Structural and physicochemical characterization of thermoplastic corn starch films containing microalgae

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ABSTRACT

This work provides an in-depth analysis on how the addition of different microalgae species (*Nannochloropsis*, *Spirulina* and *Scenedesmus*) affected the structural and physicochemical properties of thermoplastic corn starch biocomposites. Structural characterization was conducted by combined SAXS/WAXS experiments and it was correlated with mechanical and barrier properties of the biocomposites. A water vapour permeability drop of *ca.* 54% was observed upon addition of the different microalgae species. The oxygen permeability and the mechanical properties of biocomposites containing *Spirulina* or *Scenedesmus* were not improved since the presence of microalgae hindered the re-arrangement and packing of the lamellar structure of starch polymeric chains, according to the SAXS results. *Nannochloropsis* caused a great reduction of the matrix rigidity and, the oxygen permeability was also improved.

Therefore, all of these features make the *Nannochloropsis* biocomposites an alternative to generate biodegradable food packaging materials with the additional advantage that they can be easily scaled-up.

KEYWORDS: *Spirulina*, *Scenedesmus*, *Nannochloropsis*, barrier properties, tensile properties, SAXS/WAXS.

1. INTRODUCTION

Bio-based polymers are an important part of the bioeconomy and they represent an alternative towards reducing the great amount of plastic waste produced. Nowadays, bioplastics are used in an increasing number of market sectors such as packaging, catering products, agriculture/horticulture or electronic devices. Packaging represents the largest field of applications for bioplastics which is estimated in 1.6 million tonnes of the total bioplastics market in 2016 (European Bioplastics Association). Interestingly, it is expected that the global bioplastics market for the packaging industry will grow until 2022 at an annual average rate of 17% and, in terms of end-use categories, food packaging makes up the second largest end-use sector, followed by beverage bottles (Business-standard). This is the reason why biopolymers have shown enormous growth in recent years in terms of both technological developments and commercial applications.

Among bio-based polymers, polysaccharides have attracted great attention due to their large availability, renewability and biodegradability (Alcantara, Darder, Aranda, & Ruiz-Hitzky, 2014). In particular, starch-based films have been researched thoroughly for the last decades due to their attractive combination of low price, wide availability and high purity. Nevertheless, new advances are still possible through the incorporation of new bio-based resources, that could enhance the biocomposites overall performance (Basiak et al., 2017, Fabra et al., 2016, Medina-Jaramillo et al., 2016).

However, there is a need to look for alternative sources of biopolymers which do not compete with food since, according to the latest news from the Food and Agriculture Organization of the United Nations (FAO), in the first half of this century, global demand for food, feed and fibre is expected to grow by 70%. In this regard, marine resources could

serve as a source of biopolymers which could partially replace other biopolymers naturally obtained from biomass.

Even though the most recent interest in the use of algae is focused on producing biodiesel, marine resources have the potential for further exploration in a wide variety of uses and applications other than production of biodiesel. Algae and microalgae are an interesting natural resource because they proliferate quickly, they grow in a wide range of environments and they do not interfere with food production (Katiyar et al., 2017). Moreover, algae research could provide an environmentally friendly solution for serious global environmental threats like those concerning to greenhouse gas emissions.

To the best of our knowledge, little information exists in the literature in which algae have been explored as a new source for plastic production and this topic is just starting to develop among scientists who are now addressing the challenge of making high-quality and inexpensive plastics from algae (Fabra et al., 2017, Torres et al., 2015, Yan et al., 2016).

The use of macro- or microalgae as a reinforcing agent of biodegradable matrices could provide several benefits even when their use is compared to other bio-based materials. Marine natural resources could replace part of other bio-based materials, which compete with food and food applications and consume large amounts of petroleum products in their life cycle. Additionally, by using algae species in the development of bio-based packaging materials, the sense of a circular bioeconomy would increase since those resources coming from the sea would be put back into the sea where initially they were partially obtained and where they could be degraded and absorbed by the sea.

This work is aimed at understanding how the addition of different microalgae species (*Nannocloropsis*, *Scenedesmus* and *Spirulina*) modify the properties of thermoplastic corn starch matrices produced by the melt-mixing process. The effect of microalgae types on

thermal, barrier and tensile properties of the developed biocomposites have been deeply analysed.

2. MATERIALS AND METHODS

2.1 Materials

Corn starch (28% of amylose) was kindly supplied by Roquette (Roquette Laisa España, Valencia, Spain). Microalgae (*Nanochloropsis gaditana*, *Spirulina* and *Scenedesmus*) were kindly supplied by Fundación Cajamar (Almería, Spain). Glycerol was purchased from Panreac Quimica, S.A. (Castellar Del Vallés, Barcelona, Spain). All products were used as received without further purification.

2.2 Development and characterization of microalgae-containing starch films

Thermoplastic corn starch films (TPCS) were prepared by dispersing corn starch and glycerol in water, using a polymer:glycerol:water ratio of 1:0.3:0.5 (w/w/w) and, the dispersion was subsequently melt-mixed in a Brabender Plastograph internal mixer at 130 °C and 60 rpm for 4 min to obtain a homogeneous batch. The mixture was then spread evenly on Teflon and placed in a compression mould (Carver 4122, USA) at a pressure of 30,000 lbs and 130 °C for 4 min.

Similarly, films containing microalgae were prepared by adding 20% of microalgae respect to the corn starch mass to the starting solution, prior to incorporating them to the Brabender Plastograph internal mixer.

Samples were equilibrated for one week at 54% relative humidity using oversaturated solutions of magnesium nitrate-6-hydrate (Panreac Quimica, SA, Castellar del Vallés, Barcelona) and 25 °C prior to barrier, mechanical, morphological and SAXS/WAXS

analyses. The thickness of the samples was measured with a Palmer digital micrometre to the nearest 0.0025 mm at six random positions.

2.3 Scanning Electron Microscopy (SEM)

The microstructural analysis of the films was carried out by means of a Scanning Electron Microscope (Hitachi S-4800). Two different samples, 0.5 cm² in size, of each film were cryo-fractured after immersion in liquid nitrogen and randomly broken to investigate the cross-section of the samples. Cryo-fractured samples were mounted on aluminium stubs and fixed on the support using double-side adhesive tape. Finally, samples were gold–palladium coated and observed using an accelerating voltage of 10 kV and a working distance of 12 mm.

2.4 Optical properties (transparency and colour)

The transparency of the biocomposites was determined, in triplicate, through the surface reflectance spectra from 400 to 700 nm with a spectrophotometer CM-3600d (Minolta Co, Tokyo, Japan), with a 10 mm illuminated sample area, as previously described by Fabra et al., 2009. The Kubelka-Munk theory was applied in order to determine the transparency. Internal transmittance (T_i) of the biocomposites was quantified using Eq. (1) in which R_0 is the reflectance of the biocomposite on an ideal black background and parameters a and b were calculated by Eqs. (2) and (3), where R is the reflectance of the sample layer backed by a known reflectance R_g .

$$T_i = \sqrt{(a - R_0)^2 - b^2} \quad (1)$$

$$a = \frac{1}{2} \left(R + \frac{R_0 - R + R_g}{R_0 R_g} \right) \quad (2)$$

$$b = \sqrt{(a^2 - 1)} \quad (3)$$

Moreover, CIE-L* a* b* coordinates (CIE, 1986) were obtained by the infinite reflection spectra of the samples, using D65 illuminant/10 observer.

2.5 Water vapour permeability (WVP)

WVP was measured according to the ASTM E96/E96M-10 gravimetric method and using Payne permeability cups (3.5cm diameter, Elcometer SPRL, Hermelle/s Argenteau, Belgium). Oversaturated solution of sodium chloride (Panreac Quimica, SA, Castellar del Vallés, Barcelona) was placed in each cup to expose the film to 75% RH on one side. Once the films were secured, each cup was placed in an equilibrated relative humidity cabinet at 0% RH and 25°C. The cups were weighed periodically (± 0.0001 g). Cups with aluminium samples were used as control samples to estimate solvent loss through the sealing. Water vapour permeation rate was calculated from the steady-state permeation slopes obtained from the regression analysis of weight loss data vs. time, once the steady state had been reached, divided by the film area (the exposed area was 9.6×10^{-4} m²) and, the weight loss was calculated as the total cell loss minus the loss through the sealing. Permeability was obtained by multiplying the permeance by the average film thickness.

2.6 Oxygen permeability (OP)

OP was calculated from oxygen transmission rate (OTR) measurements recorded, in triplicate, using an OXTRAN Model 2/21 ML Mocon (Lippke, Neuwied, Germany). The

samples were previously purged with nitrogen in the humidity equilibrated samples, before exposure to an oxygen flow of 10 mL min⁻¹. The exposure area during the test was 5 cm² for each sample. In order to obtain the oxygen permeability, film thickness and gas partial pressure were considered in each case. Experiments were carried out at 23°C and 54% RH.

2.7 Mechanical properties

A Mecmesin MultiTest 1-í universal test machine (Landes Poli Ibérica, S.L., Barcelona, Spain) equipped with a 100-N static load cell was used for the tensile testing, according to ASTM standard method D882-09 18 (ASTM, 2010b). Tensile strength (TS), elastic modulus (EM), and elongation at break (EAB) were determined from the stress-strain curves, estimated from force-distance data obtained for the different samples (1 cm wide and 8 cm long). Eight replicates were analyzed per film formulation. Equilibrated specimens were mounted in the film extension grips of the testing machine and stretched at 50 mm min⁻¹ until breaking. The experiments were carried out 54% RH and 24°C.

2.8 Small and Wide Angle X-ray scattering (SAXS/WAXS) and X-ray diffraction (XRD)

Combined small and wide angle X-ray scattering (SAXS and WAXS, respectively) experiments were carried out in the Non Crystalline Diffraction beamline, BL-11, at ALBA synchrotron light source (www.albasynchrotron.es). The energy of the incident photons was 12.4 KeV or equivalently a wavelength, λ , of 1 Å. The SAXS diffraction patterns were collected by means of a photon counting detector, Pilatus 1M, with an active area of 168.7x 179.4 mm², an effective pixel size of 172 x 172 μm^2 and a dynamic range of 20 bits. The sample-to-detector distance was set to 6425 mm, resulting in a q range with a maximum value of $q = 0.23 \text{ Å}^{-1}$. Additionally, the WAXS diffraction patterns were collected by means

of a 3 CCD detector Rayonix LX255-HS with an active area of 85 x 255 mm², an effective pixel size of 44 x 44 μm² and a dynamic range of 16 bits. In this case, the sample-to-detector distance was set to 143.6 mm, corresponding to a maximum q value of 8.37 Å⁻¹. This detector was tilted with a pitch of 27.3 degrees. The exposure time, common to both detectors, was optimized in order to maximize the signal to noise ratio while avoiding detector saturation. After some trials, 0.1 seconds was determined as optimum. The data reduction was treated by pyFAI python code (ESRF) ¹⁴, modified by ALBA beamline staff, that is able to do on-line azimuthal integrations from a previously calibrated file. The calibration files were created from well-known standards, i.e. Silver behenate (AgBh) and Cr₂O₃ for SAXS and WAXS respectively. The intensity profiles were then represented as a function of q (SAXS) and 2θ (WAXS) using the IRENA macro suite within Igor procedures. Furthermore, the X-ray diffraction patterns of the raw microalgae were collected on a D5005 Bruker diffractometer. The instrument was equipped with a Cu tube and a secondary monochromator. The configuration of the equipment was θ–2θ, and the samples were examined over the angular range of 5°–60° with a step size of 0.02° and a count time of 200 s per step. Peak fitting was carried out in Igor using Lorentzian functions to fit the diffraction peaks. The crystallinity index X_C was determined by the method reported by Wang et al. (Wang et al.,2007):

$$X_C (\%) = \frac{\sum A_{Crystal}}{A_{Total}} \times 100 \quad (3)$$

where A_{Total} is the sum of the areas under all the diffraction peaks and $\sum A_{Crystal}$ is the sum of the areas corresponding to the crystalline peaks.

2.9 Statistical Analysis

The statistical analysis of data was carried out by means of IBM SPSS Statistics software (v.23) (IBM Corp., USA) through the analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) was used at the 95% confidence level for multiple comparison tests

3. RESULTS

3.1 Morphology of the developed composite films

The microstructure analysis of the biocomposites provides relevant information about the arrangement of the different components, which in turn has a great impact on the biocomposites' properties.

Figure 1 shows the cross-section of the neat TPCS film and its biocomposites with microalgae. Neat TPCS showed a smooth appearance, consistent with the formation of a compact arrangement of polymer chains, whereas a coarse aspect was appreciated for the microalgae-loaded films, suggesting that the polymer chain packing was hindered by other components from microalgae. In the case of *Nannochloropsis* microalgae, small spherical white spots (~ 2.6 - $2.7 \mu\text{m}$) were observed uniformly distributed throughout the cross-section, indicating that microalgae remained intact after the melt-mixing process. This particle size matches with the intact *Nannochloropsis* microalgae as it has been previously observed by SEM (see Supplementary material of Martínez-Sanz et al., 2017). In contrast, several irregularities can be discerned in the cross-section of the biocomposites containing *Spirulina* or *Scenedesmus* microalgae. These features reveal that both microalgae could have been disrupted during the melt-mixing process due to the more labile properties of their cell walls.

Figure 1. Cross-section images of neat starch (S) film and biocomposites containing *Nannochloropsis* (S-Nanno), *Spirulina* (S-Spiru) and *Scenedesmus* (S-Scen).

3.2 Optical properties

Figure 2 shows the overall appearance of the TPCS film and the obtained biocomposites. As observed, only biocomposites prepared with *Spirulina* preserved a good contact transparency, although it was slightly diminished when compared to the neat starch matrix. In contrast, either *Nannochloropsis* or *Scenedesmus* turned the materials less transparent and darker than *Spirulina*.

Figure 2. Contact transparency images of the films. From “left to right”: S, S-Nanno, S-Spiru, S-Scen.

Optical properties were also assessed quantitatively and the spectral distribution curves of internal transmittance are plotted in Figure 3. Firstly, the transparency of the biocomposites were evaluated by means of the internal transmittance (Ti), considering that an increase in the internal distribution of transmittance is ascribed to an increase in transparency. As observed, the highest Ti values and, thus, the greatest transparency, was found for the neat TPCS. The presence of microalgae significantly reduced the transparency of biocomposites, showing lower Ti values ($\leq 40\%$) in all the wavelength range considered, which indicates that biocomposite films were less homogeneous and transparent than the neat TPCS matrix due to the light scattering generated by the presence of microalgae. When biocomposite films were compared, those containing *Nannochloropsis* or *Scenedesmus* were less transparent (Ti values below 20% in all the wavelength range considered) than their

counterparts prepared with *Spirulina*, in agreement with the contact transparency images. Therefore, biocomposite films containing microalgae can be considered to have low transparency. This can be ascribed to the fact that transmittance and haze of the samples are mainly affected by differences in refractive indices between the TPCS matrix and the different compounds which constitute the film structure.

From the reflectance spectra of an infinite thickness film, Lightness (L^*), hue (h^*_{ab}) and Chroma (C^*_{ab}) were obtained as well as the total color differences (ΔE) with respect to the neat TPCS film (Table 1). As expected, color parameters significantly varied by the incorporation of microalgae species. The color of biocomposite films became darker (lower L^*), and less vivid (lower C^*) with a brown-green hue. The estimated color differences (ΔE) were calculated between TPCS films and the biocomposites containing microalgae. The values ranged between 48 and 57, thus indicating that there were relevant color differences as compared to the neat TPCS film.

Figure 3. Spectral distribution of internal transmittance (T_i) of the neat TPCS film and its biocomposites.

Table 1. Color parameters of the neat TPCS film and its biocomposites.

Sample	L*	a*	b*	C _{ab} *	h _{ab} *	ΔE
S	77.7 (0.4) ^a	14.5 (0.1) ^a	16.8 (0.0) ^a	22.2 (0.1) ^a	49.0 (0.7) ^a	-
S-Nanno	25.1 (0.5) ^b	0.3 (0.1) ^b	0.4 (0.1) ^b	0.5 (0.5) ^b	55.0 (1.4) ^a	56.5 (0.5) ^a
S-Spiru	32.5 (0.4) ^c	-4.6 (0.1) ^c	10.8 (0.1) ^c	11.7 (0.1) ^c	113.0 (0.0) ^c	48.7 (0.3) ^b
S-Scen	24.4 (0.2) ^b	0.8 (0.1) ^b	2.3 (0.2) ^d	2.5 (0.2) ^c	71.0 (0.0) ^d	56.2 (0.4) ^a

Data reported are mean values and standard deviation (in parentheses). Mean values with different letters in the same column represent significant differences ($p < 0.05$) among the samples according to ANOVA and Tukey's multiple comparison tests.

3.3 Nanostructural characterization

The nanostructure of starch in the different films was investigated by means of WAXS (to probe the crystalline structure) and SAXS (to assess the arrangement of crystalline and amorphous domains into lamellar structures).

Figure 4A shows the WAXS patterns from the neat starch and the biocomposite films loaded with the three different microalgae species. The neat starch film presents scattering peaks located at *ca.* 12.9°, 17.0°, 18.6°, 19.8°, 22.5°, 25.0° and 27.1° 2 θ . Although the native corn starch is known to possess A-type crystallinity (Martínez-Sanz et al., 2017), the WAXS profile of the starch film is characteristic of the V-type crystalline allomorph, in particular, the V_H type (Van Soest, 1996), hence suggesting that a crystalline transition from a double helical to a single helical configuration takes place as a result of the melt mixing process and subsequent storage at 54%RH. This phenomenon has been previously reported for several types of starch which were subjected to thermal processing at temperatures lower than 180°C and water contents higher than 10% (Bhatnagar and Hanna, 1994; Ortega-Toro et al., 2015, Van Soest et al., 1996). The three biocomposite films show the same crystalline patterns as the pure starch film, indicating that the presence of the microalgae does not induce any change in the crystalline allomorph. Interestingly, the samples containing *Spirulina* and *Scenedesmus* show additional scattering peaks located at *ca.* 20.7°, 29.2°, 29.6°, 31.0°, 31.7°, 35.3°, 36.2°, 38.1°, 39.6°, 41.9°, 47.2°, 49.0°, 50.4°, 52.0° and 54.9° 2 θ for *Spirulina* and 29.7°, 36.1°, 39.6°, 43.3°, 47.7°, 48.6° and 57.7° 2 θ for *Scenedesmus*. To investigate the origin of these peaks, the XRD patterns of the raw microalgae submitted to a cell wall disruption process were also collected (Fig. 1 supplementary material). By comparing the patterns from the raw microalgae with those of the biocomposite films, it is evident that the additional scattering peaks detected in the films with *Spirulina* and *Scenedesmus* arise from the crystalline components, such as fatty acids, present in the raw microalgae. In fact, it is well-known that both microalgae species are rich in lipids (Wu et al., 2013, Markou, 2012, Seyhaneyildiz Can, Koru and Cirik,

2017). Interestingly, none of the crystalline peaks detected in the disrupted *Nannochloropsis* microalgae are apparent in the corresponding biocomposite film. In agreement with previous studies (Fabra et al., 2017, Martínez-Sanz et al., 2017) and supporting the SEM characterization in this work, this result confirms that the tough cell wall in the *Nannochloropsis* species does not undergo disruption during processing and therefore, the cell components are not released towards the starch matrix during melt-mixing. The crystallinity values from the raw microalgae, calculated from the XRD patterns (Figure 1 Supplementary material), were $60.3 \pm 0.3\%$ for *Nannochloropsis*, $58.2 \pm 0.3\%$ for *Spirulina* and $56.7 \pm 0.4\%$ for *Scenedesmus*. The neat starch film presented a crystallinity value of $20.5 \pm 3.4\%$, whereas values of 22.9 ± 0.5 , 26.3 ± 2.5 , and 27.0 ± 2.2 were attained for the biocomposite films loaded with *Nannochloropsis*, *Spirulina* and *Scenedesmus*, respectively. Thus, it seems that the components released from the *Spirulina* and *Scenedesmus* microalgae have a minor effect on increasing the starch crystallinity. A similar effect has been previously reported for starch casting films loaded with *Nannochloropsis* subjected to intense sonication treatments to disrupt the cell walls (Fabra et al., 2017).

Figure 4. WAXS patterns of the neat starch film and its biocomposites containing microalgae.

The WAXS results indicate that although the crystalline structure of starch is destroyed during the melt-mixing process, starch is able to re-crystallise to some extent upon storage. To assess the effect of processing and storage on the structure at the long-range order, i.e., the lamellar structure of starch granules, SAXS experiments were also carried out and the results are shown in Figure 5. The neat starch presents a broad shoulder which is centred at $ca. 0.057 \text{ \AA}^{-1}$, corresponding to a real distance of $ca. 11 \text{ nm}$. The characteristic lamellar feature from native starch is usually detected as a well-defined peak located at q values corresponding to $ca. 9\text{-}10 \text{ nm}$ (López-Rubio et al., 2007, Vermeylen et al., 2006). The broad shoulder detected in the starch film has been previously reported for extruded starches (Chanvrier et al., 2007; López-Rubio et al., 2007) and is indicative of a re-

organisation of starch into a heterogeneous semicrystalline structure after the melt blending and storage process. This shoulder is not visible in the SAXS patterns from the biocomposite films, suggesting that somehow the presence of microalgae hinders the re-arrangement and packing of the lamellar structure of starch polymeric chains upon storage after melt mixing. In fact, a recent study on the effect of microalgae on the starch gelatinisation process showed that the presence of microalgae limited water accessibility towards the interior of starch granules, hindering the arrangement of amylose and amylopectin chains into lamellar structures (Martínez-Sanz et al., 2017). The biocomposite loaded with *Nannochloropsis* presented a very broad shoulder in the low q region (corresponding to real distances of 70-200 nm) and a sharp peak located at $0.126 \text{ \AA}^{-1}$, corresponding to a real distance of *ca.* 5 nm. A similar scattering peak has been previously observed in starch casting films loaded with *Nannochloropsis* provided that the microalgae cell walls were not disrupted (Fabra et al., 2017). Thus, it was hypothesised that this peak may arise from the arrangement of structured layers in the complex cell wall from this species. The absence of scattering features in the biocomposites with *Spirulina* and *Scenedesmus* supports the fact that cell wall rupture occurs during the melt blending process and that cell components released interfere with the re-organisation of starch chains to form semi-crystalline lamellae.

Figure 5. SAXS patterns of the neat starch film and its biocomposites containing microalgae.

3.4 Mechanical properties

The mechanical response of the neat TCPS and biocomposites is greatly affected by the glassy-amorphous state of the materials and by the presence of crystalline forms. In the studied films, tensile strength curves were obtained for biocomposite films. Fig. 4 shows the stress-strain curves for the different film samples and the corresponding values of tensile parameters are shown in Table 2. The first clear observation is that the presence of *Nannochloropsis* caused a great reduction in the matrix rigidity due to the discontinuities introduced in the biopolymer network as could be seen in the SEM image (Fig. 1). Discontinuities could infer a loss of the cohesion forces and mechanical resistance, hence

reducing the elastic modulus (E) of the biocomposite. Neat TPCS and the biocomposite film prepared with *Nannochloropsis* showed a plastic deformation, indicative of the rubbery state of the biopolymer networks. However, a glassy state of the matrices can be deduced in biocomposite films containing *Spirulina* or *Scenedesmus* since they did not show plastic deformation and they broke when elastic deformability was exceeded. This could be linked with the disruption degree of the microalgae cell wall which was greater for both *Spirulina* and *Scenedesmus* after the melt-mixing process. Similar effects have previously been reported when lipid-extracted algal biomass (LEA) obtained from *Nannochloropsis salina* was used as the filler for biocomposite synthesis with poly(vinyl alcohol) (PVA). They observed that the incorporation of LEA into PVA matrix reduced tensile strength and elongation at break of PVA/LEA biocomposites (Tran et al., 2016). While one would expect a matrix plasticization arising from lipid release from the microalgae, from the WAXS results (cf. Figure 4), V-type crystals consisting on amylose-lipid complexes (Singh et al., 2002, Jiménez et al., 2013) and other crystalline components released from the microalgae were present in the matrix. This lipid-amylose complexation had a strong impact on the structure and mechanical properties of the biocomposite films. These crystalline components resulted in increased Young modulus, but apparently reduced the interactions between the amorphous parts of the amylose and amylopectin chains required for plastic deformation. In contrast, *Nannochloropsis* microalgae have a resistant cell wall which was not disrupted during thermal processing and the intact microalgae could be discerned in the cross-section of the biocomposite film (see Figure 1). In this case, as suggested by SAXS, the presence of intact cells prevented the formation of lamellar structures at the nanoscale size range, fact which could explain the decrease in the Young

modulus, but did not interfere in biopolymer entanglements, thus not affecting extensibility of the materials.

Tensile strength (TS) values were very similar for the neat TPCS and biocomposites films containing microalgae, although the neat TPCS was slightly more resistant due to the greater cohesion forces in the more homogeneous network.

Figure 4. Stress-strain curve for neat starch (S) film and biocomposites films containing *Nannocloropsis* (S-Nanno), *Spirulina* (S-Spiru) and *Scendesmus* (S-Scen) equilibrated at 53% relative humidity.

Table 2. Tensile parameters ^(*), oxygen and water vapour barrier properties^(**) of the neat starch (S) film and biocomposites films containing *Nannocloropsis* (S-Nanno), *Spirulina* (S-Spiru) and *Scendesmus* (S-Scen).

Sample	EM (MPa)	TS (MPa)	E (%)	WVP 10 ¹³ (Kg Pa ⁻¹ s ⁻¹ m ⁻¹)	OP 10 ¹⁹ (m ³ m m ⁻² s ⁻¹ Pa ⁻¹)
S	1039 (88) ^a	14.3 (2.1) ^a	2.1 (0.2) ^a	3.45 (0.05) ^a	4.3 (0.8) ^a
S-Nanno	800 (76) ^b	11.7 (1.2) ^a	2.1 (0.3) ^a	1.68 (0.16) ^b	1.2 (0.3) ^b
S-Spiru	1276(149) ^a	13.5 (1.1) ^a	1.2 (0.1) ^b	1.66 (0.15) ^b	7.9 (0.5) ^c
S-Scen	1257 (99) ^a	10.8 (1.2) ^a	0.9 (0.1) ^b	1.58 (0.24) ^b	6.8 (0.5) ^c

^(*) Elastic modulus (EM), tensile strength (TS), and Elongation at break (EAB) of films equilibrated at 53 % relative humidity. Different superscripts within a column indicate significant differences among formulations ($p < 0.05$). Data reported are mean values and standard deviation (in parentheses).

^(**) Water vapor permeability (WVP), Oxygen permeability (OP).

3.5 Barrier properties

Table 2 summarizes the measured water vapour permeability values for the neat TPCS and its biocomposites. Water vapour permeability was in agreement with values previously reported for starch processed by melt blending (Ortega-Toro et al., 2015) but lower than those previously reported for cast films (Jiménez et al., 2012, Müller et al., 2011), as the melt compounded biocomposites produced in the present work had higher overall material density. Using the compression-moulding method, biopolymer chains become more compacted and with higher crystallinity than through the casting process (Fabra et al., 2017). All the biocomposites incorporating microalgae had significantly lower water

permeability, with an average 54% drop as compared to the neat TPCS film. In the case of the biocomposites loaded with *Spirulina* and *Scenedesmus*, this effect could be attributed to (i) the cell wall disruption upon processing, contributing to the lipid extraction and providing a more hydrophobic character and (ii) the slight crystallinity increase in the biocomposite films (as evidenced by the WAXS results). In the case of the *Nannochloropsis*-loaded films, the well-dispersed crystalline microalgae could act as filling and blocking agent, hence, reducing the water vapour transfer through the biocomposite film.

Oxygen permeability (OP) was measured at 53% RH and the results are gathered in Table 3. The OP value for the neat TPCS film is in agreement with that reported in the literature for melt-compounded TPCS films (Ortega-Toro *et al.*, 2015). The first observation to highlight is that biocomposites prepared with *Nannochloropsis* microalgae had reduced oxygen permeability as compared to the TPCS. However, OP values were higher for the biocomposites prepared with *Spirulina* or *Scenedesmus* microalgae, with no significant differences between both. These findings can be ascribed to the presence of lipids released from the cell wall. Their hydrophobic character may promote the increase in the oxygen permeability due to their chemical affinity, favouring the gas solubility in the biopolymer matrix (Park *et al.* 1996, Bertan *et al.*, 2005, Fabra *et al.*, 2012). Thus, the incorporation of both *Spirulina* or *Scenedesmus* microalgae into the TPCS matrices modifies their barrier properties, enhancing the water vapour barrier, but reducing the barrier to oxygen. In contrast, the presence of well-dispersed *Nannochloropsis* microalgae contributed to reducing the OP values by up to 72% as it did for water vapour, probably due to the tortuosity created by the intact microalgae within the polymer network.

CONCLUSION

From amongst the microalgae species used as additives, *Nannochloropsis* is the most promising alternative for generating biodegradable food packaging materials by replacing part of the corn starch matrix. Their presence led to great reduction in the oxygen and water vapour barrier properties, while *Spirulina* and *Scenedesmus* only improved water vapour permeability of the neat starch matrices. The mechanical response of the biocomposites was greatly affected by the microalgae type and, more concretely, by the disruption degree of their cell wall. Whereas, neat TPCS and the biocomposite film prepared with *Nannochloropsis* showed a plastic deformation, a glassy state of the matrices was observed in biocomposite films containing *Spirulina* or *Scenedesmus* (as a result of them breaking when the elastic deformability was exceeded). This could be linked with the disruption of the microalgae cell walls in the biocomposites with *Spirulina* and *Scenedesmus* during the melt-mixing process, as demonstrated by microscopy and scattering analysis, resulting in amylose-lipid complexes and other crystalline components which on one hand provided a stiffer matrix in terms of elastic deformation, while on the other hand, they hampered proper interchain bonding needed for plastic deformation.

All of these aspects make the biocomposites containing *Nannochloropsis* a promising alternative to produce biobased food packaging materials with the additional advantage that they can be easily scaled-up or even processed by other techniques such as extrusion.

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