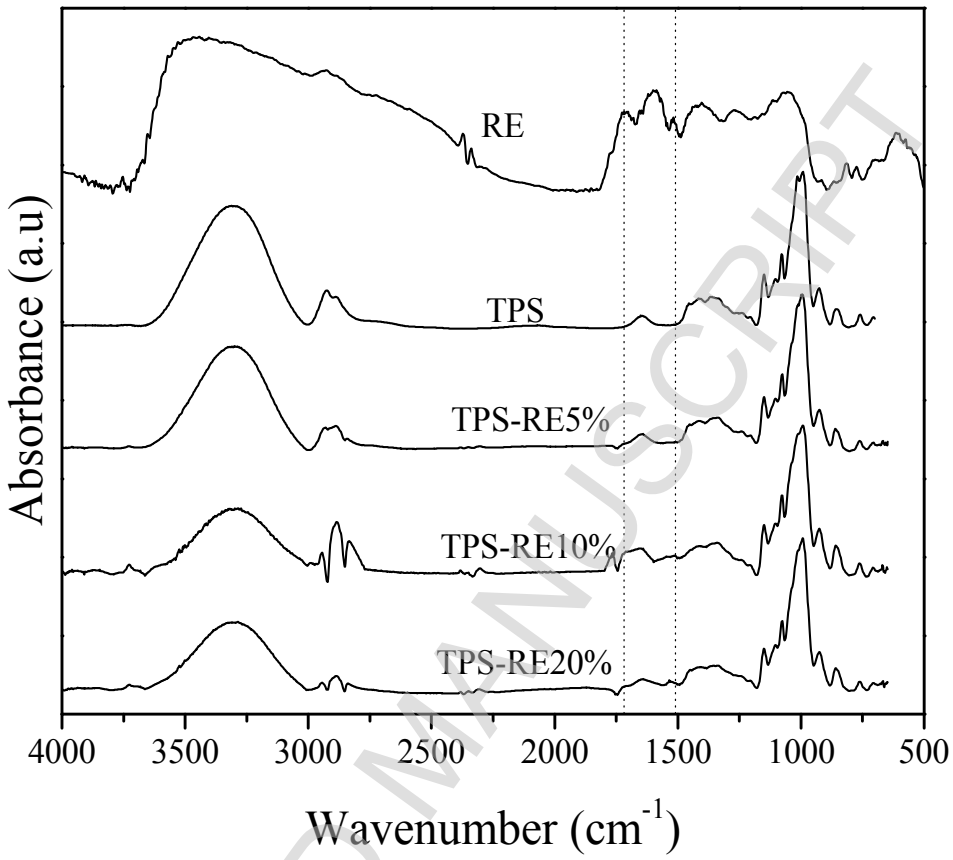

Figure 2

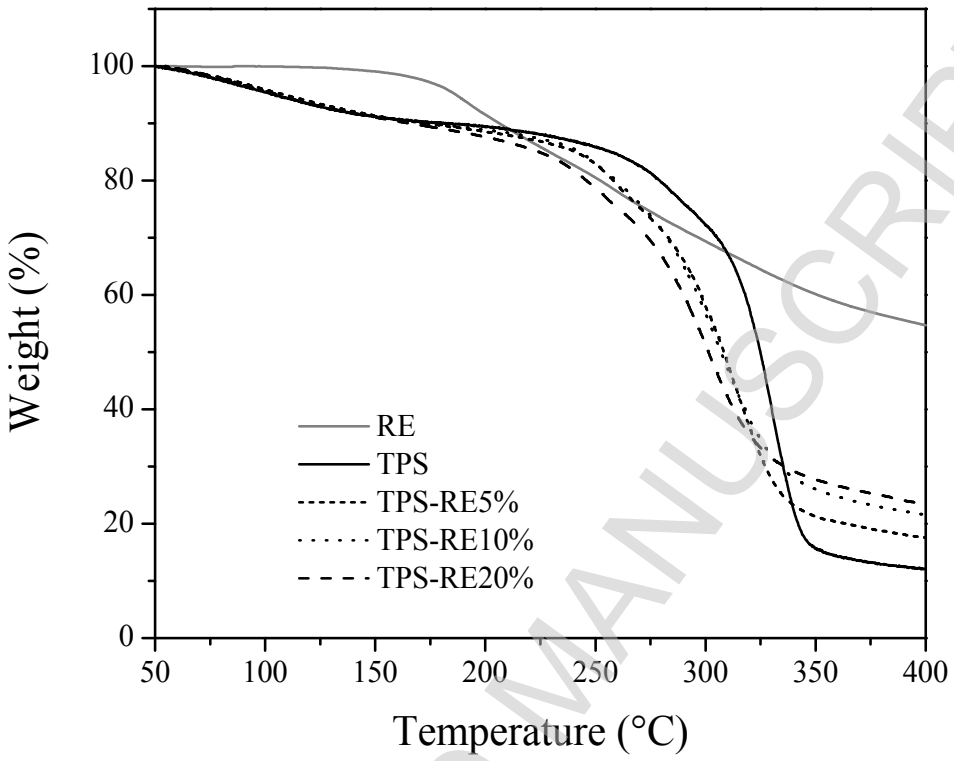

Figure 3

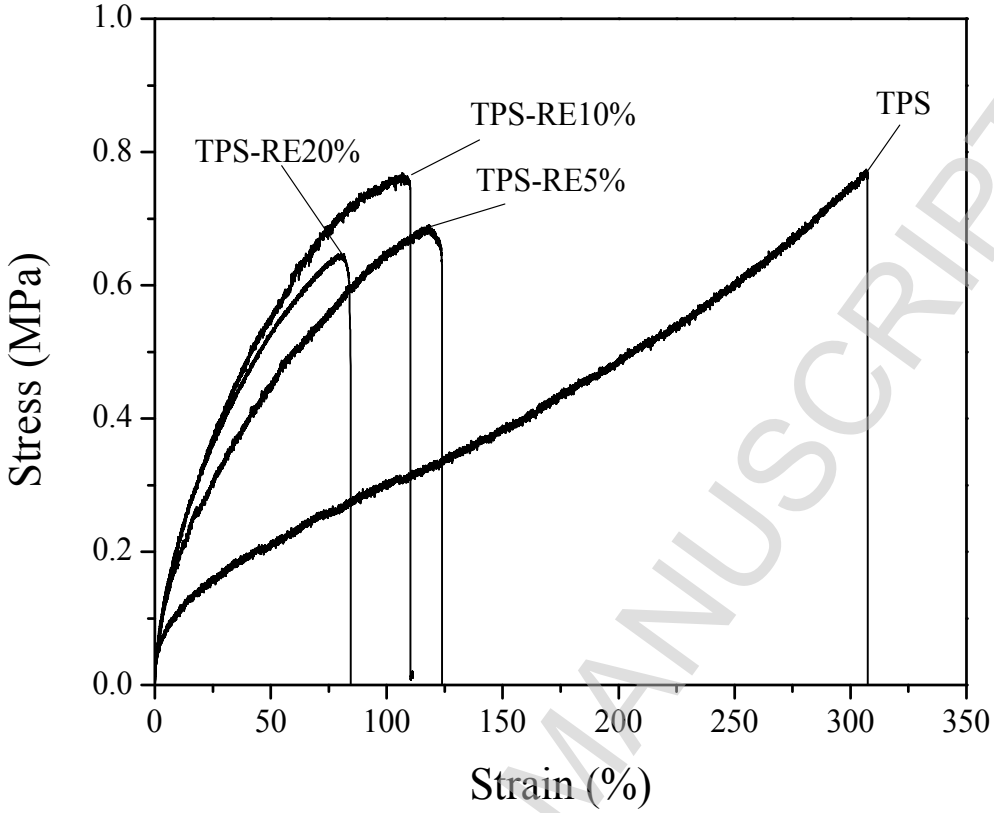

Figure 4

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Figure 2 ATR-FTIR spectra of powdered rosemary extract, control and active films

Figure 3 TGA curves of powdered rosemary extract, control films and active films carrying rosemary extract.

Figure 4 Stress-strain curves for the control and active films

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Alex

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Table 1 Contact angle and water vapor permeability (WVP) values of the control and active films

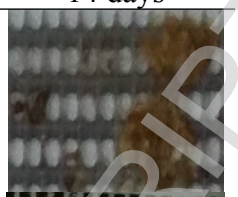
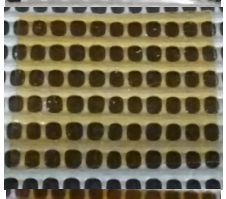
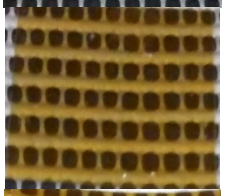
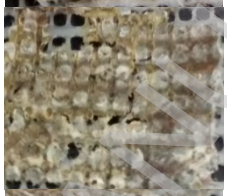
Systems	Contac angle (°)	WVP ($g\ s^{-1}m^{-1}Pa^{-1}\ x10^{-10}$)
TPS	39 ± 4^{a}	5.8 ± 0.5^{a}
TPS-RE5%	49 ± 5^{b}	6.0 ± 0.2^{a}
TPS-RE10%	52 ± 7^{b}	7.8 ± 0.1^{b}
TPS-RE20%	53 ± 4^{b}	11.0 ± 0.1^{c}

Different letters within the same columns indicate statistically significant differences

Table 2 Polyphenol content, DPPH• -scavenging activity and migration test results obtained for the active films

Film sample	mg GAE/g dried film	DPPH inhibition (%)	Migration test	
			Food simulant	mg GAE/kg simulant
TPS-RE5%	4.4±0.2	28.6±0.3	Water	40
			Ethanol 95%	<7
TPS-RE10%	8.6±0.3	54.4± 1.6	Water	83
			Ethanol 95%	<7
TPS-RE20%	13.6±0.4	81.9 ±1.7	Water	140
			Ethanol 95%	<7

Table 3 Visual appearance of edible films recovered at different composting times.

Systems	Composting times		
	Initial time	7 days	14 days
TPS			
TPS-RE5%			
TPS-RE10%			
TPS-RE20%