



Use of Residual Yeast Cell Wall for New Biobased Materials Production: Effect of Plasticization on Film Properties

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Abstract

The use of renewable resources to develop food contact materials, such as proteins or polysaccharides, and the use of industrial residues for alternative applications are trending topics for researchers and the industry. Yeast cell wall (YCW) is a very rich waste from the yeast extract industry. Due to this, the aim of this work is to develop new biodegradable films based on residual YCW and the study of the effect of plasticization on films properties. Residual YCW was used as base matrix and different concentrations of glycerol (0, 15, 25 and 35 wt%) were tested to obtain casted films. Homogeneous and yellow-brownish films, which allow seeing through them, were obtained from the YCW. Total soluble matter demonstrated that glycerol enhanced solubility of films but glycerol was retained in the polymer matrix. TGA studies indicated that YCW films exhibited substantial degradation at temperatures above 180 °C. FTIR spectra of the casted films were representative of yeast cell wall material and SEM photographs showed that cell wall maintained their shape after film formation. As expected, Young's modulus and tensile strength values were decreased with the increasing amount of glycerol. However, elongation at break was not increased further with higher concentration of plasticizer and the addition of 15 wt% of glycerol seemed to be enough to improve mechanical properties. The linear increment of water vapour permeability with glycerol concentration was produced by the increase in water solubility in the film. Therefore, based on solubility in water, mechanical, and barrier properties, it is possible to propose yeast cells residues as film-forming material for biodegradable film developments.

Keywords Industrial residues · Yeast cell wall · Biodegradable films · Plasticization · Characterization · Water vapour permeability

Introduction

Biopolymers extracted directly from biomass, such as polysaccharides and proteins, have received much attention in recent years for the development of biodegradable materials for food packaging and are considered as potential substitutes of non-biodegradable polymers, due to their renewable origin and

biodegradability (Song et al. 2011). The most successful systems to perform biodegradable materials by using these biopolymers are casein, gelatine, starch, alginates, chitosan, soy protein, and cellulose, among others (Arrieta et al. 2014; Jost and Stramm 2016; Han et al. 2017). Indeed, blends of proteins and polysaccharides were also described, such as blends of quinoa protein-chitosan films (Abugoch et al. 2011) or carboxymethyl cellulose/soy protein (Su et al. 2010), among other mixtures, where the best properties of each compound are gained, giving rise to a complex network with specific properties (Moine et al. 2004). Yeast biomass films were also described as polymer base for film multicomponent formulations (Delgado et al. 2016). These microorganisms are unicellular fungi composed by proteins (40.5%), carbohydrates (40.2%), lipids (7%) and nucleic acids (6.9%) (Sceni et al. 2009) and are used mostly for food-grade applications such as ethanol production and bakery. However, yeast industries are not only focused on the production of yeast for such applications but for the production of yeast extract. This product is obtained after mechanical or enzymatically hydrolysis of yeast

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cells and then, the intracellular material is isolated and used for food supplements, flavour enhancement or for culture media. The broken cell wall is discarded as waste or used for animal feeding. Figure 1 shows a scheme of yeast extract production. Thus, the use of the discarded cell wall (rich in polysaccharides and proteins) in alternative applications, such as biodegradable films production could be a solution to waste generated by the agro industries, giving added value to the residues.

Yeast cell wall (YCW) represents almost 40% of the cell and is conformed mostly by β -glucan and mannoproteins. β -1,3-glucans are present in microorganisms and have the same structural function as cellulose in plants. Fungal glucans are conformed by a main chain of β -(1,3)-D-glucose residues to which β -(1,6)-D-glucose side groups are attached (branched), which give to the molecule interesting properties like its bio-activity. In the yeast cell wall, β -glucans present a triple helix conformation, unique in the polysaccharide family (Lipke and Ovalle 1998; Peltzer et al. 2018). There are many sources of β -glucan, such as bacteria, fungi, yeast, mushrooms and higher plants (Peltzer et al. 2018). Morgan (2002) suggested the vegetal origin β -glucan potential to be used as film-forming agent and a matrix for encapsulation of active compounds. While Skendi et al. (2003) confirmed the ability of cereals β -glucan to form films with mechanical properties comparable to conventional synthetic films. These authors found that mechanical properties were affected by the amount of plasticizer (water, polyols) and the molecular weight of the polysaccharide. Literature related to the use of microbial β -glucan as a source for biodegradable films is scarce; however, the potentiality of this biopolymer for these purposes is getting the interest of many researchers. It already described the development of biofilms based on β -glucan from yeast with promising characteristics (Novák et al. 2012). Mechanical deformation tests were performed to yeast β -glucan-casted films in order to understand the influence of amount of plasticizer, relative humidity and the stretching rate on mechanical properties (Blahovec et al. 2011). However, the isolation of β -glucan is quite expensive or many solvents should be applied for its isolation and purification. Indeed, during the glucan isolation, mannoproteins are discarded, losing their functionality and total yield. For this reason, it would be interesting to avoid β -glucan isolation and use the whole cell wall of yeast for multicomponent applications, keeping proteins from cell wall. Some applications of the YCW, isolated after breaking the cell and removing the cytoplasm, were described for pharmaceutical applications by the development of edible coatings. These coatings were characterized and presented high tensile strength and good barrier properties against oxygen (Kasai et al. 2000). Vegetal origin β -glucan was also studied in combination with protein from same biomass for the development of biodegradable films. Razzaq et al. (2016) produced biodegradable films from barley biopolymers (β -glucan and

proteins) and demonstrated that there was a strong network established through interactions between polysaccharide and proteins chains when the extraction was performed in alkaline media. Thermal properties were increased due to this interaction and mechanical properties were comparable to those of many commodities materials such as polyethylene.

Many additives could be added to film-forming dispersion in order to modify mainly mechanical and final properties of biodegradable films. Plasticizers are the most important additives to be added to film formulation because they can efficiently enhance the properties of films conformed by biopolymers. Films based on biopolymers are very brittle without plasticizers, though they are necessary for film manipulation after drying or avoid cracking and defects (Jost and Stramm 2016; Arrieta et al. 2013). The most used plasticizers in these matrices are polyols such as sorbitol, glycerol, propylene glycol and ethylene glycol due to their affinity to polysaccharides and proteins because of their hydrophilic nature (Vanin et al. 2005). The solution casting method is only adequate for laboratory use and if the production of films should be scaled to perform food packaging such as food wraps or pouches, the addition of plasticizers allows to undergo glass transition of the biopolymers, facilitates deformation, and so, the processability of the material without thermal degradation (Hernandez-Izquierdo and Krochta 2008). However, the addition of plasticizer can also affect film characteristics such as gas and water vapour permeability and solute diffusion (García et al. 2000), as well as sorption characteristics (Farahnaky et al. 2013). Therefore, it is important to study the effect of plasticizers in polymer matrices.

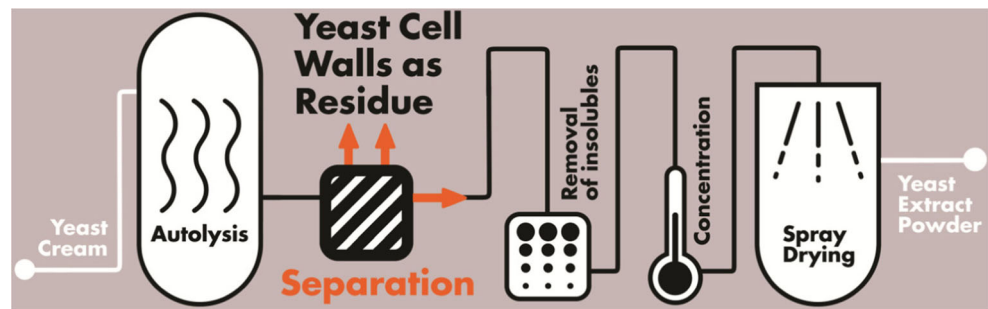
The aim of this work is to develop new biodegradable films based on residual YCW and characterize them in order to find the best formulation related to glycerol concentration.

Materials and Methods

Sample Preparation

YCW was supplied by Calsa (Lanús, Argentina) and it was obtained as a residue from the yeast extract industry. The YCW was washed with distilled water to eliminate any soluble residue. A dispersion of 5 wt% was prepared in distilled water and the pH was adjusted to 11 with NaOH 0.1 N. Dispersion was thermally treated at 90 °C during 20 min and cooled quickly. Then, dispersion was homogenized at 12000 rpm during 3 min with Ultraturrax T-25 (IKA, Germany). Glycerol was added at different concentrations to obtain formulations with 0, 15, 25 and 35 wt% dry basis (db). Films were casted in Petri dishes at 35 °C and 50% relative humidity (rh) during 20 h. Finally, films were peeled and conditioned at 43% rh for further characterization.

Fig. 1 Scheme of yeast extract production



Thickness, UV-Visible Measurements and Visual Appearance

Films thickness was measured with a digital micrometer (3109-25E, Insize Co., China). Measurements were taken at ten different locations of the films. The mean thickness was used in mechanical properties calculations and permeability. The visual appearance of films was checked by taken photographs. The absorption spectra in the 200–700 nm region of plasticized YCW films were investigated by means of a UV-Visible spectrophotometer (T60, PG Instruments, UK).

Total Soluble Matter

Total soluble matter (TSM) was expressed as the percentage of dry film matter solubilized after 24-h immersion in distilled water. Therefore, TSM (%) express the solubility in water of the YCW films. Measurements were performed by the immersion of a film sample (2 cm × 2 cm) in 30 mL of distilled water containing sodium azide 0.01% at 25 ± 2 °C for a period of 24 h with continuous agitation at 100 rpm. Before the immersion, the samples were dried for 1 week in silica to determine the initial dry mass. The final dry mass was determined by drying the samples at 105 °C for 1 h or constant weight once the immersion experiment was finished. All determinations were performed in triplicate. TSM (%) was calculated by using Eq. 1.

$$TSM(\%) = 100 \times \left(\frac{m_0 - m_f}{m_0} \right) \quad (1)$$

where m_f is the final constant weight after drying at 105 °C and m_0 is the initial dry mass.

Thermal Characterization

Thermogravimetric analyses (TGA) were performed in a TA Instrument Q-500. Approximately, 8 mg of each sample were weighed in platinum balance and heated from 30 to 550 °C at 10 °C min⁻¹, under nitrogen atmosphere (flow rate 60 mL min⁻¹). Initial degradation temperature (T_{ini}) was determined at 15% of weight, while temperatures at the

maximum degradation rate (T_{max}) for each degradation stage were determined from peaks of derivative curves. All determinations were performed in duplicate.

Fourier Transformed Infrared Spectroscopy

Fourier transformed infrared spectroscopy (FTIR-ATR) spectra were recorded in the range of 4000–400 cm⁻¹ by using a Shimadzu IR-Affinity (Shimadzu Co., Japan) equipped with an attenuated total reflectance diamond module (GladiATR, Pike Technologies, USA). Spectra were obtained, in duplicate, at 48 scans and with 4-cm⁻¹ resolution. A blank spectrum was obtained before each test to compensate the humidity effect and the presence of carbon dioxide in the air by spectra subtraction.

Scanning Electron Microscopy of YCW Films

Surface of YCW films was analysed by using a TableTop Microscope (TM3000, Hitachi, Japan) at 5 kV, with no further preparation. Images were registered at ×300, ×2000, and ×4000 magnification.

Mechanical Properties

Mechanical properties were carried out at room temperature by using a Universal Test Instrument Megatest TC-500 series II (Megatest, Argentina) equipped with a 30 N cell load and experiments were performed at 5 mm min⁻¹. Specimens 46 mm × 18 mm were tested and deformation at break (EB, %); elastic modulus (E, MPa) and tensile strength (TS, MPa) were calculated from the resulting stress–strain curves as the average of eight measurements. Before testing, samples were conditioned at 53% rh.

Experimental Water Vapour Permeability

Experimental water vapour permeability (P_w^{exp}) of the YCW-films was measured using the cup method described in ASTM-E96 (2016). Films were sealed on the cups containing a saturated solution of BaCl₂ providing 90% rh. Test cups were placed in desiccators maintained at constant temperature of 24 °C and 10% rh provided by a saturated solution of

NaOH. A fan was used to maintain uniform conditions inside the desiccators over the films according to recommendations from previous authors (McHugh et al. 1993; Gennadios et al. 1994). Weight loss measurements were taken by weighing the test cup using an analytical balance ($\pm 10^{-3}$ g) and plotted versus time, when steady state (straight line) was reached, 10 h further of experiment were registered (Delgado et al. 2018). The P_w^{exp} ($\text{g s}^{-1} \text{m}^{-1} \text{Pa}^{-1}$) was calculated according to Eq. 2.

$$P_w^{\text{exp}} = \frac{1}{A} \left(\frac{\Delta m}{\Delta t} \right) \frac{L}{\Delta p_w} \quad (2)$$

where $\frac{1}{A}$ is the water vapour flux through the film was calculated from the slope of a linear regression of weight loss versus time and A is the effective area of exposed film ($2.2 \times 10^{-3} \text{ m}^2$). L is the film thickness, and $\Delta p_w = (p_{w2} - p_{w1})$ (in units of Pa) is the differential water vapour partial pressure across the film. p_{w1} is the partial pressure of water vapour at the film surface outside the cup, and p_{w2} is the partial pressure of water vapour at the film surface inside the cup corrected for air gap distance of $5 \times 10^{-3} \text{ m}$ between saturated solution of BaCl_2 level and the film position (Gennadios et al. 1994). Experiments were repeated three times for each type of film.

Water Sorption Isotherms

Water sorption isotherms allow to study the equilibrium water uptake as a function of rh or water activity ($a_w = \% \text{ rh}/100$) of the environment. Sorption isotherms of films were determined gravimetrically according to standard procedure described by Delgado et al. (2016), using saturated salts. The evolution to equilibrium at each moisture condition was monitored by weighting periodically the samples until the constant weight was achieved. Weight of samples was made using analytical balance ($\pm 10^{-4}$ g). The water content h , given in units of grams of water per gram of dried mass was evaluated as function of a_w . Experiments were performed in duplicates. Isotherms were fitted using the Guggenheim-Anderson-DeBoer (GAB) model through Eq. 3 (Guggenheim 1966):

$$h(a_w) = \frac{N \cdot c \cdot k \cdot a_w}{\left[(1 + (c-1)k \cdot a_w)(1 - k \cdot a_w) \right]} \quad (3)$$

where N is the monolayer water content (g of water per g of dried mass) related to the number of primary binding sites of water molecules, c is a parameter related to the difference between the chemical potential of the water molecules in the upper layers and in the monolayer, and k is a factor related to the difference between the chemical potential of the water in pure liquid state and in the upper layers. c can also be interpreted as a parameter related to the force of the water

binding to monolayer, and k as the capability to water binding to multilayer (Salvay et al. 2003).

Kinetics of Water Sorption

Hydration kinetics experiments permit to obtain solubility of water in the matrix film and the diffusion coefficient. Fully dried film samples were placed in Petri dishes and located into the sorption containers at 90% rh. Hydration experiments were performed with one surface of the film exposed to the wet atmosphere. A fan was used to maintain uniform conditions inside the desiccators. The samples were removed at specific intervals of time and weighed for data collection. Experiments were carried out in duplicates. The water content h as function of time t was fitted with Fick's mass transport differential law solution in Fourier series, for one-dimensional diffusion of water in a plane infinite sheet as was described by Crank (1975) and done by Eq. 4.

$$h(t) = h_\infty \left\{ 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp \left[-D_w^{\text{eff}} (2n+1)^2 \frac{\pi^2 t}{4L^2} \right] \right\} \quad (4)$$

where h_∞ is the water content at equilibrium at 90% rh, L is the film thickness in units of metres, and D_w^{eff} is the apparent diffusion coefficient in units of square metres per second.

According to the Henry's law, the solubility of water S_w in the film at 90% rh is given by Eq. 5:

$$S_w = \frac{h_\infty}{P_s} \rho_{d.f} \quad (5)$$

where S_w is given in units of grams of water per cubic metre of dried mass per Pascal, $\rho_{d.f}$ is the dry matter density of the film, and $p_s = 2687.3 \text{ Pa}$ is the vapour saturation pressure corresponding to the atmosphere of 90% rh at 24 °C.

In the initial period of sorption, the water uptake as function of time $h(t)$ can be expressed as displayed in Eq. 6 (Shankar 1981):

$$h(t) = 2h_\infty \left(\frac{D_w^i t}{\pi L^2} \right)^{1/2} \quad (6)$$

Short time lapse (STL) model done by Eq. 6 was proposed to fit sorption data when $h(t)/h_\infty < 2/3$. This equation gives a value of initial diffusion coefficient D_w^i that take into account the diffusion of water in the film during the initial period of sorption (first 12 h of the experiments).

Theoretical Water Vapour Permeability

The water transport through polymeric films do not occur through pores but through a mechanism that consists in the water sorption on the polymeric matrix surface followed by diffusion of water molecules and finally desorption from the

other surface of the film (Debeaufort et al. 1994; Roy et al. 2000). Therefore, water transport through polymeric films depends on the hydration or solubility of water in the film, as well as the water mobility within itself. When there are no pores, faults or film punctures, water vapour permeability P_w is defined as displayed in Eq. 7 (Rogers 1985; Roy et al. 2000).

$$P_w = D_w S_w \quad (7)$$

where P_w is given in units of grams per second per metre per Pascal, and S_w and D_w can be obtained by Eqs. 5 and 6, respectively (Delgado et al. 2018).

Statistical Analysis

Results were expressed as mean \pm standard deviation and were analysed by analysis of variance (ANOVA) using R Software (R Foundation for Statistical Computing, Vienna, Austria). Means were tested with the honestly significant differences Tukey's test for mean comparison, with a significance level of $p < 0.05$.

Results and Discussion

Film Thickness, Visual Aspect and Optical Properties

The average thickness of the films was around $200 \pm 20 \mu\text{m}$. Yellow-brownish films, which allow seeing through them, were obtained from the yeast cell wall as it is showed in Fig. 2a, for YCW plasticized with 25 wt% of glycerol. No differences were found in the visual aspect for the rest of the formulations. Homogeneous and no cracks films were obtained for all plasticizer concentration and control sample (non-plasticized). However, non-plasticized samples were more brittle than the plasticized ones as expected for biobased films. Figure 2b shows the UV spectra of YCW-25%Gly sample as an example of the rest. Visible and UV light absorption by materials in contact with food is crucial to maintain its quality, mainly in those products containing vitamins, lipids or edible oils which must be protected from UV radiation due to the sensitivity of these compounds (Auras et al. 2004). Ultraviolet (UV) band is between 100 and 400 nm, while the visible spectrum is between 400 and 700 nm. YCW films showed almost no transmission in the UV-B region (315–280 nm), and also in the UV-A (400–315 nm) the transmission was very low. Most of the films in contact with food tend to be transparent since they are more attractive for consumers due to the possibility to see the product through the package. However, these transparent films present some drawbacks regarding UV and visible absorption/transmission and some UV absorbers should be added to the film formulation to protect the packed

product. Films obtained in this work produced a UV blocking effect in the UV-A, UV-B and visible spectra region, with no need to add any compound in this respect.

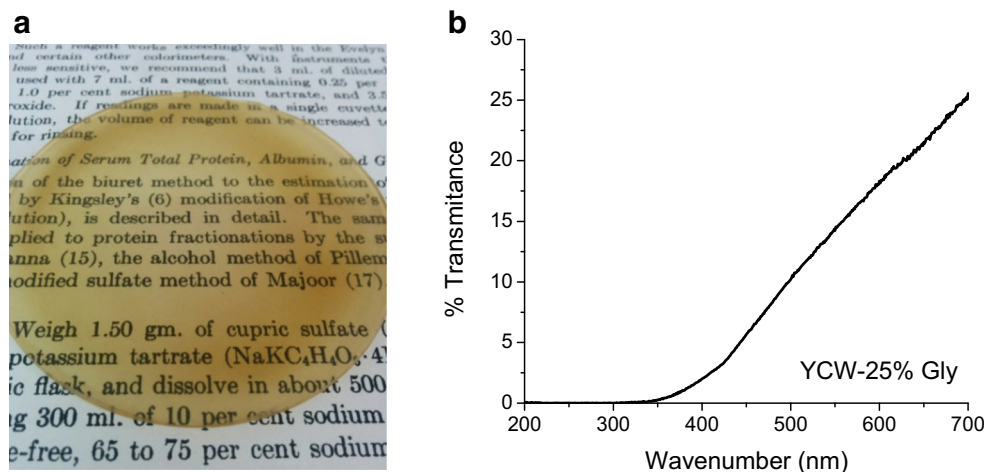
Total Soluble Matter

TSM (%) in water of YCW films is similar to the concept of films' solubility in water. This parameter is important since it would be necessary to keep the integrity of the films during use when they are in contact with high-humidity products. On the other hand, there are applications where the solubilisation of the films in water is needed like encapsulation of drugs and flavours (Bertuzzi et al. 2007; Farahnaky et al. 2013), this is the reason of the importance of this determination. As shown in Fig. 3a, TSM (%) was increased significantly, as expected, with the addition of plasticizer ($p < 0.05$). Total soluble matter was 18% for non-plasticized YCW films and increased to 25% in sample YCW-15%Gly, reaching higher values in plasticized samples with 25 and 35% of glycerol (YCW-25%Gly and YCW-35%Gly, respectively). No significant differences were observed for the highest glycerol concentrations ($p > 0.05$). Compared to the solubility in water to other biobased films, YCW present lower values of this parameter, for example TSM(%) values reported for plasticized gelatin was around 100% and about 75% for wheat starch with 25% of glycerol (Tanada-Palmu and Grosso 2003). However, glycerol enhanced solubility of films in water due to the solubility in water of the plasticizer itself, or maybe due to the fact that high concentrations of plasticizers reduced cohesiveness polymer matrix, exposing polymer chains to water interaction, enhancing its solubility (Ghanbarzadeh et al. 2011). Due to the linearity of the correlation between the differences of TSM (%) versus the glycerol concentration (Fig. 3b), it could be said that the increment in solubility is due to the solubilization of the plasticizer itself (Hu et al. 2009). Considering glycerol solubility in water is infinite, differences in TSM (%) of plasticized samples respect the unplasticized one suggested that not all the glycerol is solubilized, since the amount of solubilized material is increased as the half of the concentration of glycerol. This could be explained as the glycerol is strongly interacting with polymer chains or the plasticizer is retained and encapsulated inside the cell wall, as it is well known that yeast cells are able to encapsulate different types of compounds (Bishop et al. 1998; Tanada-Palmu and Grosso 2003).

Thermal Characterization

TGA curves presented a multistep degradation profile as shown in Fig. 4a, b. Degradation of plasticized samples occurred at lower temperatures than non-plasticized one, this is due to the increase in the chain mobility and by the high vapour pressure of glycerol (Guerrero et al. 2010). There are

Fig. 2 Yeast cell wall film with 25% glycerol. **a** Photo of the film. **b** UV-Vis spectra of edible films



three main degradation zones. The first one, from 30 to 120 °C, corresponds to moisture evaporation and small molecules degradation (this zone is not shown in the graph). Second zone, from 120 to 350 °C, with T_{max} centred between 310 and 312 °C, which corresponded to the complete degradation of proteins and pyrolysis of β -glucan skeleton (Wu et al. 2013). This zone also present a small shoulder, around

220–250 °C, related to the initial degradation of proteins. Third zone, between 350 and 550 °C, with T_{max} at 388 °C, is not specified but may correspond to the final degradation of macromolecules. The first zone, YCW-35%Gly sample started before the first zone, due to the higher water retention of these samples. TGA results indicated that YCW films exhibit substantial thermal degradation at temperatures above 180 °C.

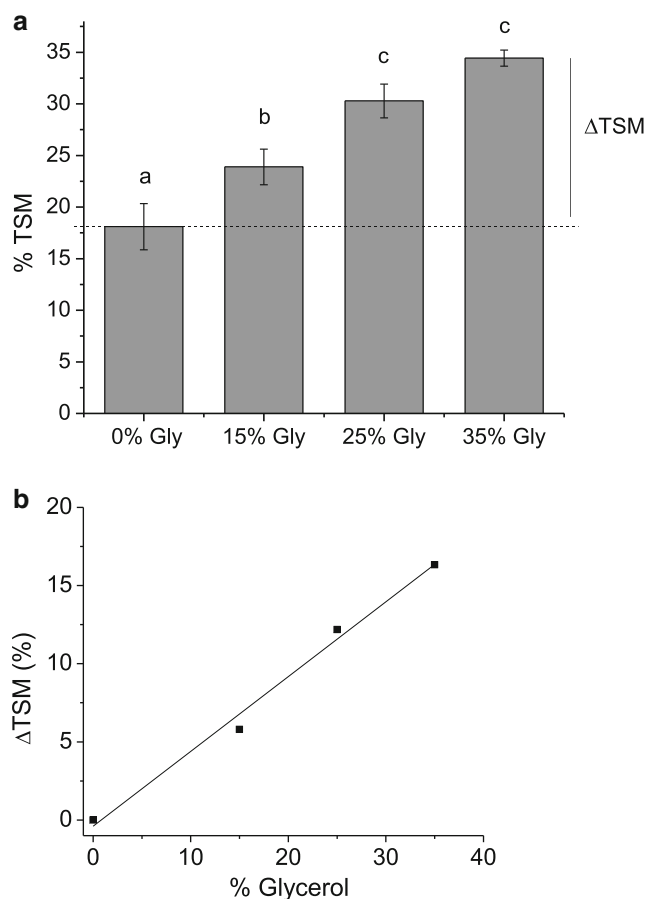


Fig. 3 **a** Total soluble matter (%) tested in distilled water of YCW films. **b** Δ TSM (%) respect glycerol concentration

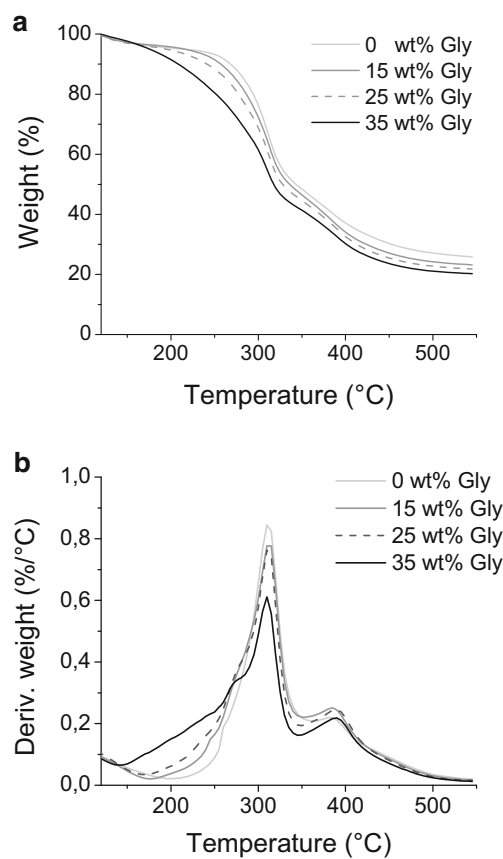


Fig. 4 Thermogravimetric curves. **a** Mass loss versus temperature. **b** Derivate mass loss versus temperature

FTIR-ATR Spectroscopy

Figure 5 shows the spectra of plasticized and non-plasticized YCW films. As it can be seen, FTIR spectra are representative of yeast cell wall material (Novák et al. 2012; Bzducha-Wróbel et al. 2014; Šandula et al. 1999), i.e. the spectra showed the absorption band from stretching of C–C and C–O–C bonds around 1160 cm^{-1} . The big absorption band centred at 1030 cm^{-1} and shoulders at 1060 and 972 cm^{-1} could be attributable to the ring and C–OH side groups stretching vibration of sugars and the band at 891 cm^{-1} , only observed for unplasticized film and is assigned to β -glycosidic (C–H) bond (Hromádková et al. 2003). Plasticized samples showed a band at 918 cm^{-1} due to the stretching of C–OH of glycerol (Wu et al. 2013; Ahmed et al. 2010). From the spectra, it is possible to confirm the presence of proteins in the cell wall by the occurrence of amide I and II absorbance at 1637 and 1540 cm^{-1} , respectively. The broad band centred at 3275 cm^{-1} indicates OH stretching vibration of hydroxyls (polysaccharides and glycerol) and water. Bands at 2923 and 2857 cm^{-1} correspond to CH, CC and CH_2 from CH_2OH stretching vibrations from sugars. FTIR-ATR spectra presented differences in plasticized and non-plasticized samples in the amide I and II regions may be explained as a change in protein conformation with the addition of the plasticizer (Gao et al. 2006). FTIR spectra showed that amide I and II decreased their intensity with the addition of plasticizer due to the amount of protein in plasticized samples is lower and NH group may interact with glycerol, changing its conformation (Gao et al. 2006). In addition, the absorption intensity ratio of both bands was modified with the incorporation of the plasticizer, the absorption intensity in the amide II band decreased with respect to the amide band I, due to the sensitivity of NH group from amide II to hydrogen bonding. Shoulder at 1128 cm^{-1} is shifted to lower wavenumbers may be due to the interaction of the polysaccharides with glycerol. The increase in the absorption bands between 1450 and 1200 cm^{-1} , corresponds to the presence of glycerol (Arrieta et al. 2013). A band at 1746 cm^{-1} was observed in all the samples and can be associated to the carbonyl from phospholipids. The same was observed in alkaline oat β -glucan films and the authors suggested that correspond to the formation of ester bonds between carbonyl and hydroxyl group in the films (Razzaq et al. 2016).

Scanning Electron Microscopy

Figure 6 shows the microscopy images of YCW plasticized with 25% of glycerol. It was possible to observe that the shape, oval to elliptical, of the cell wall was maintained after thermal and casting treatments. However, at $\times 4000$ (Fig. 6c), some distorted shapes and agglomerations were observed. Same results were described by other authors that subjected

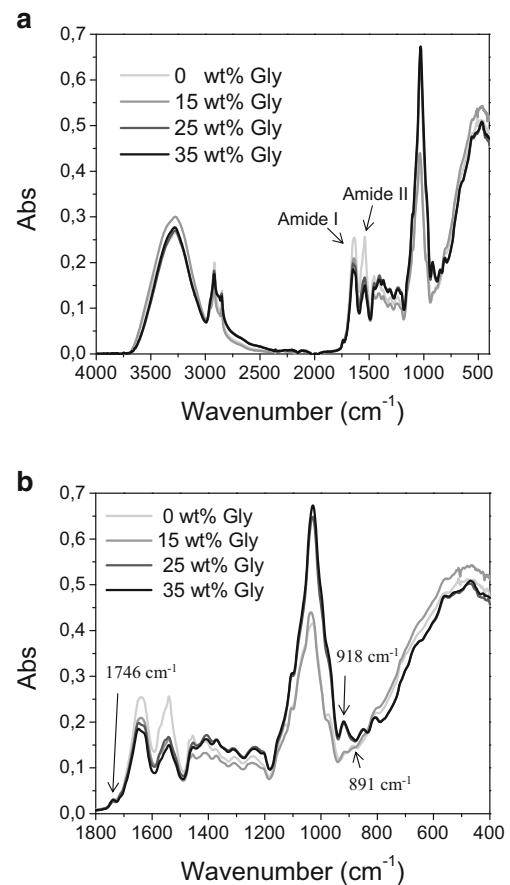


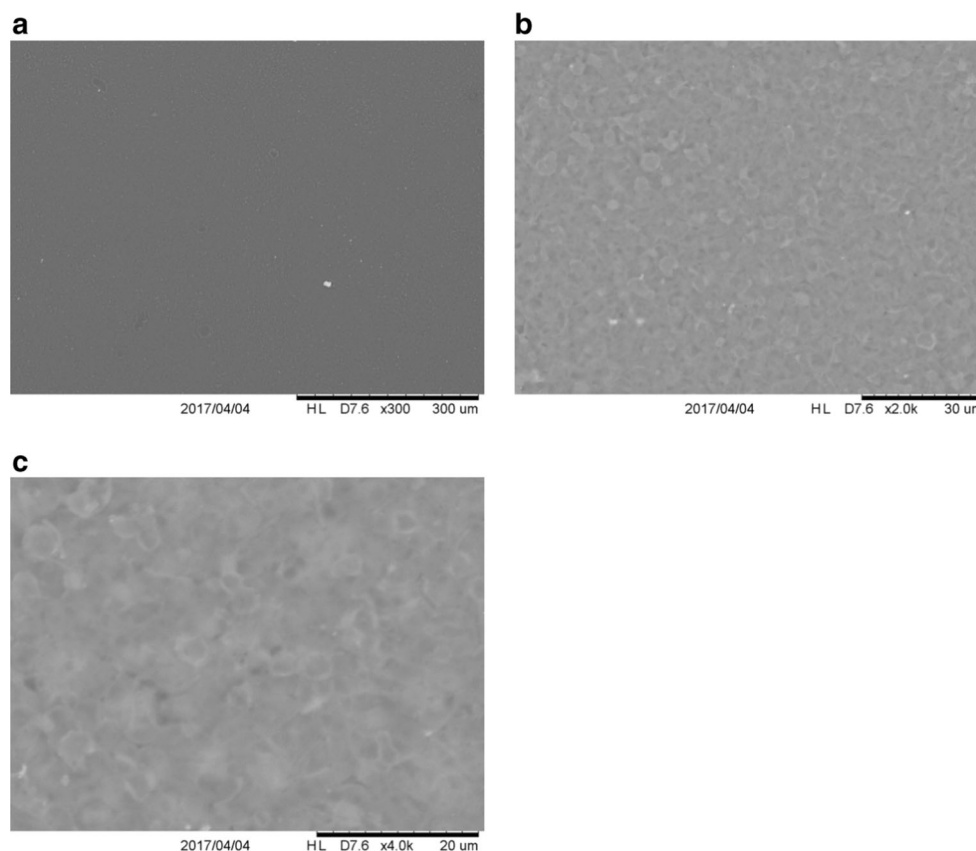
Fig. 5 FTIR spectra of yeast cell wall films (YCW) plasticized with different amount of glycerol. **a** Complete spectra. **b** Spectra from 1800 to 400 cm^{-1}

cell wall to different drying processing (Zechner-Krpan et al. 2010). The mean size of the cell wall was around $3\text{ }\mu\text{m}$.

Mechanical Properties

The addition of plasticizing agent to the biofilms is required to overcome film fragility caused by intermolecular forces. Plasticizers as glycerol reduce these forces, thereby improving flexibility and extensibility of the films; therefore, the chain mobility is increased. Tensile test results are showed in Table 1, and the decrease in tensile strength (TS) and Young's modulus (YM) was observed with the addition of plasticizer, but elongation at break (EB; %) was enhanced. No differences were observed on EB (%) values for 25 and 35 wt% of plasticizer and the addition of 15 wt% of glycerol seemed be enough to improve mechanical properties, since the EB (%) was enhanced without altering so much the values of TS and YM due to plasticization. Some authors also described good mechanical properties for β -glucan films with 15 wt% of sorbitol, reaching values of TS similar to those from conventional polymers such as LDPE and EB (%) values were enhanced (Skendi et al. 2003). YCW film presented

Fig. 6 Micrographs of YCW film plasticized with 25 wt% Gly. **a** $\times 300$. **b** $\times 2000$. **c** $\times 4000$



mechanical properties comparable to those films based on proteins and polysaccharides, non-plasticized and plasticized with glycerol. Most of the differences were observed in EB (%) values, where low values of this parameter in YCW films found may be due to the intrinsic structure of the matrix, as shown in SEM micrograph displayed in Fig. 6, where the shape of the cell wall was maintained. Plasticized films with 25 wt% of glycerol based on pea protein and gluten films presented values of EB (%) of $219 \pm 41\%$ and $116 \pm 15\%$, respectively (Jost & Stramm 2016), clearly higher than those values obtained for YCW films at the same plasticizer concentration.

Films based on sodium caseinate which presented values of TS (MPa) of 1.50 ± 0.2 , 0.62 ± 0.19 and 0.14 ± 0.06 for samples plasticized with 15, 25 and 35% w/w of glycerol, respectively, and they were lower than those from YCW films (Arrieta et al. 2013), this may be due to the presence of polysaccharide in the matrix which give strength to the film as reported by Al-Hassan and Norziah (2012). Starch films presented values of TS (MPa) between 37 ± 1 and 9 ± 3 for films plasticized with glycerol from 0 to 35% w/w. However, values of elongation at break were lower than proteins films (Jost and Stramm 2016). In general, films based on polysaccharides are more rigid than proteins films, which are more extensible and mechanical properties of mixtures of biopolymers depend on the ratio between them. Results demonstrated that YCW films presented mechanical properties given by a mixture of protein

and polysaccharides, since these films are based on a multi-component matrix. In addition, mechanical properties might be influenced by the intrinsic structure of the matrix, as shown in Fig. 6.

Experimental Water Vapour Permeability

Experimental water vapour permeability P_w^{exp} was obtained through Eq. 2 and the values are described in Table 2. It is possible to see that these values were increased with the increasing amount of glycerol since it produced structural modifications of the film network. Higher amount of glycerol tend to get more water in the matrix due to its hygroscopic character and increase of hydration water and this water also acts as a plasticizer for hydrophilic films producing a modification in the matrix structured and exposition of hydrophilic groups (Delgado et al. 2018). This behaviour is common in hydrophilic materials and was also observed in other biobased films such as protein and polysaccharide based films (Gennadios et al. 1994; Coupland et al. 2000; Bertuzzi et al. 2007; Jost and Stramm 2016). The measured P_w^{exp} for the films studied showed values were comparable to films obtained from other sources, for example films based on pea protein plasticized with 0, 15 and 25 wt% of glycerol presented values of water vapour permeability of 1.9 ± 0.2 , 8.7 ± 0.6 and $15 \pm 1 \times 10^{-10} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}$, respectively, showing values in the

Table 1 Tensile properties of YCW films

Formulation	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)
YCW-0%Gly	23 ± 3 ^a	662 ± 101 ^a	5 ± 1 ^a
YCW-15%Gly	6 ± 1 ^b	110 ± 16 ^b	30 ± 3 ^b
YCW-25%Gly	1.6 ± 0.3 ^c	28 ± 5 ^{bc}	26 ± 4 ^{bc}
YCW-35%Gly	0.8 ± 0.1 ^c	11 ± 1 ^c	23 ± 2 ^c

The mean and standard deviation ($n = 8$) are reported. The same letters in the data reported in a column means non-significant differences ($p < 0.05$)

same order of those from YCW. However, when the amount of plasticizer increased in protein films, the permeability values increased in a higher amount than the observed for YCW films. As explained in previous sections, it is possible that the glycerol may be encapsulated in yeast cell and is not available to interact with water vapour as it does for other biobased films. Then, non-plasticized starch films presented values around $4.7 \pm 0.6 \times 10^{-10} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}$ and for plasticized with 25 wt% of glycerol the value raised to $14 \pm 1 \times 10^{-10} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}$ (Jost and Stramm 2016). It is well known that the addition of plasticizers in biobased matrices is necessary to decrease brittleness of the formed films and enhance mechanical properties; however, plasticizers could affect water vapour and gases permeability (García et al. 2000). Though the addition of 15 wt% of glycerol is the best concentration to be added regarding mechanical properties and P_w^{exp} .

Water Sorption Isotherms

Sorption isotherms of YCW films at percentages of glycerol of 0, 15, 25 and 35 wt% dry basis are shown in Fig. 7 and experimental points were fitted with GAB model (Eq. 4). It is possible to see that the addition of glycerol increased the amount of hydration water, preserving the shape of the isotherms. All isotherms show a slight increase in the hydration water content at low values of a_w and a sharp increase for $a_w > 0.6$. This shape of sorption isotherms suggests the existence of a small amount of water directly bounded to the polymeric matrix, forming the monolayer, and then, most of the hydration water is forming multilayers, which is indirectly bounded to the polymeric matrix (Salvay et al. 2003; Peltzer et al. 2017). As follows, hydration water is not strongly bounded to the matrix film and is susceptible to be moved by diffusion mechanism. The shape of the isotherm of the YCW film without glycerol displayed in Fig 7 was similar to other films without plasticizer as whole yeast biomass based films (Delgado et al. 2016), cellulose-based edible films (Chinnan and Park 1995), starch-based films (Bertuzzi et al. 2007), hydroxypropyl methylcellulose-based films (Pastor Navarro 2010), myofibrillar protein-based films (Cuq et al. 1997) and sodium caseinate-based film (Kristo et al. 2008). However, the water content of YCW films without glycerol is low compared with these biopolymeric films reported in the literature, revealing that

films prepared by yeast cell wall are less hydrophilic. On the other hand, the effect of glycerol increasing the amount of hydration water without changes on the shape of the isotherms as is showed in Fig. 6 was also observed in other polysaccharide and proteins films such as, cassava starch film (Mali et al. 2005) and whey protein films (Coupland et al. 2000), and peanut protein films (Jangchud and Chinnan 1999), respectively.

Table 3 displays the parameters obtained after fitting sorption isotherms with the GAB model. Parameter N related to the number of primary binding sites of hydration increased with the glycerol content in the films, while parameter c linked to the force of the water binding to these primary sites decreased. Then, parameter k related to the capability of water to be bounded to the multilayer, slightly increased when glycerol was added to the formulation, but is independent of glycerol content. Similar behaviour of GAB parameters as function of glycerol was observed in cassava starch film (Mali et al. 2005) and whey protein films (Coupland et al. 2000).

Kinetics of Water Sorption

Figure 8 describes the results obtained from kinetics of water sorption experiments which gives information about the mobility of the water in the film by determining the diffusion coefficient D_w . Indeed, at the equilibrium, kinetics experiments give values of solubility of water S_w in the matrix. Since the barrier properties to water vapour depend on both D_w and S_w , it is particularly important to characterize the hydration of the film through kinetics of water uptake. Figure 8a

Table 2 Fit parameters of water sorption isotherms fitted with the GAB model (Eq. 4). Units of N are grams H₂O per gram of dm

%Glycerol	P_w^{exp}
0	3.0 ± 0.02 ^a
15	3.7 ± 0.2 ^b
25	5.8 ± 0.3 ^c
35	6.8 ± 0.2 ^d

The mean and standard deviation ($n = 3$) are reported. The same letters in the data reported in a column means non-significant differences ($p < 0.05$)

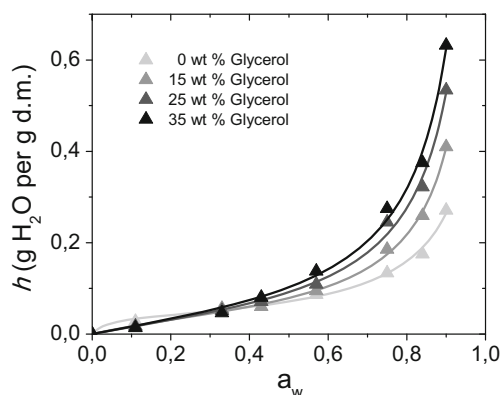


Fig. 7 Water sorption isotherms of yeast cell wall films with different glycerol contents. Experimental data was fitted with Eq. 4

displays the plots representing the kinetics of water sorption at 90% rh of YCW films at percentages of glycerol of 0, 15, 25 and 35 wt% dry basis. The curves connecting the experimental data in Fig. 8a are the best fittings using Eq. 5 and Table 4 shows the fitting parameters. Kinetics data of Fig. 8a clearly shows that the hydration water h increased when glycerol content increased. It was found that the addition of glycerol produced an increase in h_{∞} , the water content at equilibrium at 90% rh. The glycerol is an alcohol with three hydroxyl groups, highly hygroscopic and soluble in water. When glycerol is added to polymeric matrices as plasticizer, it reduces the attractive forces between polymer chains spacing them and exposing more hydrophilic groups to be hydrated (Jangchud and Chinnan 1999; Delgado et al. 2018). In this way, the incorporation of glycerol appeared to increase the number of water molecules hydrating the film. Values of the apparent diffusion coefficient D_w^{eff} obtained by Crank model are showed in Table 4. During hydration, the film undergoes to gradually transformations in the mobility of polymer chains (in some cases from glassy to rubber state at room temperature) due to the advance of the water front (Delgado et al. 2018). Therefore, D_w^{eff} represents an average value throughout the hydration process. Table 4 also shows values of the initial diffusion coefficient D_w^i obtained from STL model, fitting the initial period of sorption with Eq. 7. D_w^i takes into consideration the diffusion of water in the film during the initial 12 h of sorption and it can be considered constant during the

Table 3 Fit parameters of water sorption isotherms fitted with the GAB model (Eq. 4). Units of N are grams H_2O per gram of dm

% Glycerol	R^2	Number	c	k
0	0.990	0.040 ± 0.004	28 ± 4	0.95 ± 0.02
15	0.997	0.051 ± 0.004	4.0 ± 0.9	0.98 ± 0.01
25	0.993	0.067 ± 0.009	2.5 ± 0.4	0.98 ± 0.02
35	0.994	0.076 ± 0.008	2.2 ± 0.4	0.98 ± 0.02

hydration at this period of time (Shankar 1981). According to values displayed in Table 4, D_w^{eff} is lower than D_w^i and both were not altered by the content of glycerol in the films. It has been reported that a similar behaviour of D_w^{eff} and D_w^i for whole yeast-based films (Delgado et al. 2018). On the other hand, in films obtained with cassava starch (Müller et al. 2008) and keratin (Rocha Plácido Moore et al. 2006), the authors calculated diffusion coefficient indirectly through values measured of P_w^{exp} and S_w via $D_w = P_w^{\text{exp}}/S_w$, and also observed that D_w was not altered by the addition of glycerol. From values of h_{∞} and Eq. 6, the solubility of water in the matrix film at 90% rh, S_w , was obtained. Figure 8b shows S_w and D_w^i both as function of glycerol content. It can be observed that S_w increased linearly with the glycerol content in the film. An analogous linear behaviour of S_w with glycerol content was observed in whole yeast biomass based films (Delgado et al. 2018). On the other hand, glycerol content did not cause any perceivable change in D_w^i considering experimental errors.

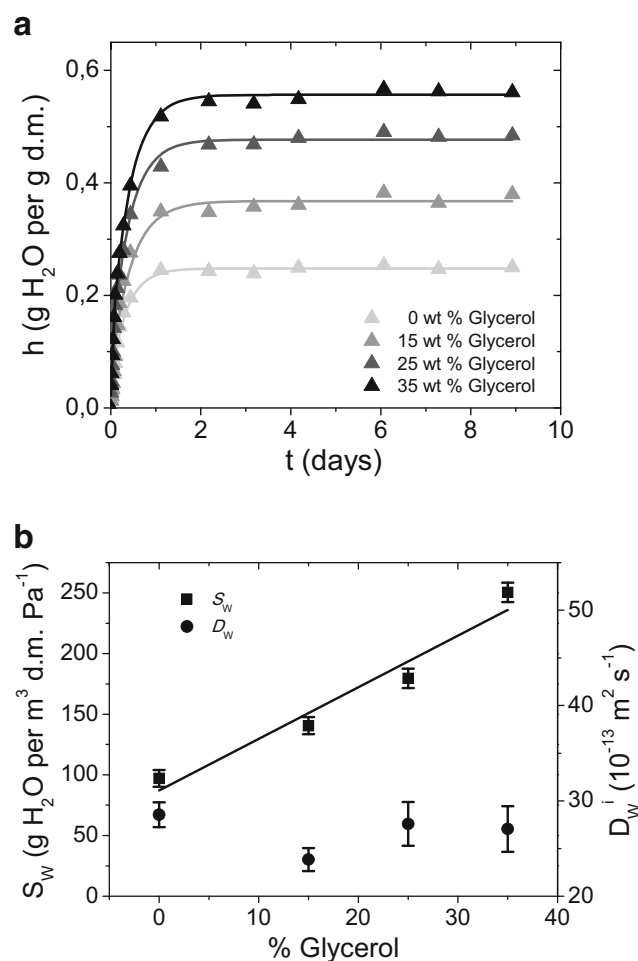


Fig. 8 a Kinetics of water sorption at 90% rh of yeast cell wall films with different glycerol content. Experimental data were fitted with Eq. 4. b Dependency of S_w and D_w^i with glycerol content of films

Table 4 Values of the parameters h_{∞} (g of H₂O per g d.m.), D_w^{eff} (10^{-13} m² s⁻¹), and D_w^i (10^{-13} m² s⁻¹) determined by using Crank model and short time lapse (STL) model. S_w (g m⁻³ Pa⁻¹) was calculated fromEq. 6 using h_{∞} . The theoretical water vapour permeability $P_w = S_w D_w^i$, in units of 10^{-10} g s⁻¹ m⁻¹ Pa⁻¹

% Glycerol	Crank model				STL model		Water vapour permeability $P_w = S_w D_w^i$
	R^2	h_{∞}	S_w	D_w^{eff}	R^2	D_w^i	
0	0.989	0.25 ± 0.01	97 ± 6	6.4 ± 0.6	0.985	29 ± 2	2.8 ± 0.2
15	0.990	0.37 ± 0.01	141 ± 6	4.9 ± 0.6	0.993	24 ± 2	3.4 ± 0.2
25	0.991	0.48 ± 0.01	180 ± 8	5.5 ± 0.5	0.995	28 ± 3	5.0 ± 0.5
35	0.995	0.56 ± 0.01	250 ± 8	5.4 ± 0.3	0.995	27 ± 3	6.8 ± 0.6

The reported values of R^2 indicate a very good acceptance of the fit model. Errors are estimated from the fit analysis of data of Fig. 7a and propagated according to the rules of errors

Theoretical Permeability

Water vapour permeability is a measure of how easily a film could be penetrated by water vapour (Rogers 1985). Permeability should not be confused with transport through pores. In the permeation process, water vapour dissolves on one side of the film, then diffuses through to the other side, and finally emerges to the atmosphere (Rogers 1985; Ro et al. 2000). Therefore, water vapour permeability P_w is defined by Eq. 7. Micrograph of YCW films showed in Fig. 6 exhibit a continuous and homogenous matrix, without pores, faults or film punctures. These studies indicated that the water transport in YCW films did not occur through pores but by means of the mechanism of sorption-diffusion-desorption. In previous permeability experiments, sorption occurs on the side of the film exposed at 90% rh and desorption on the other side exposed at 10% rh. In order to compare the experimental value P_w^{exp} with the theoretical one, $P_w = S_w D_w^i$, the values of S_w and D_w^i obtained from kinetics of hydration experiments performed at 90% rh were used. D_w^i was determined taking into account the diffusion of water in the film during the initial period of sorption (first 12 h in our experiments). The reason of the use of D_w^i is because represent the diffusion process that

occurs at the steady state during the experimental permeability test performed (Delgado et al. 2018).

Figure 9 shows the values of $P_w = S_w D_w^i$ and P_w^{exp} as function of the glycerol content in the film and a good agreement between both values (P_w^{exp} and P_w) was observed, considering experimental errors. This agreement allows studying the effect of the glycerol on the permeability in more comprehensive way seeing how the addition of plasticizer affects to S_w and D_w^i .

Observing Fig. 8b, we can see that S_w increased linearly with the amount of glycerol, while D_w^i was not affected by the content of plasticizer added. This suggests that the growth of water vapour permeability due to the addition of glycerol is given only by the increase in water solubility. An identical behaviour of permeation process with the glycerol content was observed in films obtained from whole yeast biomass based films (Delgado et al. 2018).

Conclusion

A sustainable application for the reutilization of industrial waste was described in this work. Residual yeast cell wall was used to prepared biodegradable films as food contact materials. The obtained films were homogeneous and no cracks were observed after the casting process. Solubility of YCW films in water was between 15 and 35% depending on the amount of plasticizer and values were lower than other biobased films. This could be an advantage if the pretended use is in contact with humid food products or in humid environments. Glycerol enhanced solubility of films in water due to the solubility of glycerol in water itself and due to the fact that high concentrations of plasticizers reduced cohesiveness of the YCW matrix. This was in agreement with mechanical properties of YCW films, where TS and E were decreased and EB (%) increased with the increasing amount of plasticizer. Values of TS and YM were similar to other biobased polymers. However, most of the differences with films from other sources were observed in EB (%) values, maybe due to the

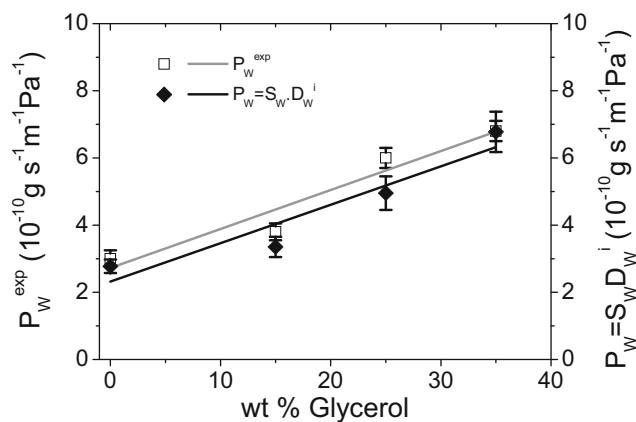


Fig. 9 Dependency of P_w^{exp} and $P_w = S_w D_w^i$ with glycerol content in the films

structure of the matrix, were the shape of the cell was maintained after treatments. TGA results indicated that YCW films exhibit substantial thermal degradation at temperatures above 180 °C. The measured water vapour permeability for residual YCW films showed values comparable with edible films based on conventional biobased sources and was increased by the presence of glycerol. This increment is only due to the increase in water solubility in the film matrix. Therefore, depending on the application pursued, it is possible to play with the amount of plasticizer. In this study, 15 wt% of glycerol seemed to be the optimum amount of plasticizer regarding mechanical, thermal, and hydration properties. Based on the results obtained in this study, it is possible to propose the residual yeast cells wall as film-forming material for sustainable applications, such as dry food packaging.

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