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Additional Information

Improving properties of thermoplastic starch films by incorporating active extracts and cellulose fibres isolated from rice or coffee husk

Sofía Collazo-Bigliardi[a*], Rodrigo Ortega-Toro[b] and Amparo Chiralt[a]

[a]Instituto de Ingeniería de Alimentos para el Desarrollo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. socol@alumni.upv.es; dchiralt@tal.upv.es

[b]Programa de Ingeniería de Alimentos, Facultad de Ingeniería, Universidad de Cartagena, Carrera 6 # 36-100, Cartagena de Indias D.T y C, Colombia. rortegap1@unicartagena.edu.co

*Corresponding author: Sofía Collazo-Bigliardi. Instituto de Ingeniería de Alimentos para el Desarrollo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. Phone: 34-963877924. Fax: 34-963877926. socol@alumni.upv.es

ABSTRACT

Hydrothermal (60 min, 180ºC) extracts and cellulose fibres from coffee and rice husks were obtained to be incorporated into corn starch films, in order to improve the film functional properties as food packaging material and confer them active properties. Extracts exhibited antioxidant properties (EC_{50}: 5.37-5.29 mg extract solids/mg DPPH) and antibacterial activity against *Listeria innocua* and *Escheriquia coli* (MIC values: 35-45 and 34-35 mg extract solids/mL, respectively). The active extracts improved tensile properties of the starch films; elastic modulus increased by about 350% and films become less stretchable. The cellulosic fibres from both residues were more

effective as reinforcing agents in films containing extract solids than in net starch films. Extracts also provoked 30 % reduction in the WVP of starch films and 50-85 % reduction in the oxygen permeability, depending on their amount in the films, but no effect of cellulose fibres was observed on barrier properties.

Keywords: Active compounds, rice husk, coffee husk, cellulose fibres, thermoplastic starch, composites.

1. Introduction

In food packaging, the development of adequate packaging materials for the purposes of prolonging the shelf life of food represents a challenge. Oxidative reactions and microbiological alteration are the main processes that cause undesirable changes in the quality and safety attributes of foodstuffs (Talón, Trifkovic, Vargas, Chiralt, & González-Martínez, 2017a), and the use of active packaging materials with antimicrobial and/or antioxidant properties can control these deterioration processes, helping to extend the product shelf life.

Lignocellulosic agro-waste, such as rice or coffee husk, is rich in polyphenols linked to the hemicellulose or lignin fractions, which exhibit active properties for controlling microbial or oxidative processes (Aguiar, Estevinho, & Santos, 2016; Balasundram, Sundram, & Samman, 2006; Wanyo, Meeso, & Siriamornpun, 2014). Different kinds of polyphenols, such as lignans, stilbenes, flavonoids or phenolic acids have been classified on the basis of their structure or phenol units (Cong-Cong, Bing, Yi-Qiong, Jian-Sheng, & Tong, 2017; Shavandi et al., 2018). The antioxidant and antimicrobial properties of natural polyphenols have been widely studied. They are biosynthesised naturally by plants, and have been isolated from different plant products, such as spices or aromatic herbs, plant-food by-products and agro-waste (Balasundram et al., 2006;

Talón et al., 2017a). Phenolic compounds have been successfully extracted from thyme (Talón et al., 2017a), garlic waste (Kallel et al., 2014), guarana seeds, boldo leaves, cinnamon barks, rosemary leaves (Bonilla & Sobral, 2016), rice hulls, almond hulls, buckwheat hulls or oat hulls (Balasundram et al., 2006), among others. The antioxidant character of polyphenols is associated with their ability to act as free radical scavengers, to inhibit lipoxygenase enzyme activity and to chelate metals (Talón, Trifkovic, Vargas, Chiralt, & González-Martínez, 2017b). The antimicrobial nature of polyphenols is associated with their capacity to inhibit extracellular microbial enzymes, to destabilise the cytoplasmic membrane and to provoke a deficit of the substrates required for microbial growth (Guil-Guerrero et al., 2016). In phenolic acids, the protonated form spreads across the membrane, which produces the acidification of the cytoplasm and, usually, cell death. Guil-Guerrero et al. (2016) reviewed the antimicrobial behaviour of several polyphenols (simple phenolic acids, flavonoids, tannins) extracted from plant-food by-products, and reported effective antibacterial action against pathogens like *E. coli, Lactobacillus* spp., *Staphylococcus aureus, P. aeruginosa*, and *Listeria* spp. strains, among others.

Lignocellulosic wastes, such as rice and coffee husk, are potential sources of active compounds, as well as cellulosic fractions that can be exploited as reinforcing agents (Collazo-Bigliardi, Ortega-Toro, & Chiralt, 2018a). Rice husk is one of the main renewable by-products of rice milling operations, and coffee husk (endocarp of coffee beans) is the residue obtained after de-hulling in coffee dry processing, both of them being rich in cellulosic (~55-57%) and lignin (~22-35%) components (Collazo-Bigliardi, Ortega-Toro, & Chiralt, 2018b). These kinds of lignocellulosic wastes have been used to extract polyphenols, which have been mostly evaluated as to their antioxidant activity, mainly using organic solvents such as ethanol or methanol (Kallel

et al., 2014; Vadivel & Brindha, 2015). Nevertheless, the polyphenol extraction by hydrothermal treatments is a better option because hot-water high-pressure extraction is an environmentally-friendly process, with a low cost, non-toxic solvent. During this treatment, a better preserved fraction of hemicelluloses and linked phenols can be obtained (Piñeros-Castro & Otálvaro, 2014) while a part of the lignin is degraded providing a great variety of phenolic compounds, such as cinnamic, benzoic, ferulic, gallic, syringic or vanillinic acids, tannins, syringaldehyde or flavonoids (Piñeros-Castro & Otálvaro, 2014; She et al., 2012).

Thermoplastic starch (TPS) has been widely studied for food packaging applications because of its biodegradability, low cost, abundance and suitability for food contact. TPS exhibits excellent filmogenic properties with high barrier capacity to oxygen and gases (Collazo-Bigliardi et al., 2018b; Ortega-Toro, Bonilla, Talens, & Chiralt, 2017). However, TPS films have some drawbacks, such as their high water sensitivity, retrogradation phenomena throughout time and low barrier capacity to water vapour. Different strategies have been used to improve these properties (Ortega-Toro et al., 2017), such as the addition of plasticisers the compatibilised blending with other polymers, the incorporation of cross-linking agents (Ortega-Toro, Collazo-Bigliardi, Talens, & Chiralt, 2016) or of different kinds of fillers (Brinchi, Cotana, Fortunati, & Kenny, 2013; Ng et al., 2015). The incorporation of cellulose micro-fillers and active extracts coming from lignocellulosic by-products could improve the starch film properties, while the use of these components allows for the valorisation of these residues in the context of circular economy.

The aim of this work was to improve the properties of thermoplastic starch films by incorporating active fractions, extracted from rice and coffee husk using a hydrothermal method, and cellulose fibres, also coming from these by-products, incorporated as

reinforcing agents. The aqueous extracts were characterized as to their antioxidant and antimicrobial properties. The effect of the incorporation of active extracts and reinforcing agents on the mechanical, thermal, barrier, optical and microstructural properties of thermoplastic corn starch matrices was analysed.

2. Materials and methods

2.1. Materials

Rice and coffee husks were provided by the Universidad Jorge Tadeo Lozano (Bogotá, Colombia). Maltodextrin (MD) 18 DE used in spray drying of extracts was from Tecnas S.A., Colombia.

For the characterization of active compounds (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2-Diphenyl-1-pikryl-hydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid and methanol was obtained from Sigma-Aldrich (Madrid, Spain). For the antimicrobial activity analysis, stock cultures of *Escherichia coli* (CECT 101) and *Listeria innocua* (CECT 910) were supplied by the Spanish Type Culture Collection (CECT, Burjassot, Spain). Tryptone Soy Broth and agar bacteriological were provided by Scharlab (Barcelona, Spain)

Corn starch was purchased from Roquette (Roquette Laisa, Benifaió, Spain). Glycerol, sodium hydroxide, sodium carbonate, phosphorus pentoxide and magnesium nitrate-6-hydrate were obtained from Panreac Química, S.A. (Castellar del Vallès, Barcelona, Spain). Sodium chlorite and acetate buffer were provided by Sigma Aldrich (Madrid, Spain). All chemicals used were reagent grade and underwent no further purification.

2.2. Extraction of active compounds

Rice and coffee husks were ground in a bladed mill (Model SK100, Retsch, Germany) until 0.75mm in size to promote the extraction, which was performed in a 5L pilot scale reactor (A2423 model, Amar Equipment, India) by pressurized hot water. To this end, 750g (rice husk) or 650g (coffee husk) and 3L of distilled water were used and the operation was carried out for 60 min at 180°C, 9.5 bar, according to previous studies (Piñeros-Castro & Otálvaro, 2014). Then, the extracts were separated from the solid fraction, which was dried for the purposes of subsequently extracting the cellulose fibres. Rice and coffee extracts were concentrated at 90°C under continuous stirring, obtaining about 6.5 and 7g ss/mL extracts, respectively. To obtain a powdered product, the aqueous extracts were spray dried by using a Vibrasec pilot dryer model Pasalab 1.5 (Universidad Nacional de Colombia, Medellin), operating at 180ºC and 90ºC outlet temperature, with atomizer disk speed of 24,000 rpm. Maltodextrin (18 DE) at 32.1 and 29.8 wt% was added to the rice and coffee extracts, respectively, as drying coadjuvant.

2.2.1. Measurement of antioxidant activity, EC_{50} parameter, total phenolic content and antimicrobial activity

The antioxidant capacity of the extracts was determined by using a 2,2-Diphenyl-1-pikryl-hydrazyl (DPPH) reduction method (Brand-Williams, Cuvelier, & Berset, 1995). To this end, 30µL of water diluted samples (1:5 for the aqueous extract or 1:10 for the powdered samples) were mixed with 1mL of a 0.1 mM DPPH in methanol. The mixture was vortexed and left to stand at room temperature in darkness (40 min) before reading the absorbance at 517 nm. The results were expressed as mg Trolox equivalents per g of extract solids (mg TE/g extract solids) by using the corresponding calibration curve for Trolox.

149 Likewise, the EC_{50} parameter corresponding to the amount of sample required to reduce
150 the DPPH concentration by 50%, once the stability of the reaction has been reached (t=
151 40 min), was determined following the methodology described by Talón et al. (2017a).
152 The water-diluted samples (0.025 to 0.175 mL) were mixed with the 0.1 mM DPPH
153 methanol solution to a final volume of 1mL. The DPPH concentration (mM) in the
154 reaction medium was calculated from the calibration curve, determined by linear
155 regression of DPPH concentration vs Absorbance at 517 nm. The EC_{50} values were
156 obtained by plotting $\%[DPPH]_R$ *versus* the mass ratio of solid extract to DPPH (mg
157 extract solids/mg DPPH), where $\%[DPPH]_R = ([DPPH]_{t=40}/ [DPPH]_{t=0})x100$; $[DPPH]_{t=40}$
158 is the concentration of DPPH when the reaction was stable and $[DPPH]_{t=0}$ is the
159 concentration at the beginning of the reaction.
160 Total phenolic content was analysed using Folin-Ciocalteu reagent, as described by
161 Singleton & Rossi (1965) with some modifications. For this purpose, 0.05 mL of Folin-
162 Ciocalteu reagent was mixed with 1 mL of Na_2CO_3, 0.5 mL of diluted sample (solids-
163 water ratio was 1:20), at 37ºC. After 50 minutes of incubation in darkness, the
164 absorbance at 765nm was measured. The total phenolic content was determined
165 applying the equation fitted to the standard curve prepared with gallic acid. The results
166 were expressed as mg of gallic acid equivalents (GAE)/ g extract solids.
167 To determine the antimicrobial activity of the active powdered extracts against *E. coli*
168 and *L. innocua*, an aliquot of each culture was transferred to a tube with 10mL of TSB
169 and incubated at 37°C for 24h. Then, 10 µL aliquots were taken from these cultures and
170 transferred to new 10 mL tubes of TSB, which were incubated at 37ºC for 24h. In this
171 way, work cultures in exponential growth phase were obtained, which were diluted to a
172 concentration of 10^5 colony forming units (CFU)/mL. From this bacterial suspension,
173 aliquots of 100µL were deposited in each well of the plate. Then, different

concentrations of the active compound diluted in water were added to each well and completed up to 100µL with TSB. The whole plate was incubated at 37°C for 24h. After 24h of incubation, the MTT reagent was reconstituted in PBS (5 mg/mL) and 10µl was incorporated in each well of the plate. The plate was re-incubated for 4h at 37ºC and the visual colour of the wells was registered. Those wells in which change of colour from yellow to purple is observed, indicate the presence of viable bacteria. In this sense, the MIC (minimum inhibitory concentration) of each active extract was considered as the lowest concentration at which no change in colour in the well was observed.

2.3. Extraction of cellulose fibres

The extraction process of cellulose fibres from rice and coffee husks was carried out according to the methodology reported by Collazo-Bigliardi et al. (2018a). Rice or coffee husks (solid residue from the hydrothermal treatment) was alkali treated with 4 wt% of NaOH at 80ºC for 3h, at 1:15 solid:liquid ratio under continuous stirring. The samples were washed with distilled water until the alkali solution was removed. Following alkali treatment, the bleaching process was completed by adding equal parts of acetate buffer solution, sodium chlorite (1.7 wt%) and distilled water mixed with the alkali treated solid (at 1:15 solid:liquid ratio) and submitted to reflux temperature (about 100ºC) for 4h under mechanical stirring. This process was repeated as many times as necessary (3 and 4, respectively for rice and coffee husks) until the samples were completely white. Then, the samples were washed with distilled water several times, dried and ground, in a Moulinex grinder DJ200031 350W, to be incorporated in the films.

2.4. Experimental design and film preparation

Thermoplastic corn starch films were obtained with glycerol as plasticiser (1:0.3 starch:glycerol ratio) by melt blending and compression moulding. To incorporate dry active extracts into the starch films, the total glycerol was partially substituted by the solid extracts in different proportions (glycerol:powdered extract ratios of: 80:20, 70:30 and 60:40), assuming that the extract compounds could also exert a plasticising effect. Then, seven film formulations were initially prepared, identified as S (starch-glycerol) and S-80:20C, S-70:30C, S-60:40C, S-80:20R, S-70:30R, S-60:40R, where C and R specify the origin of the incorporated extract (coffee (C) or rice (R) husks) and the figures reflect the glycerol:extract ratios. Since the films with the 70:30 ratio exhibited the best functional properties, these were selected to incorporate cellulose fibres, at 5% of the total blend, as reinforcing agents, based on previous studies (Collazo-Bigliardi et al., 2018b), in comparison with the reinforced S formulation. Therefore, four additional film formulations were obtained and identified with the label CF (coffee husk fibre) or RF (rice husk fibre) added to the initial sample code. All materials were hand-blended before the melt blending process. The mass fractions of each component in the different film formulations are reported in Table 1.

The melt blending process was carried out in an internal mixer HAAKE™ PolyLab™ QC, Thermo Fisher Scientific, Germany) at 130°C, rotor speed 50 rpm, for 12 min. After processing, blends were cut and conditioned at 25ºC and 53% relative humidity (RH) for one week. Four grams of the conditioned pellets were put onto Teflon sheets and preheated for 4 min in a hot plate press (Model LP20, Labtech Engineering, Thailand). Films were obtained by compressing at 160 ºC for 2 min at 30 bars, followed by 6 min at 130 bars and a final cooling cycle for 3 min (Ortega-Toro, Contreras, Talens, & Chiralt, 2015). The obtained films were conditioned at 25ºC and 53% RH for 1 week before their characterisation.

224

225 **2.5. Film characterisation**

226 **2.5.1. Microstructural properties**

227 The microstructural analysis of the surface and cross-sections (fractured samples) of the
228 films was carried out by using a Field Emission Scanning Electron Microscope
229 (FESEM Ultra 55, Zeiss, Oxford Instruments, U.K). The film samples were maintained
230 in desiccators with P_2O_5 for 2 weeks at 25°C. Film samples were adequately mounted
231 on support stubs and platinum coated. Observations were carried out at 1.5 kV.

232

233 **2.5.2. Physico-chemical properties**

234 The mechanical properties were determined using a universal test machine (TA.XTplus
235 model, Stable Micro Systems, Haslemere, England) according to the ASTM standard
236 method D882 (ASTM, 2001). Conditioned samples (2.5cm x10cm) were mounted in the
237 film-extension grips of the testing machine and stretched at 50 mm/min until breaking.
238 The tensile strength (TS), the elastic modulus (EM), and the elongation at break (ε) of
239 the films were determined from the stress-strain curves, estimated from the force-
240 distance data obtained for different films. The conditioned film thickness was measured
241 using a Palmer digital micrometer at six random positions around the film.
242 The water content of conditioned films at 53% RH and 25ºC was determined
243 gravimetrically by drying for 24h at 60ºC using a convection oven (J.P. Selecta, S.A.
244 Barcelona, Spain) and their subsequent conditioning in a desiccator at 25ºC with P_2O_5
245 (a_w=0) for 2 weeks.
246 The ASTM E96-95 (ASTM, 1995) gravimetric method was used to determine the Water
247 Vapour Permeability (WVP) of the films, with the modification proposed by McHugh,
248 Avena-Bustillos, & Krochta (1993). Payne permeability cups, 3.5 cm in diameter

(Elcometer SPRL,Hermelle/s Argenteau, Belgium) were filled with 5mL of distilled water (100% RH). Each cup was placed in a cabinet equilibrated at 25ºC and 53% RH, with a fan placed on the top of the cup in order to reduce the resistance to water vapour transport. The cups were weighed periodically (±0.0001g), and the water vapour transmission rate (WVTR) was determined from the slope obtained from the regression analysis of weight loss data versus time. From this data, WVP was obtained according to Ortega-Toro et al. (2016).

The oxygen permeability (OP) was determined using an OX-TRAN Model 2/21 ML (Mocon Lippke, Neuwied, Germany), in samples conditioned at 25ºC and 53% RH. The oxygen transmission values were determined every 10 min until equilibrium was reached. The film area used in the tests was 50 cm^2. The film thickness was considered in all cases to obtain the OP values.

The optical properties were determined by the reflection spectra of the samples from 400 to 700 nm using a spectro-colorimeter CM- 3600d (Minolta Co., Tokyo, Japan). The transparency was measured by the internal transmittance (Ti), applying the Kubelka-Munk theory of multiple scattering (Hutchings, 1999), using the film reflection spectra obtained on both black and white backgrounds. The CIEL*a*b* colour coordinates were obtained from the reflectance of an infinitely thick layer of the material by considering illuminant D65 and observer 10º, as reference. The psychometric coordinates, Chroma (Cab*) and hue (hab*), were also evaluated (Talón et al., 2017a).

The film gloss was determined at an incidence angle of 60º using a flat surface gloss meter (Multi.Gloss 268, Minolta, Germany), according to the ASTM standard D523 method (ASTM, 1999). The results were expressed as gloss units (GU), relative to a highly polished surface of black glass standard with a value near to 100 GU.

2.5.3. Thermal analysis

The thermal stability of the different samples was analysed using a Thermogravimetric Analyser TGA 1 Star^e System analyser (Mettler-Toledo, Inc., Switzerland) under nitrogen atmosphere (gas flow: 10 mL min^{-1}). Samples (about 4-5 mg) were heated from 25 to 600ºC at 20ºC/min. Initial degradation temperature (T_{Onset}) and peak temperature (T_{Peak}) corresponding to the maximum degradation rate, were obtained from the first derivative of the resulting weight loss curves using the STAR^e Evaluation Software (Mettler-Toledo, Inc., Switzerland).

A Differential Scanning Calorimeter (DSC 1 Star^e System, Mettler-Toledo Inc., Switzerland) was used to analyse the phase transitions in the polymer matrices. Samples (8-10 mg) were placed into aluminium pans and sealed. The thermograms were obtained by heating from 25ºC to 160ºC at 10ºC/min; then the samples were cooled until 25ºC, and heated in a second step to 160ºC at the same rate. In the first scan, the bonded water in the film was eliminated and in the second heating scan, the glass transition of starch was analysed.

2.5.4. Antioxidant activity

The antioxidant capacity of the films was determined using a 2,2-Diphenyl-1-pikryl-hydrazyl (DPPH) reduction method, following the methodology described in section 2.2.1. In this case, films (~1.5g) were dissolved in 100 mL of distilled water under continuous stirring in dark bottles. A final volume of 1mL was obtained by mixing samples (0.05 to 0.35 mL) with methanol solution of 0.1 mM DPPH. The EC_{50} parameter was determined as described in section 2.2.1.

299 **2.6. Statistical analysis**

300 Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD) was used for
301 carrying out statistical analyses of data through analysis of variance (ANOVA). Fisher's
302 least significant difference (LSD) was used at the 95% confidence level.

303

304 **3. Results and discussion**

305 **3.1. Properties of coffee and rice husk extracts**

306 Rice and coffee husks were used to extract active compounds with potential antioxidant
307 and antimicrobial activity through the hydrothermal process; the high temperatures and
308 pressures modify some physical properties of the water that give it particular
309 characteristics as solvent (subcritical water extraction). The aqueous extract, the
310 subsequent concentrate and the powdered form obtained by spray-drying were analysed
311 to know how the process steps affected phenolic content or antioxidant capacity (Table
312 2). The antioxidant activity was quantified in terms of Trolox equivalent (TE) of the
313 extract solids, as well as the EC_{50} values (amount of extract solids necessary to reduce
314 the initial DPPH concentration by 50%).

315 The total phenolic content of rice and coffee husk extracts ranged between 60-67 mg
316 GAE/g extract solids, this being slightly affected by the process steps. After the
317 concentration step, an increase in the quantified phenolic compounds was observed for
318 both samples, whereas a decrease in this content was determined after the spray drying
319 step. The increase after the concentration step could be due to the partial hydrolysis of
320 some linked phenols, which could contribute to the increase in the spectrophotometric
321 response. The reduction in the value of the powder samples, in comparison with the
322 concentrated extract, could be associated with the partial oxidation of some components
323 during the spray-drying process. The obtained contents referred per mass unit of dry

husks ranged between 10.7-17.3 mg GAE/g dry husks and were higher than that reported for other lignocellulosic waste. Kallel et al. (2014) reported 2.97 mg GAE/g dry husk for garlic husk treated with boiling water for 45 min. Other authors (Wanyo et al., 2014) reported that the phenolic content of rice husk was affected by different pre-treatments such as hot air drying at 120ºC for 30 min (1.70 mg GAE/g dry husk), Far-Infrared Radiation at FIR intensity of 2 kW/m^2 (3.14 mg GAE/g dry husk) or enzyme hydrolysis (2.21 mg GAE/g dry husk). The main phenolic acids found in rice husk aqueous extract were gallic, protocatechuic, vanillic and ferulic acids, although chorogenic, caffeic, syringic, p-coumaric and ferulic acids were also found in small quantities (Piñeros-Castro & Otálvaro, 2014; Wanyo et al., 2014). In contrast, caffeic and chlorogenic acids were the main phenolic compounds of coffee husk, and vanillic, gallic, tannic and protocatechuic acids were found in minor proportion (Aguiar et al., 2016; Andrade et al., 2012).

The evaluation of the antioxidant activity by DPPH assay has been widely used. DPPH is a stable free radical compound used to determine the free radical scavenging ability of different kinds of samples, such as pure compounds, plant extracts, fruit, vegetables, cereals, or lignocellulosic agro-waste (Dorta, Lobo, & Gonzalez, 2012; Lapornik, Prosek, & Wondra, 2005; Meneses, Martins, Teixeira, & Mussat, 2013). Coherently with the determined phenol content, the antioxidant activity in terms of TE (Table 2) slightly increased in the concentrated extract (30-40 % with respect to the initial extract) and decreased in the dried extract (5-8 % with respect to the concentrated extract). In contrast, the EC_{50} parameter revealed a slight increase in the antioxidant capacity (lower EC_{50} values) for both concentrated and dried samples, for both coffee and rice husk extracts. In this sense, it is remarkable that, although the antioxidant activity increases as the amount of phenolic compounds rises, other compounds present in the extracts

may also affect this capacity. Thus, γ-oryzanol and tocopherol (Butsat & Siriamornpun, 2010; Wanyo et al., 2014), HMF (hydroxymethylfurfural) resulting from the decomposition of hexoses and pentoses derived from cellulose and hemicellulose (Piñeros-Castro & Otálvaro, 2014), or some proteins and peptides, could be present in the extracts at different concentrations (Narita & Inouye, 2012), affecting the total antioxidant activity.

The values of the EC_{50} parameter expressed in terms of the total solids of powdered extracts (also containing MD) that were used for the film production were 7.66 and 7.76 mg powder/mg DPPH for coffee and rice husk extracts, respectively. Andrade et al. (2012) reported similar antioxidant activity for coffee husk extracts obtained by supercritical fluid extraction with CO_2 and 8% of ethanol (5.25 mg extract solids/mg DPPH), and soxhlet extraction with dichloromethane (5.70 mg extract solids/mg DPPH). Other authors also reported antioxidant activity for the rice husk extracts in terms of the % inhibition of DPPH, with a wide range of values, depending on the extraction method and solvent used. For instance, 74.3-87.7% of DPPH inhibition was reported for rice husk treated with hot air, cellulase and FIR (Wanyo et al., 2014), about 25% for 25:75 water:ethanol extraction, about 78% for alkali extracts with NaOH or about 76% by acid hydrolysis with 2% of H_2SO_4 (Vadivel & Brindha, 2015).

Plant extracts containing polyphenols have been widely studied as to their antimicrobial activity against different Gram-positive and Gram-negative bacteria, yeast, fungi, and moulds (Guil-Guerrero et al., 2016). Some phenolic acids, flavonoids and tannins can destabilise the cytoplasmic membrane of the microorganisms, provoking the inhibition of microbial growth and cell death (de Oliveira et al., 2015; Guil-Guerrero et al., 2016; Sánchez-Maldonado, Mudge, Gänzle, & Schieber, 2014). The use of lignocellulosic waste as a source of potentially antimicrobial extracts is of great interest in order to

374 exploit these kinds of by-products while providing them with potential food
375 applications. This is in response to the growing interest in the use of natural
376 antibacterial products for food preservation. In this sense, Kallel et al. (2014) found
377 antimicrobial activity against *S. aureus* and *B. subtilis* for the 50:50 methanol:water
378 extract from garlic. Likewise, Bonilla & Sobral (2016) found that the boldo leaf extract
379 was effective against *E. coli* and *S. aureus*.
380 The antibacterial activity of the powdered extracts from coffee and rice husks against *L.*
381 *innocua* and *E. coli* are shown in Table 2, in terms of their minimal inhibitory
382 concentration (MIC, mg/mL). The coffee sample exhibited the greatest inhibitory effect
383 against *E. coli,* which could be due to the expected presence of caffeic and chlorogenic
384 acids, which are highly effective against this pathogen, as reported by Kallel et al.
385 (2014). This action has been attributed to the diffusion of the undissociated acid through
386 the membrane causing the acidification of the cytoplasm. However, no significant
387 differences were observed in the MIC values of rice and coffee husk extracts against *L.*
388 *innocua,* both being equally as active against this bacterium, at the same level as coffee
389 husk extract against *E. coli.*
390
391 **3.2. Properties of starch-based films containing active extracts and cellulosic fibres**
392 This section discusses the effect of the incorporation of different proportions of coffee
393 and rice husk extracts on the properties of the starch films, by substituting a determined
394 fraction of the plasticizing glycerol. Likewise, the effect of adding cellulosic fibres,
395 obtained from coffee or rice husk residue, to the best film formulation containing extract
396 solids was analysed.
397
398 **3.2.1. Microstructural analysis**

This analysis allows for a better understanding of the differences in the physical properties of the films, since the microstructural arrangement of the film components greatly determine the final physical and functional properties of the material (Talón et al., 2017a). Fig. 1 shows the FESEM micrographs of the surface and cross section of the starch films containing or not extract solids and/or cellulosic fibres from coffee or rice husks. The net starch films showed the typical smooth structure, which was not apparently altered when the extract solid was incorporated at different proportions. In fact, the appearance of both the surface and cross section of the films containing extracts was even more homogenous. The ability of these kinds of active compounds to contribute to the formation of a more compact, homogeneous and ordered matrix has previously been observed in other studies (Montero, Rico, Rodriguez-Llamazares, Barral, & Bouza, 2017; Talón et al., 2017a).

The cellulose fibres can be clearly observed at the film surface in all formulations when these were incorporated. In these films, the quasi-parallel distribution of the fibres at surface level is remarkable, mainly in starch films without extract solids. This was also previously observed in these kinds of films (Collazo-Bigliardi et al., 2018b), which indicates a certain tendency of fibres to adsorb at the film surface, principally the finest ones. This was less marked in the films containing extract solids, where a smoother surface was observed, with the fibres better embedded in the matrix. This could be associated with the contribution of the extract compounds to the overall interactions in the matrix, which favoured the fibre integration within the polymer. In the film cross section, individualised uncoated fibres and infiltrated fibres are observed in all cases, but once again the presence of extract solids seems to enhance the fibre integration into the starch matrix. No notable differences at microstructural level could be observed between samples containing components from rice or coffee husks. Kargarzadeh, Johar,

& Ahmad (2017) also showed that starchy materials can be infiltrated inside the fibre bundles when cellulose fibres from rice husk were incorporated into cassava starch films.

3.2.2. Tensile properties

Table 3 shows the tensile properties of the films (EM: Elastic Modulus; TS: Tensile Strength and E: Elongation at break point) conditioned at 53% RH and 25ºC for 1 week. The elastic modulus of the films increased as the ratio of extract solids grew. Thus, although the presence of these components in starch matrices allowed more homogeneous and stiffer matrices to be obtained, they were more brittle because the elongation at break was considerably reduced in comparison with the control sample (96 and 92 %, respectively, for samples with 60:40 glycerol:extract solid ratio from coffee and rice samples). The larger the amount of glycerol substituted by the extract solids, the greater the enhancement of the film's stiffness and brittleness. This could be caused by the weak plasticizing effect of the different extract solids, but also by the formation of crosslinking effects between the starch hydroxyl groups and phenol or other groups of the extract compounds. The equilibrium moisture content, and thus its plasticising effect, also changed as a consequence of the incorporation of both extracts and fibres, ranging from 9.56 g/100 g dry film in S sample to 7.24-7.28 in samples with the highest amount of extracts, and 7.76-8.22 in samples with extracts and fibres.

The tensile strength at break (film resistance), also increased in proportion with the level of extract solids in the films, but was limited by the increase in the film's brittleness. Thus, films with a glycerol:extract solid ratio of 60:40 were the least resistant due to their very low degree of stretchability. Other authors (Bonilla, Talón, Atarés, Vargas & Chiralt, 2013) also obtained an increase of more than 15% in the EM with the addition

449 of phenols from basil essential oil in starch-chitosan matrices. However, Talón et al.
450 (2017a) incorporated thyme extract into pure starch films, obtaining a decrease of ~30%
451 in stiffness.
452 On the basis of the tensile behaviour of the films with extract solids, the best
453 formulation was selected as that containing a 70:30 glycerol:extract solid ratio, since the
454 films in which a higher of glycerol was substituted were excessively brittle. Then, the
455 reinforcing effect of cellulosic fibre on these films was analysed and compared with its
456 reinforcing effect on the net starch films. The addition of cellulose fibres also increased
457 the EM of the material, mainly when extract solids were present in the films. In this
458 sense, it is remarkable that rice husk fibres have a greater reinforcing capacity than
459 coffee husk fibres in both the net starch matrix and the starch matrix with extract solids.
460 When both components were incorporated, the EM increased, with respect to the control
461 sample (S), by 600% for rice husk solids and 400% for coffee husk solids. Similar
462 behaviour was observed by other authors after the incorporation of cellulose fibres into
463 starch films (Kargarzadeh et al., 2017; Montero et al., 2017; Zainuddin, Ahmad,
464 Kargarzadeh, Abdullah, & Dufresne, 2013). Some authors explain this increase in
465 stiffness as the result of the interaction between the amylopectin chains and the
466 cellulose in the matrix, while others relate it to the crystallinity associated with the
467 hydrogen bonds of the cellulosic fraction (Montero et al., 2017; Zainuddin et al., 2013).
468 The greater reinforcing capacity of both kinds of fibres (CF and RF) in the starch matrix
469 containing extract solids (12 against 8 % EM increase for CF and 57 against 35 % for
470 RF) revealed the better integration of fibres into the matrix containing extracts, as
471 deduced from the qualitative FESEM observations. On the other hand, fibre (CF and
472 RF) incorporation reduced the film's stretchability by about 50% in the net starch films,
473 as previously reported by Kargarzadeh et al. (2017) for cassava starch films with rice

474 husk cellulose fibres, but did not have a significant effect on this property for matrices
475 containing extracts. In contrast, CF slightly enhanced the film's resistance to break in
476 the matrices containing extracts, whereas there was no significant impact of fibre
477 addition on this parameter for the other cases. Then, the reinforcing of starch matrices
478 containing extracts with cellulosic fibres was more effective than that of net starch
479 matrices, which could be associated with a compatibilizer effect of the extract
480 compounds in the matrix. This supposes additional advantages in the formulation of
481 active films, since better mechanical properties were obtained when cellulosic fibres
482 were added.
483
484 **3.2.3. Barrier properties**
485 The water vapour permeability and oxygen permeability of the films are shown in Table
486 3. The addition of extract solids into starch matrices caused a significant decrease in the
487 WVP and in OP. In comparison with the control film (S), the WVP of films containing
488 extracts was reduced by near 30% regardless of the extract and its ratio in the film. In
489 contrast, the reduction in the OP values was dependent on the amount of extract solids,
490 ranging from 50 to 85% respect to the value of the S films, this being similar for both
491 kinds of extracts (C or R). The lower degree of plasticization of the films with extract
492 solids, due to the lower total glycerol content, could also contribute to the improvement
493 in WVP, as well as the previously commented on crosslinking effect in the matrix. The
494 great reduction in the OP values can be associated with the oxygen scavenging effect of
495 the compounds with antioxidant capacity, in addition to the lower degree of
496 plasticization of the films and the increase in the tortuosity factor associated with the
497 crosslinking effect, which hinders mass transport. Similar behaviour (~50% reduction in

498 OP) was found by Bonilla et al. (2013) in starch-chitosan matrices when 11% of α-
499 tocopherol was added as antioxidant.
500 The incorporation of cellulose fibres did not have a significant effect on the water
501 vapour and oxygen permeabilities. Although an increase in the tortuosity factor in the
502 matrix could be expected from the dispersion of the fibres, the high water affinity of the
503 cellulose could enhance the transport of water molecules through the polymer matrix.
504 However, Wattanakornsiri, Pachana, Kaewpirom, Traina, & Migliaresi (2012) showed a
505 reduction of ~63% in the WVP of cassava starch films after the addition of 8% cellulose
506 fibres from recycled paper. This could be attributed to the differences in the
507 amylose:amylopectin ratio, which could play a key role in the nanostructure of the
508 matrix, producing a different effect of the fillers on the WVP of the films.
509
510 **3.2.4. Optical properties**
511 Table 4 presents the values of lightness (L*), chroma (C*), hue (h*) and gloss of the
512 different film formulations, as well as their internal transmittance (Ti) values at 460nm.
513 Likewise, the Ti spectra for the different film formulations are shown in Fig. 2. The
514 lightness value of the matrix decreases when fibres are added, especially after the
515 addition of the extract solids. The presence of immiscible compounds generates
516 heterogeneity in the refractive index in the samples, which causes greater light
517 dispersion and opacity. The incorporation of coloured components (extract solids)
518 causes the selective absorption of light and the reduction of the transmission at low
519 wavelengths. This also caused changes in the chromatic attributes, chroma and hue. The
520 colour saturation (chroma) increased as with the extracts were incorporated, but
521 decreased when their ratio rose in the films. Likewise, the hue values fell as the extract
522 ratio rose in the formulation. These changes differed depending on whether they were

coffee or rice extracts, the latter provoking a greater decrease in hue and less colour saturation, according to the colour difference of the extracts. Films containing rice husk extract also exhibited lower lightness values than those containing coffee husk extract.

Fibre addition reduced the film transparency in both films (net starch and those containing extracts) and slightly modified the colour attributes, especially in coloured films with extracts. In general, fibre addition reduced the lightness, chroma and hue of the films, more markedly for films containing extracts due to the overlapped effect of light scattering. Therefore, films containing both solid extracts and fibres exhibited the lowest values of lightness and Ti, while presenting a brown coloration associated with the coloured extracts, with differences between coffee and rice husk extracts. Talón et al. (2017a) observed similar behaviour when thyme extracts were incorporated into starch matrices.

As regards the gloss, a decrease of about 40-60% with respect to the net starch film was observed when fibres and extract solids were incorporated into the films. However, fibre only led to a gloss reduction of about 27 % compared to the respective film containing extracts, because extract solids also reduced the film's gloss. This effect can be attributed to the changes in the roughness of the surface of the films because of a heterogeneous distribution of the non-miscible components (Ortega-Toro et al., 2016).

3.2.5. Thermal behaviour

The temperature values of onset thermal degradation (T_{onset}) and maximum degradation rate (T_{peak}), obtained from TGA, as well as glass transition temperature (Tg; second heating scan), obtained from DSC analyses, are shown in Table 5. The Tg of starch was about 100ºC, similar to that reported by other authors for glycerol-plasticized corn starch films (Ortega-Toro et al., 2015 and 2016). When extract solids from rice or

548 coffee husk were incorporated in different ratios, the Tg of the starch did not exhibit
549 notable changes, which suggests the extract solids exert a similar plasticizing effect to
550 that of the glycerol, since the latter was partially substituted to a different extent by the
551 extracts in the different formulations. As expected, the addition of cellulose fibres did
552 not provoke any notable changes in the starch Tg either, when compared with the
553 control sample, since they are non-miscible in the polymer. Wattanakornsiri et al.
554 (2012) report that the small variations in the starch Tg in cellulose composites could be
555 attributed to the interaction between fibres and plasticizer, the composites becoming less
556 plasticized than the pure matrix.
557 As regards the thermogravimetric analysis, Fig. 3 shows the TGA and DTGA curves of
558 the different formulations. A small weight loss occurred in every case at between about
559 70-150ºC, which can be attributed to the evaporation of bonded water in the polymer.
560 This peak in the DTGA curves was slightly more marked in films containing fibres,
561 according to the greater water binding capacity of cellulosic material. Likewise, a
562 progressive, slow weight loss was observed until the start of the main degradation peak
563 (mainly associated with the polymer degradation), which can be attributed to the
564 thermal degradation of glycerol (Valencia-Sullca, Vargas, Atarés, & Chiralt, 2018)
565 and/or the extract solids. In this sense, a more marked shoulder was detected at a
566 temperature lower than the onset of the main thermo-degradation step for film
567 formulations containing extract solids, both from coffee and rice husk products. This
568 was reflected in the values registered for the onset temperature of the main peak, which
569 notably decreased with respect to the films without extracts. However, no marked
570 differences were observed for the main peak temperature, due to the addition of extracts
571 or fibres. The main peak occurred at about 300°C and it is associated with the division
572 of the main chains of starch (Zainuddin et al., 2013). The thermal degradation of

cellulose fibres occurred at between 290-360ºC (Collazo et al. 2018a), and the small shoulder exhibited by the main peak at the higher temperature edge in samples containing fibres can be attributed to their final degradation. No remarkable differences can be observed for the thermal behaviour of coffee or rice husk products; however, coffee husk extracts seemed slightly more unstable than rice husk products (onset of degradation at a lower temperature).

3.2.6. Antioxidant capacity

The antioxidant activity of the films containing different ratios of glycerol:extract solids, expressed in term of EC_{50} values, are shown in Table 6. As expected, the increase in the amount of active extracts added to the formulations led to films with better antioxidant capacity, associated with lower EC_{50} values. Formulations with coffee husk extracts presented greater antioxidant activity than samples with rice husk extracts, which was more clearly evidenced when the EC_{50} values were referred as mg of extract solids per mg DPPH (Table 6). In this case, a constant value would be expected for films with different extract solid ratios, which was observed for films with rice husk extract, but not for films with coffee husk solids. The decrease in the EC_{50} values, in terms of mg of solids, in line with the increase in the extract solid ratio, reveals that a part of the minor antioxidant compounds could be degraded during the thermal processing of the films. This would exert a milder effect on the total antioxidant capacity when a greater proportion of extract solids was present in the films. Likewise, a greater antioxidant activity was obtained for films with coffee husk extract than that expected from the values obtained for the isolated extract (Table 2). This suggests changes in the extract composition during the film processing which enhanced the overall antioxidant activity of the extract in the films.

598 Several authors analysed the antioxidant capacity of starch films incorporating active
599 extracts. Starch-chitosan matrices with thyme extract or tannic acid and thyme extract
600 showed EC_{50} values of 3.5 kg film/mol DPPH and 0.9 kg film/mol DPPH, respectively
601 (Talón et al., 2017a). Cassava starch films with 5, 10 and 20% of rosemary extract
602 exhibited a DPPH inhibition of 28.6, 54.4 and 81.9%, respectively (Piñeros-Hernandez
603 et al., 2017). The antioxidant capacity of the films depended on the kind and content of
604 the different active compounds, since their activity varies widely: e.g. the EC_{50} values of
605 tannic acid, resveratrol, ascorbic acid, gallic acid and caffeic acid were 0.0131, 0.7,
606 0.27, 0.08 and 0.1 mol/mol DPPH, respectively (Talón et al., 2017a).
607
608 **4. Conclusions**
609 The incorporation of aqueous extracts and cellulose fibres from rice and coffee husks
610 into thermoplastic starch films leads to improved functional properties as packaging
611 materials, while exploiting these by-products. Both hydrothermal aqueous extracts
612 exhibited antioxidant and antibacterial activity against *L. innocua* and *E. coli*, which
613 provide the films with active properties. The active extracts improved the tensile
614 properties of the starch films, mainly when they were incorporated by substituting 30 %
615 of the plasticizing glycerol. Although the films became less stretchable, a relevant
616 reinforcing effect was observed, with the EM increasing by about 350% for rice and
617 coffee husk extracts. The incorporation of cellulosic fibres from both residues was more
618 effective in films containing extract solids than in net starch films in terms of the
619 reinforcing effect (EM increased by 600% for rice husk solids and 400% for coffee husk
620 solids, respect to net starch films). This can be attributed to a certain compatibilizer
621 effect of the extract compounds that allows for a better integration of the fibres in the
622 starch matrices. Likewise, active extracts led to a 30 % reduction in the WVP of starch

623 films and a 50-85 % reduction in the oxygen permeability, depending on the amount of
624 extract. However, cellulose fibres at 5 % were observed to have no effect on barrier
625 properties. So, the incorporation of extracts and fibres produced films with improved
626 tensile and barrier properties, which, in turn, were less transparent and brown. Then,
627 they could have specific applications in the preservation of foods from light induced
628 oxidation, which may be enhanced by their antioxidant activity. Specific *in vivo* tests
629 would be required to assess their antibacterial action in different food matrices.
630

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639

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Table 1. Mass fraction (Xi, g compound/g dried film) of the different components (Starch: S, Glycerol: Gly, Active extract: A, from coffee (C) or rice (R) husks and cellulose fibres: F, from coffee (CF) or rice husks (RF)) in the different film formulations.

Formulations	X_S	X_{Gly}	X_A	X_F
S	0.7692	0.2308	-	-
S-80:20C	0.7692	0.1846	0.0462	-
S-70:30C	0.7692	0.1615	0.0692	-
S-60:40C	0.7692	0.1385	0.0923	-
S-80:20R	0.7692	0.1846	0.0462	-
S-70:30R	0.7692	0.1615	0.0692	-
S-60:40R	0.7692	0.1385	0.0923	-
S-CF	0.7308	0.2192	-	0.0500
S-70:30C-CF	0.7308	0.1535	0.0658	0.0500
S-RF	0.7308	0.2192	-	0.0500
S-70:30R-RF	0.7308	0.1535	0.0658	0.0500

Table 2. Mean values and standard deviation of antioxidant activity, in terms of Trolox Equivalent (TE), EC_{50} parameter and polyphenol content of the coffee and rice husk hydrothermal extracts (Extract), after the concentration step (Concentrated) and after spray drying of the concentrates (Powder). Minimum inhibitory concentrations (MIC) of coffee and rice husk spray-dried extracts for *E.coli* and *L. innocua* were also included.

		Antioxidant activity (mg TE/g extract solids)	**EC_{50} (mg extract solids/mg DPPH)**	**EC_{50} (mg powder /mg DPPH)**	**Polyphenol content (mg GAE/ g extract solids)**
Coffee husk	Extract	10.5 ± 0.6^{b}	6.41 ± 0.08^{c}	-	62 ± 9^{ab}
	Concentrated	13.6 ± 0.9^{d}	5.63 ± 0.03^{b}	-	70 ± 2^{b}
	Powder	12.5 ± 0.3^{c}	5.37 ± 0.09^{a}	7.66 ± 0.11^{a}	65 ± 5^{ab}
Rice husk	Extract	9.2 ± 0.5^{a}	6.44 ± 0.02^{c}	-	60 ± 3^{a}
	Concentrated	13.0 ± 0.2^{cd}	6.36 ± 0.04^{c}		67 ± 7^{b}
	Powder	12.4 ± 0.4^{c}	5.29 ± 0.04^{a}	7.76 ± 0.05^{a}	66 ± 5^{ab}
		MIC (mg extract solid/mL)		**MIC (mg powder/mL)**	
		E.coli	***L. innocua***	***E.coli***	***L. innocua***
Extract powder	Coffee	35	34	50	48
	Rice	45	35	66	52

Different superscript letters within the same column indicate significant differences among formulations ($p < 0.05$)

805 **Table 3.** Mean values and standard deviation of tensile properties (Elastic modulus:
806 EM, tensile strength: TS and elongation at break: E), water vapour permeability (WVP)
807 and oxygen permeability (OP) of starch films (S) with different ratios of
808 glycerol:extract solids from coffee (C) or rice (R) husks, or cellulose fibres from coffee
809 (CF) or rice (RF) husks, conditioned at 53% RH and 25ºC.

Formulation	EM (MPa)	TS (MPa)	E (%)	WVP ($g \cdot mm \cdot kPa^{-1} \cdot h^{-1} \cdot m^{-2}$)	OP x10^{14} ($cm^3 \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$)
S	77 ± 15^{a1}	5.2 ± 1.6^{a12}	64.9 ± 0.5^{d4}	14.9 ± 0.4^{c34}	10.4 ± 0.1^{e2}
S-80:20C	224 ± 20^{b}	7.0 ± 1.2^{b}	23.5 ± 4.6^{c}	11.3 ± 0.1^{ab}	4.8 ± 0.1^{d}
S-70:30C	344 ± 21^{c3}	9.2 ± 0.4^{cd3}	14.2 ± 1.1^{b1}	11.6 ± 0.4^{b1}	2.4 ± 0.2^{b1}
S-60:40C	516 ± 25^{e}	4.1 ± 1.2^{a}	2.5 ± 1.8^{a}	11.7 ± 1.0^{b}	1.4 ± 0.1^{a}
S-80:20R	234 ± 18^{b}	10.0 ± 1.0^{de}	24.3 ± 4.7^{c}	10.7 ± 0.2^{a}	4.7 ± 0.1^{d}
S-70:30R	348 ± 5^{c3}	12.1 ± 1.1^{e4}	18.3 ± 3.1^{bc2}	10.9 ± 0.4^{ab1}	2.7 ± 0.1^{c1}
S-60:40R	481 ± 22^{d}	7.6 ± 1.4^{bc}	5.4 ± 1.5^{a}	11.1 ± 0.1^{ab}	1.5 ± 0.2^{a}
S-CF	83 ± 6^{12}	4.5 ± 0.2^{1}	30.7 ± 2.7^{3}	14.8 ± 0.9^{34}	11.41 ± 0.3^{2}
S-70:30C-CF	386 ± 25^{4}	11.2 ± 1.2^{4}	12.9 ± 2.6^{1}	13.7 ± 1.7^{23}	2.4 ± 0.2^{1}
S-RF	104 ± 15^{2}	5.8 ± 0.5^{2}	29.7 ± 3.5^{3}	15.6 ± 0.9^{4}	10.5 ± 0.7^{2}
S-70:30R-RF	541 ± 16^{4}	12.1 ± 0.8^{4}	16.4 ± 3.6^{12}	12.2 ± 0.4^{12}	2.1 ± 0.9^{1}

810 Different superscript letters and numbers within the same column indicate significant differences among
811 formulations ($p < 0.05$)
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820 **Table 4.** Lightness (L*), chroma (C*), hue (h*), internal transmittance at 460 nm (Ti)
821 and gloss (60º) values of the films with different ratios of glycerol:extract solids from
822 coffee (C) and rice (R) husks and/or with cellulose fibres from coffee (CF) or rice (RF)
823 husks.

Formulation	L*	Cab*	hab*	T_i (460nm)	Gloss (60º)
S	73.8 ± 1.8^{f5}	12.7 ± 0.9^{a1}	88.8 ± 1.4^{f45}	0.82 ± 0.01^{f5}	28 ± 2^{e5}
S-80:20C	51.5 ± 0.6^{e}	29.5 ± 0.4^{f}	72.5 ± 0.4^{e}	0.49 ± 0.02^{e}	28 ± 3^{e}
S-70:30C	44.1 ± 0.8^{d3}	27.2 ± 1.0^{e6}	68.1 ± 0.5^{d3}	0.32 ± 0.03^{d3}	22 ± 2^{d34}
S-60:40C	36.3 ± 1.4^{a}	21.9 ± 1.6^{d}	58.0 ± 3.5^{a}	0.13 ± 0.05^{a}	16 ± 2^{bc}
S-80:20R	41.1 ± 1.2^{c}	21.9 ± 1.0^{d}	69.7 ± 1.0^{d}	0.34 ± 0.03^{d}	18 ± 3^{c}
S-70:30R	38.3 ± 3.0^{b2}	19.6 ± 1.9^{c4}	65.0 ± 3.0^{c2}	0.26 ± 0.07^{c2}	15 ± 3^{b2}
S-60:40R	35.1 ± 0.7^{a}	17.0 ± 0.8^{b}	61.6 ± 10^{b}	0.17 ± 0.01^{b}	10 ± 3^{a}
S-CF	70.4 ± 0.6^{4}	12.8 ± 0.3^{1}	89.3 ± 0.5^{5}	0.80 ± 0.01^{45}	24 ± 2^{4}
S-70:30C-CF	39.5 ± 1.0^{2}	22.9 ± 2.0^{5}	65.4 ± 1.4^{2}	0.25 ± 0.02^{2}	16 ± 2^{2}
S-RF	68.9 ± 1.2^{4}	14.9 ± 0.4^{2}	87.4 ± 0.6^{4}	0.78 ± 0.01^{4}	20 ± 3^{3}
S-70:30R-RF	35.2 ± 1.3^{1}	17.4 ± 0.9^{3}	63.0 ± 1.9^{1}	0.17 ± 0.02^{1}	11 ± 2^{1}

824 Different superscript letters and numbers within the same column indicate significant differences among
825 formulations ($p < 0.05$)
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Table 5. Mean values and standard deviation of onset and peak temperatures for thermal degradation of TPS films (conditioned at 53% RH and 25 ºC) with different ratios of glycerol: solid extract from coffee (C) and rice (R) husks, with cellulose fibres from coffee (CF) or rice (RF) husks. Mean values and standard deviation of glass transition temperature (Tg) of dry samples were also shown.

	[40-126]ºC		[235-330]ºC		Second heating scan
Samples	**Onset (ºC)**	**Peak (ºC)**	**Onset (ºC)**	**Peak (ºC)**	**Tg (ºC)**
S	45 ± 2^{a1}	88 ± 3^{a12}	264 ± 2^{d3}	299 ± 4^{b2}	96 ± 4^{ab1}
S-80:20C	71 ± 7^{b}	104 ± 4^{c}	250 ± 2^{c}	297 ± 2^{b}	91 ± 6^{a}
S-70:30C	101 ± 8^{c2}	119 ± 8^{d3}	237 ± 2^{a1}	285 ± 8^{a1}	94 ± 11^{a}
S-60:40C	95 ± 4^{c}	124 ± 2^{d}	247 ± 7^{bc}	294 ± 2^{b}	95 ± 3^{ab}
S-80:20R	45 ± 1^{a}	93 ± 3^{ab}	247 ± 5^{bc}	295 ± 1^{b}	108 ± 5^{ab}
S-70:30R	44 ± 1^{a1}	92 ± 2^{ab2}	252 ± 2^{c2}	294 ± 1^{b12}	101 ± 5^{ab}
S-60:40R	44 ± 1^{a}	97 ± 1^{bc}	240 ± 2^{a}	294 ± 1^{b}	108 ± 12^{ab}
S-CF	42 ± 1^{1}	87 ± 2^{12}	265 ± 1^{3}	297 ± 1^{2}	111 ± 10^{2}
S-70:30C-CF	48 ± 4^{1}	99 ± 8^{2}	250 ± 2^{2}	295 ± 1^{2}	95 ± 8^{1}
S-RF	42 ± 1^{1}	77 ± 1^{1}	263 ± 2^{3}	300 ± 1^{2}	97 ± 7^{1}
S-70:30R-RF	47 ± 3^{1}	95 ± 8^{2}	244 ± 8^{12}	333 ± 7^{3}	101 ± 4^{12}

Different superscript letters and numbers within the same column indicate significant differences among formulations ($p < 0.05$)

855 **Table 6.** Antioxidant activity of films containing different ratios of glycerol: extract
856 solids from coffee (C) and rice (R) husks expressed in terms of EC_{50} values.

Formulation	EC_{50} (mg film /mg DPPH)	EC_{50} (mg extract solids /mg DPPH)
S	-	-
S-80:20C	125.1 ± 9.8^{c}	4.7 ± 0.6^{c}
S-70:30C	79.1 ± 6.6^{b}	3.8 ± 0.3^{b}
S-60:40C	48.4 ± 1.3^{a}	3.21 ± 0.09^{a}
S-80:20R	172.5 ± 3.2^{d}	5.7 ± 0.3^{d}
S-70:30R	114.0 ± 0.7^{c}	5.47 ± 0.16^{cd}
S-60:40R	84.8 ± 3.6^{b}	5.3 ± 0.3^{cd}

857 Different superscript letters within the same column indicate significant differences among formulations
858 ($p < 0.05$).
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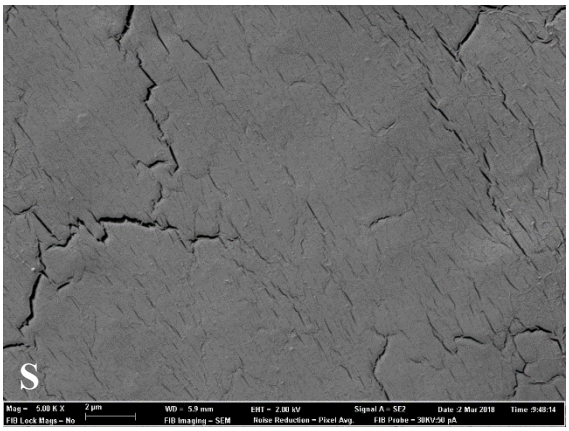
Figure captions

Fig. 1. FESEM micrographs of the surface (left, 5.000X) and cross section (right, 500X) of starch films with extract solids from coffee (S-70:30C) or rice (S-70:30R) husk, cellulose fibres from coffee (S-CF) or rice (S-RF) husk, or both components (S-70:30C-CF; S-70:30R-RF).

Fig. 2. Internal transmittance of studied formulations.

Fig. 3. TGA and DTGA curves of TPS films with different ratios of glycerol:extract solids from coffee (C) and rice (R) husks and with cellulose fibres from coffee (CF) or rice (RF) husks.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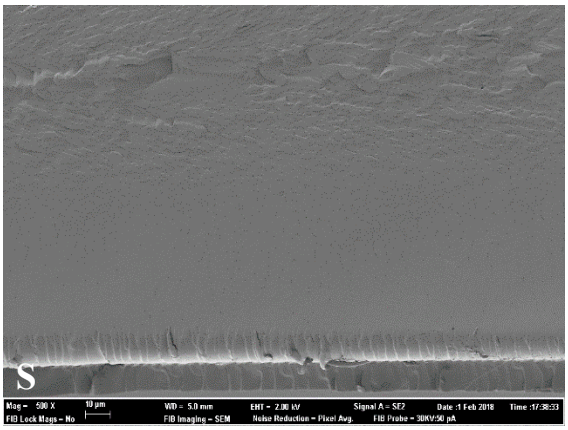
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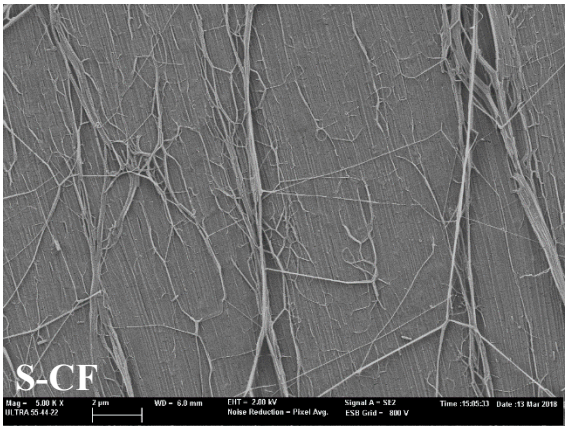
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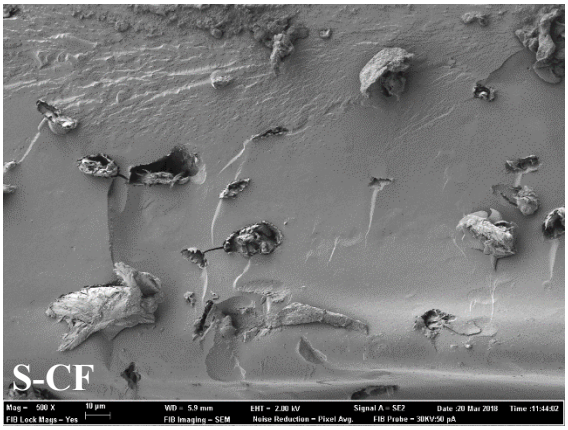
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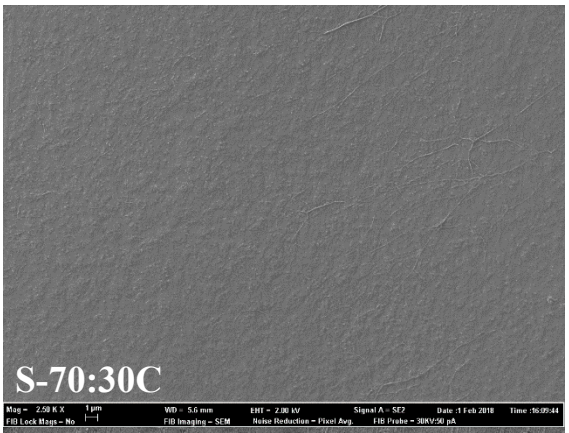
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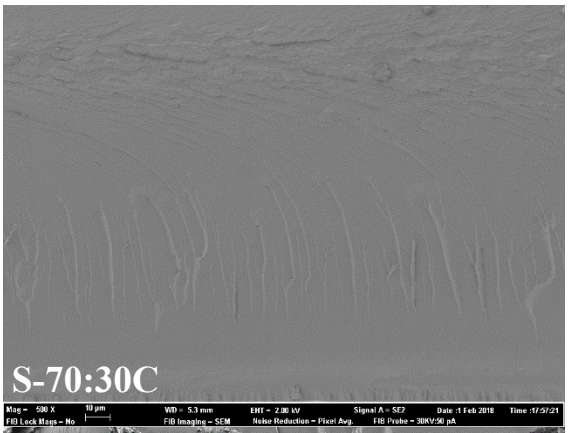
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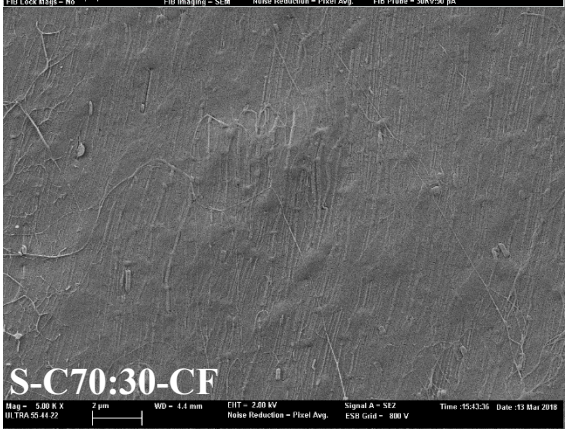
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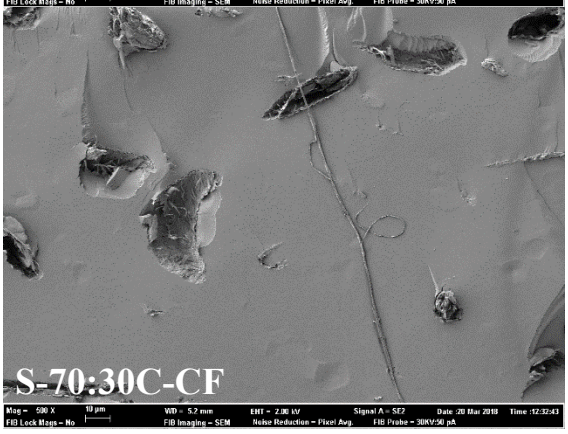

S-70:30C-CF

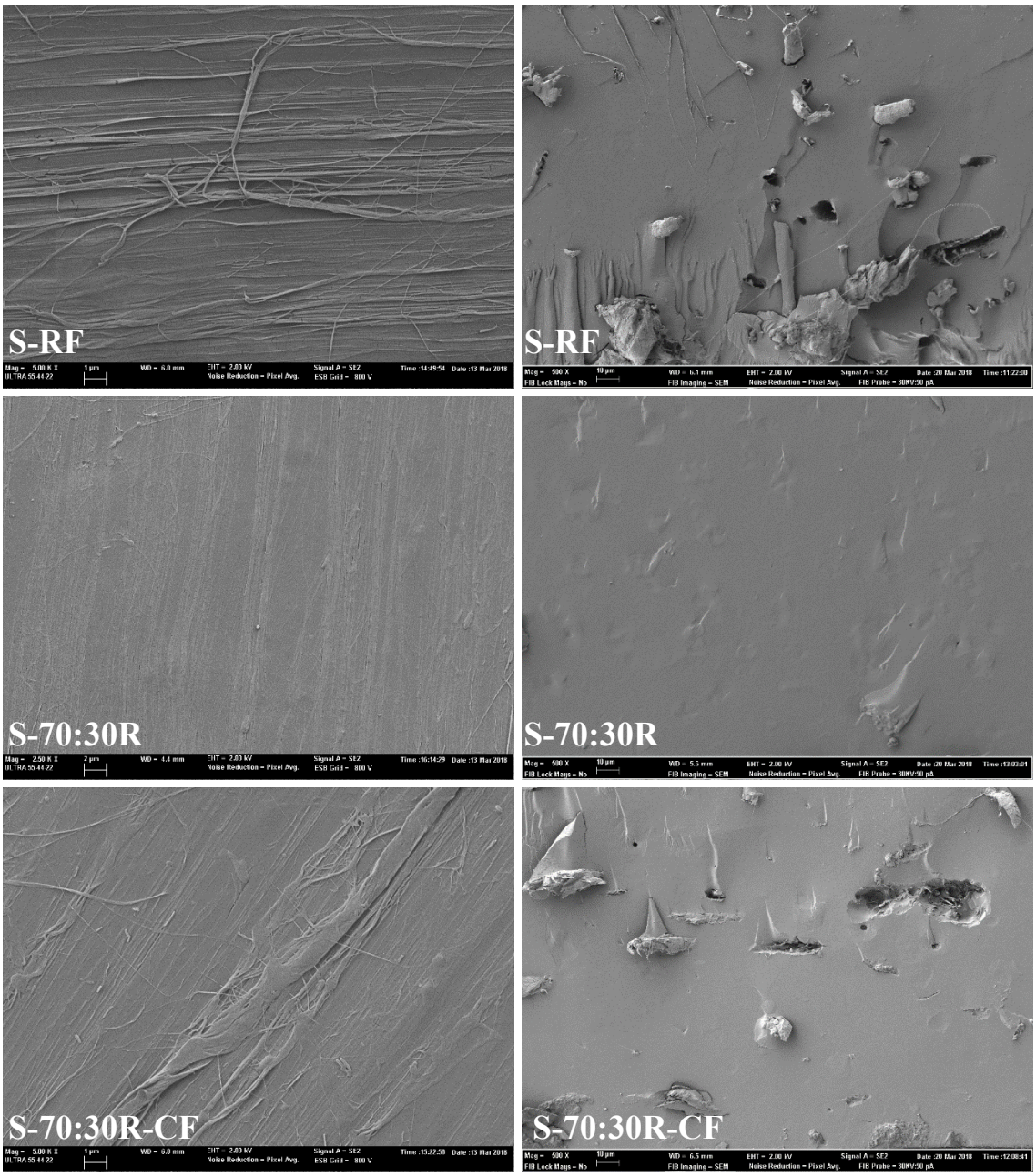
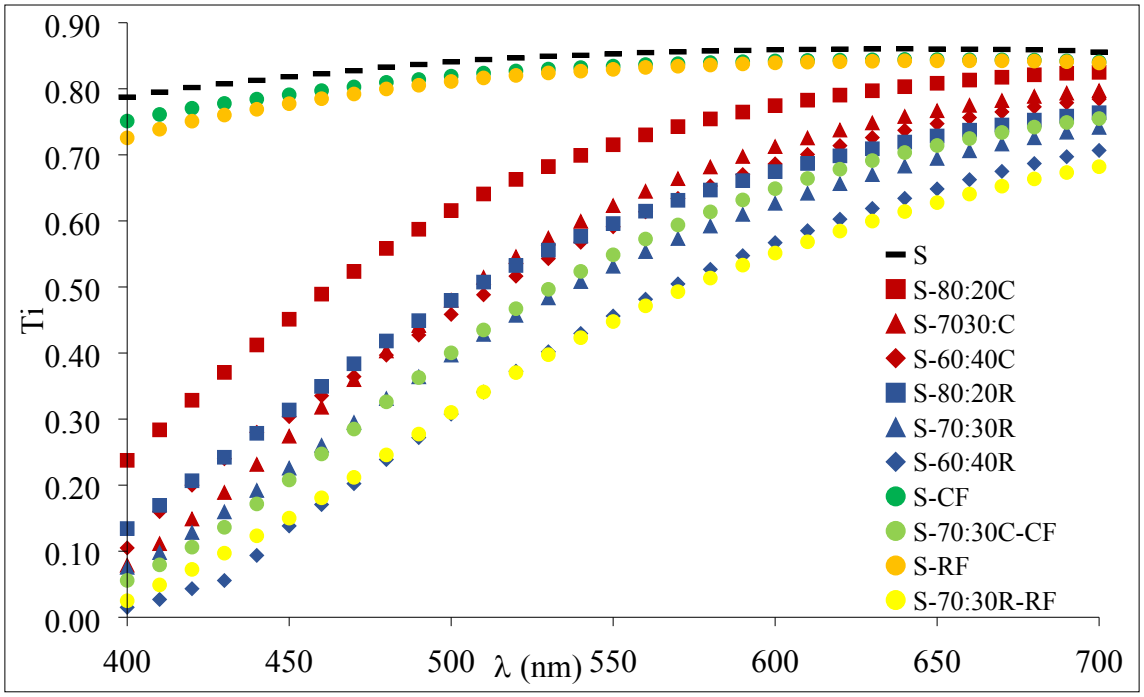


Fig.1.


0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Ti
400
450
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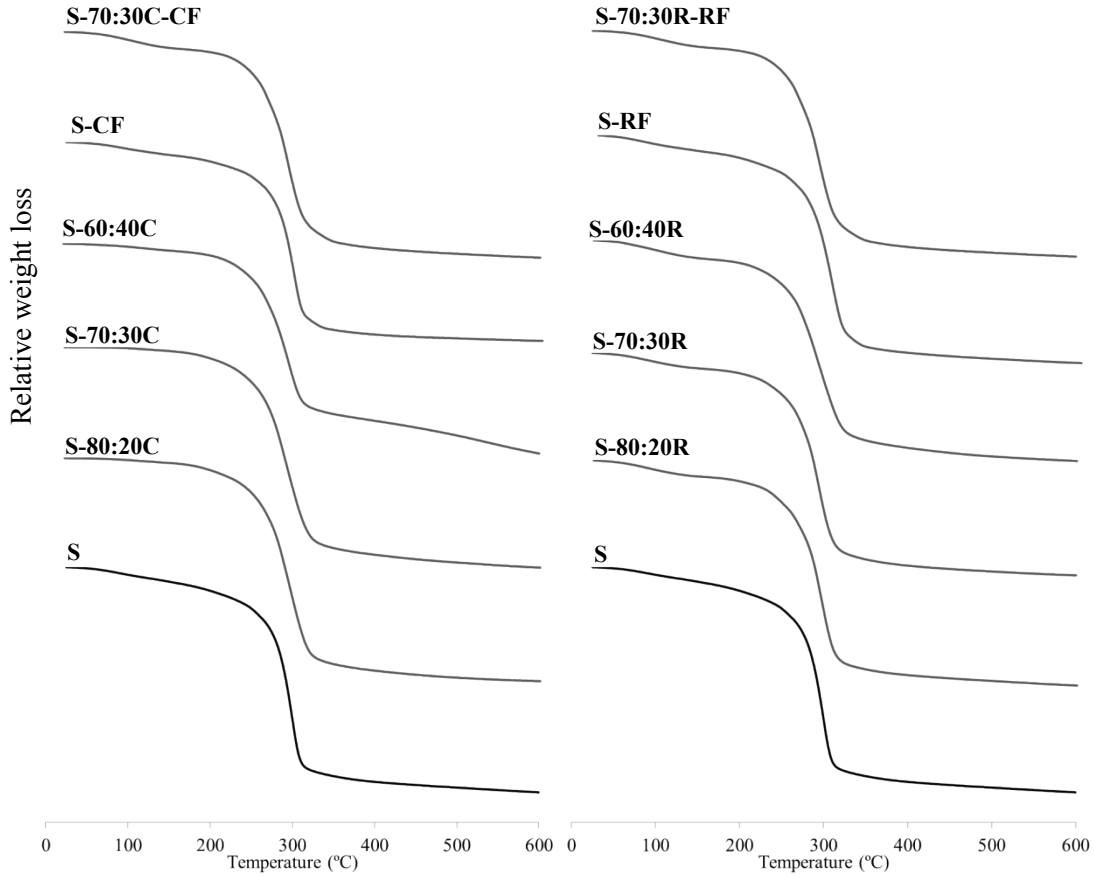
λ (nm)
550
600
650
700
S
S-80:20C
S-7030:C
S-60:40C
S-80:20R
S-70:30R
S-60:40R
S-CF
S-70:30C-CF
S-RF
S-70:30R-RF

Fig. 2.


S-70:30C-CF
S-CF
S-60:40C
S-70:30C
S-80:20C
S
S-70:30R-RF
S-RF
S-60:40R
S-70:30R
S-80:20R
S
Relative weight loss
0
100
200
300
400
500
600
Temperature (ºC)
0
100
200
300
400
500
600
Temperature (ºC)

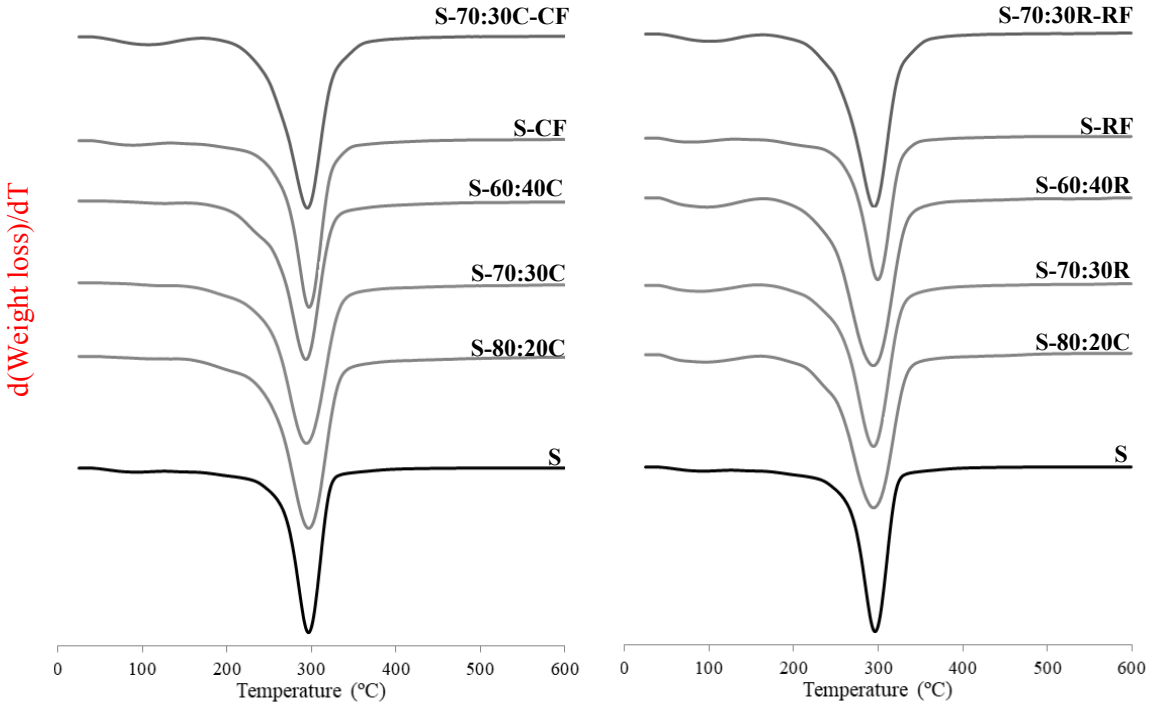

d(Weight loss)/dT
S-70:30C-CF
S-CF
S-60:40C
S-70:30C
S-80:20C
S
0
100
200
300
400
500
600
Temperature (°C)
S-70:30R-RF
S-RF
S-60:40R
S-70:30R
S-80:20C
S
0
100
200
300
400
500
600
Temperature (°C)

Fig. 3.