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THE EFFECT OF GLYCEROL ON THE FORMATION OF CHITOSAN/POLYCAPROLACTONE AND CHITOSAN/CYCLOOLEFIN COPOLYMER BILAYER FILMS

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ABSTRACT

Chitosan (CH) films were prepared with/without glycerol as a plasticizer and coated with polycaprolactone (PCL) and cycloolefin copolymer (COC) to form bilayer films. The effect of the second layer coating on the physicomechanical properties of CH films was determined. The adhesion of PCL and COC solutions, when coated on CH film, was investigated by contact angle measurement. The contact angle of PCL film solutions was lower than COC film solutions ($P < 0.05$). Scanning electron microscopy analysis revealed better compatibility between CH and PCL, while a phase separation was observed for CH/COC bilayers. Bilayers showed higher resistance to water when compared to neat CH film samples ($P < 0.05$), while a reduction was found in tensile properties for bilayer films. Bilayer films had similar transmittance values with monolayer films ($P > 0.05$). The physicomechanical properties of CH films significantly influenced by the addition of glycerol and application of the second layer.

Keywords: Polycaprolactone, cycloolefin copolymer, chitosan, bilayer film, glycerol

GLİSEROLUN KİTOSAN BAZLI İKİ KATMANLI FİLMLER ÜZERİNE ETKİSİ

ÖZ

Kitosan (CH) filmler, bir plastikleştirici olarak gliserol ile ve gliserolsüz hazırlanmış ve iki katmanlı filmler oluşturmak için polikaprolakton (PCL) ve sikloolefin kopolimer (COC) ile kaplanmıştır. İkinci bir katman olarak PCL ve COC uygulamasının CH filmlerinin fiziko-mekanik özellikleri üzerine etkileri belirlenmiştir. CH film üzerine kaplandığında PCL ve COC film çözeltilerinin yüzey adezyonu temas açısı ile araştırılmıştır. PCL film çözeltilerinin temas açısı COC film çözeltilerinden daha düşük bulunmuştur ($P < 0.05$). Taramalı elektron mikroskopi analizi, CH ve PCL arasında daha iyi bir adezyon olduğunu gösterirken, CH/COC iki katmanlı filmlerde faz ayrımı gözlenmiştir. İki katmanlı filmler, CH filmlere oranla suya karşı daha yüksek direnç gösterirken ($P < 0.05$), iki katmanlı filmlerin mekanik özelliklerinde bir azalma gözlenmiştir. İki katmanlı filmler, tek katmanlı filmler ile benzer optik özellikler göstermiştir ($P > 0.05$). CH filmlerin fizikomekanik özellikleri, gliserol ilavesinden ve ikinci katmanın uygulanmasından önemli ölçüde etkilenmiştir.

Anahtar kelimeler: Polikaprolakton, sikloolefin kopolimer, kitosan, iki katmanlı film, gliserol

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INTRODUCTION

Bio-based plastics have gained more attention because of their promising attributes, such as being alternative to conventional petroleum-based plastics, which have detrimental effects on the environment, as well as meeting the consumer demands for high-quality foods by edible films and coatings. Bio-based plastics have been widely used as edible films (Hassan et al., 2018). Among these bio-based plastics, chitosan (N-acetyl-D-glucosamine), which is naturally occurring biodegradable and biocompatible material, shows better properties such as being antimicrobial, having better resistance to water and mechanical stress (Bonilla et al., 2014). Nevertheless, chitosan presents good film-forming ability; its water resistance and tensile properties are not comparable with conventional synthetic polymers (Kurita, 2001). Chitosan films have also shown high brittleness due to the cohesive forces. Thus, food-grade plasticizers such as polyols have been added to chitosan films to decrease intermolecular forces and to increase mobility, leading to enhanced flexibility and toughness (Sothornvit and Krochta, 2005). Glycerol is one of the most common plasticizers used in biopolymer formulations. Suyatma et al. (2005) studied the effect of different hydrophilic plasticizers on the physicomechanical properties of chitosan films. They found that glycerol at 20% (w/w) is more suitable as a chitosan plasticizer to obtain flexible films. Besides, Lavorgna et al. (2010) reported that the addition of glycerol with fillers presented the best mechanical properties in chitosan films. Therefore, in this study, glycerol was chosen as a plasticizer to improve the flexibility of chitosan films.

The blending of hydrophilic polymers with hydrophobic ones has also been accepted as a way to enhance these limited properties of bio-based plastics. Chitosan has been blended with starch (Tuhin et al., 2012), beeswax (Velickova et al., 2013), polyvinyl alcohol (Bonilla et al., 2014), and gelatin (Shahbazi et al., 2017) to increase the resistance of polymers involved in a blend. Nevertheless, the blending strategy is not adequate to obtain stable composite films since low affinity between polymers results in phase separation due to lower adhesion between polymers, leading to a decrease in properties of films (Avella et al., 2000; Armentano et al., 2015). Fabrication of multilayer films composed of both bio-based and synthetic polymers is another strategy, which allows better adhesion between polymers at the interface region (Matzinos et al., 2002; Ortega-Toro et al. 2015). Researchers have shown that formation of multilayer films composed of chitosan and bio-based plastics such as beeswax (Velickova et al., 2013), carboxymethyl cellulose (Arnon et al., 2014), cassava starch (Valencia-Sullca et al., 2018), and multilayer films composed of chitosan and synthetic plastics such as polycaprolactone (Sharmin et al., 2012; Sogut and Seydim, 2018a; Sogut and Seydim, 2020), cycloolefin copolymer (Sogut and Seydim, 2018b) have enhanced the tensile properties of chitosan.

Polycaprolactone is a synthetic biodegradable polymer that shows high elasticity and low gas permeability (Sharmin et al., 2012). Researchers have identified the potential of polycaprolactone to be used as food packaging materials. Swapna et al. (2011) blended chitosan and poly-(ε-caprolactone) to increase tensile strength and lower the permeability of chitosan to be used for vegetables and fruits. On the other hand, cycloolefin copolymer is another synthetic thermoplastic that has superior properties such as high transparency, low barrier permeability, and high chemical resistance (Liu et al., 2007).

A multilayer packaging material is a single-layered structure including two or more polymer with desired properties. Petroleum-based multilayer packaging materials are already used in the food industry (Mensitieri et al., 2011). Up to now, bilayer films were obtained by using both bio-based sources (Ferreira et al., 2016; Liu et al., 2017; Valencia-Sullca et al., 2018) or a combination of synthetic biodegradable polymers with bio-based ones (Sharmin et al., 2012; Ortega-Toro et al., 2015). However, the studies about the combination of synthetic non-biodegradable with bio-based sources have been limited. The fabrication of bilayer films, including chitosan and polycaprolactone and chitosan and cycloolefin copolymer layers, could have the potential to be used as alternatives to petroleum-based polymers. Therefore, in the present study, coating chitosan films in the presence or absence of a plasticizer with other polymer matrices, polycaprolactone, and cycloolefin copolymer, has been proposed to enhance the properties of chitosan films. Resulting bilayer films were then compared with self-standing chitosan and plasticized chitosan films based on the morphological, barrier, mechanical, and optical properties.

MATERIAL AND METHODS

Materials

Chitosan (CH) (850000 Daltons, high molecular weight) and Polycaprolactone (PCL) (molecular weight of 80000 Daltons) were purchased from Sigma-Aldrich (Madrid, Spain). Cycloolefin copolymer (COC) was kindly provided by TOPAS Advanced Polymers (Frankfurt-Höchst, Germany). All chemicals were of analytical grade and supplied from Sigma-Aldrich (Madrid, Spain).

Film preparation

CH film solution at 1.5% (w/w) was obtained after dissolving CH powder in aqueous acetic acid solution (3%, w/w). Two groups of CH films were prepared: plasticized (CH-gly) and non-plasticized (CH). Glycerol at 30% (w/w, based on the weight of CH powder) was added to CH film solution to form plasticized CH film group (CH-gly). The air bubbles in CH film solutions were removed by a vacuum pump, and then film solutions were cast onto a Teflon coated plate (40 g/Petri). CH films were dried under ambient conditions. Dried CH films were treated with potassium sorbate solution (0.1 g of potassium sorbate/g CH film) to improve the layer adhesion when coated with the second layer. The aqueous potassium sorbate solution might modify the surface tension of CH films (Ortega-Toro et al., 2015). PCL and COC film solutions at 5% (w/w) were dissolved in chloroform. PCL and COC film solutions were used to form bilayer films, whereas PCL and COC based monolayer films were formed by the casting method. Bilayer films were fabricated by pouring PCL and COC film solutions on the surface of dried CH films. Uniform film distribution was obtained by a coating rod (bar thickness 200 μm), which passed over the film surface for once. The amount of glycerol was selected as 30% because film samples became weaker and unstable above the saturation value for plasticizer. In our preliminary studies, coating of CH surface was performed by using PCL, and COC film-forming solutions with different concentrations and 5% of film solutions showed better results when compared to the results obtained for film solutions having higher or lower concentrations. The thickness of all film samples was controlled with a digital micrometer (Palmer-Cometa, Spain, ±0.001 mm) by measuring the thickness at random positions. All film samples were conditioned for one week at 25°C inside a desiccator having 53% relative humidity (RH). The film preparation steps are shown in Figure 1.

Morphological properties of film samples

Conditioned film samples were stored at a desiccator containing P_2O_5 to remove the residual moisture and then were fractured under cryogenic temperatures by using liquid nitrogen. Cross-section images of film samples were obtained by a scanning electronic microscope (JEOL, JSM-5410, Japan) using a magnification of 500-2000x with an accelerating voltage of 10 kV. Copper stubs were used to adjust film samples for coating with gold.

Contact angle

The suitability of CH based film surface to be coated with PCL and COC based film solutions were determined by contact angle measurements. After mounting the air contact side of CH films on a glass slide by facing up, PCL and COC film solutions were dropped on the CH film surface and waited for a proper contact (< 20 s) between surfaces. The images of every drop (0.015 mL) were obtained by the sessile drop fitting method. A video-based contact angle meter (OCA 20 Dataphysics, Filderstadt, Germany) and SCA20 software were used to analyze the images.

Moisture content and barrier properties of film samples

The residual moisture content of film samples was determined at 60°C by using a vacuum oven (J.P. Selecta, S.A., Barcelona, Spain) until constant weight reached.

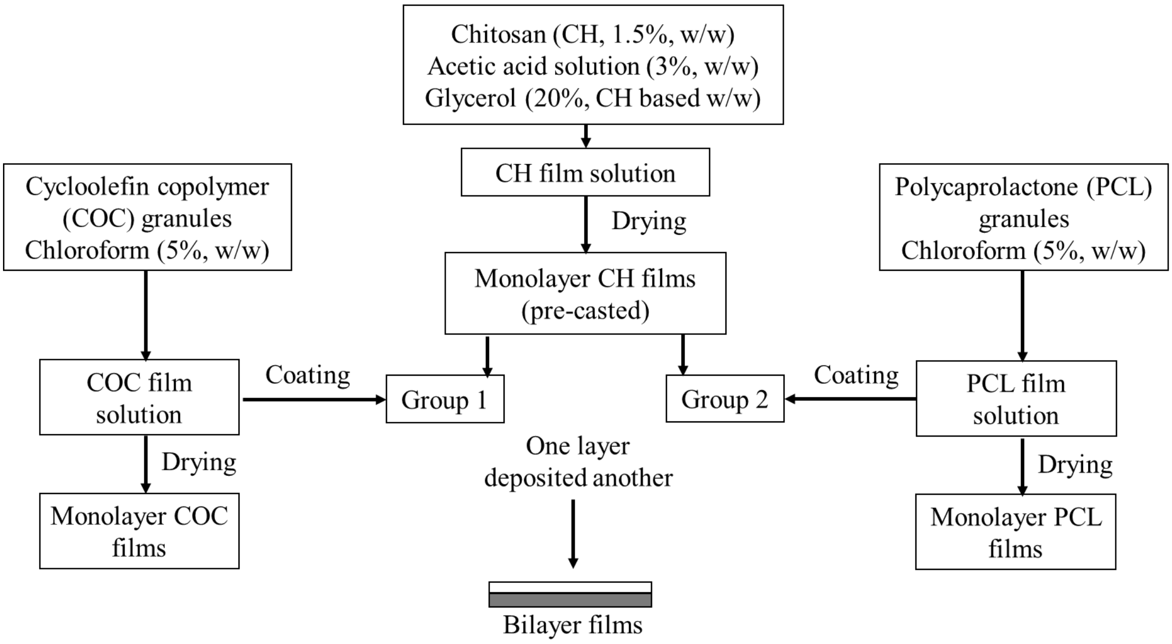

Figure 1. Schematic representation of film preparation

The gravimetric method of ASTM (E96-95) was used to measure the water vapor permeability (WVP) of film samples (ASTM, 1995). Film samples were placed onto permeability cups (Payne-Elcometer, Manchester, UK), including 5 mL of bidistilled water for the weight loss measurements under 53% RH. The measurements were performed periodically during 48 h.

The oxygen permeability (OP) of film samples was determined according to ASTM D3985-95 standard method (ASTM, 2002) using Oxtran1/50 (Mocon, Minneapolis, USA) at 25°C and 53% RH. The measurements with an exposure area of 50 cm^2 were performed every 20 min until the equilibrium.

Mechanical properties of film samples

The tensile strength (TS) and elongation (E) at breakpoint were estimated by the stress-strain curves obtained from a universal test Machine (TA.XTplus, Stable Micro Systems, Haslemere, England) using ASTM standard method D882 (ASTM, 2001). These values were measured by stretching the film samples (2.5 cm wide and 5 cm long) at 50 mm/min using an appropriate preload.

Optical properties of film samples

A Minolta spectro-colorimeter (CM-3600d model, Minolta Co., Tokyo, Japan) was used to determine the internal transmittance (T_i) values of the films. The transparency was measured with a reflectance spectrum between 400-700 nm using both white and black backgrounds.

The gloss values of the film surface were determined according to the D523 standard method (ASTM, 1999) with a surface gloss meter (Multi Gloss 268, Minolta, Germany) at 60° incidence angle and results were expressed as gloss units (GU).

Statistical analysis

The results were evaluated through an analysis of variance (ANOVA) and compared with Tukey's multiple comparison tests at a 95% confidence level using Minitab 17 software (Minitab Inc., Brandon, UK). Experiments were replicated two times with six observations, at least.

RESULTS AND DISCUSSION

Morphological properties of film samples

The adhesion between layers of each film on a microscopic scale was observed by SEM analysis

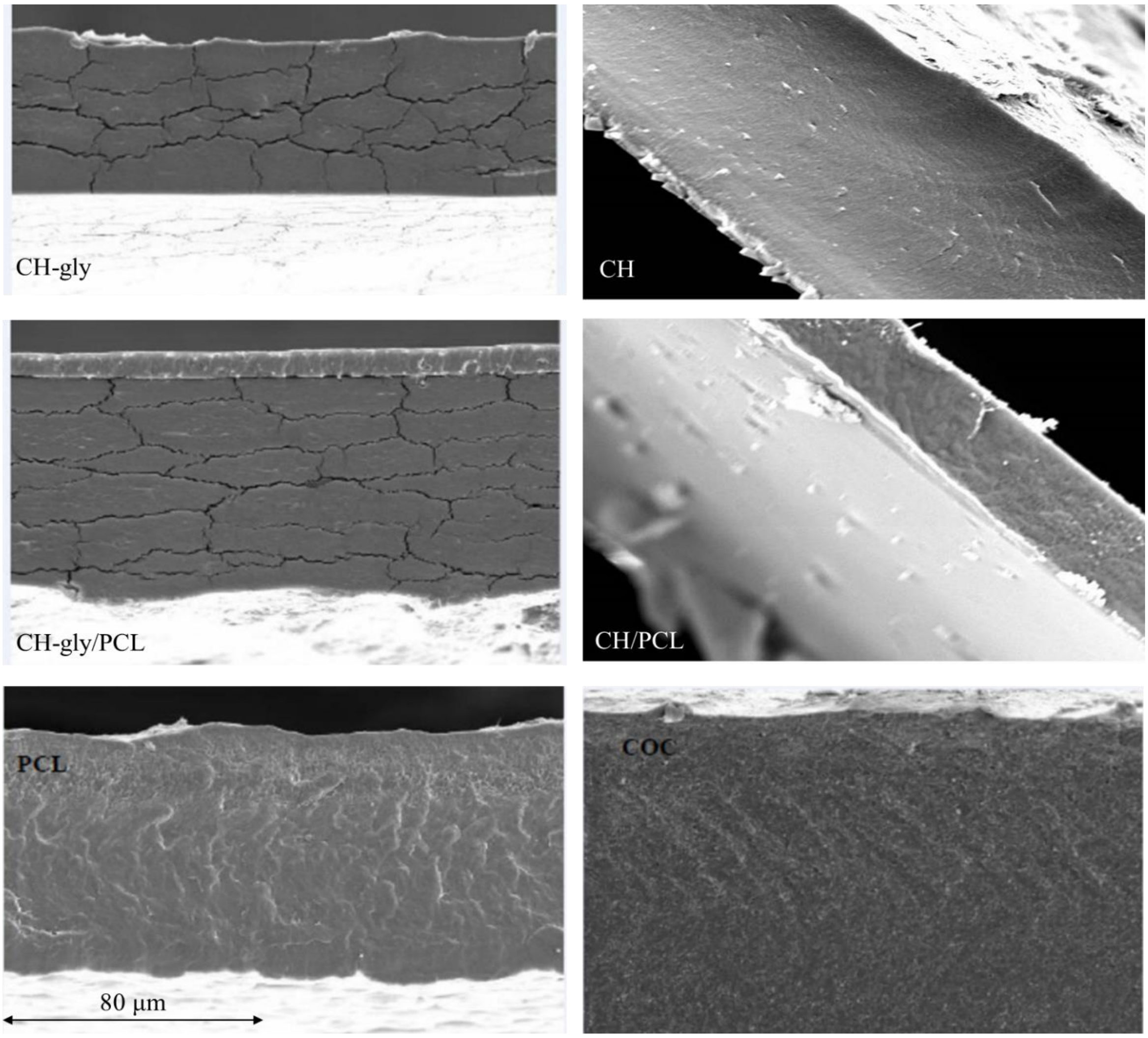

Figure 2. SEM micrographs of the cross section of film samples (500-2000X magnification) (The SEM images of COC based films were not obtained because of layer separation)

The resulting CH films were translucent with a yellowish color, and CH-gly films had more rubbery structure than CH films. CH-gly monolayer films had a more cracked region when compared to CH films. Glycerol affected the structure of CH films. On the other hand, PCL and COC monolayers presented a well dispersed, porous structure, which may be due to the rapid solvent evaporation during drying. In the bilayer films, each layer presented its structure, where CH-gly exhibited homogeneous characteristics with cracked regions, COC showed more porous structure, and PCL presented well-dispersed characteristics. The second layer (PCL and COC) was thinner than that of CH layers. PCL layers exhibited better adhesion, whereas COC layers separated during observation. The presence of the second layer caused some discontinuities on the film structure consistent with the film opacity. The coating CH films with PCL and COC as film solutions might alter the film structure at the interface due to the volatile form of chloroform and its migration through layers. In the formation of bilayer films, the adhesive-cohesive forces between layers should be considered. Similar structures were observed for CH monolayer films (Vargas et al., 2009) and starch-PCL (Ortega-Toro et al., 2015) bilayer film structures by other researchers.

Contact angle

The contact angle values of PCL and COC film solutions on the surface of CH and CH-gly films are shown in Figure 3.

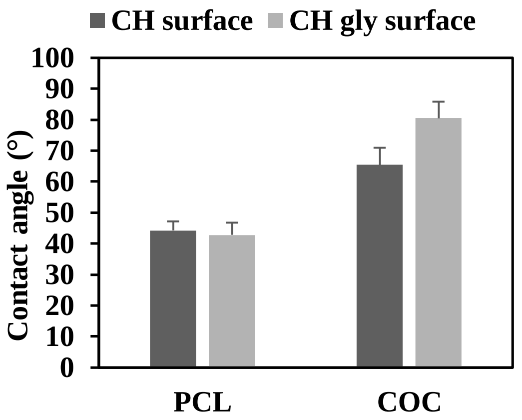

Figure 3. Contact angle values of PCL and COC solutions on the surface of CH and CH-gly films.

The compatibility between PCL and CH and COC and CH films were studied with the contact angle analysis, and it was observed that all values were below 90^{o} as an indication of surface compatibility between PCL and COC film solutions and CH films (Farris et al., 2011). The application of potassium sorbate solution on the surface of CH films might change the cohesive forces leading to better adhesion, or the functional groups of CH might have increased the spreadability of second film solution on the surface of CH films. The surface smoothness of plasticized and non-plasticized CH films was not the same, thus affecting the adhesion of coating solutions when contacting (Farris et al., 2011). PCL and COC film solutions showed significantly different behaviors consistent with SEM analysis. Higher contact angle values were found in the CH film surface when compared to the CH-gly surface for PCL film solutions (P >0.05). However, higher values were obtained for CH-gly surfaces for COC film solutions (P <0.05). The contact angle values for PCL solutions on the CH-gly surface were found to be lower than those of COC solutions on both CH and CH-gly surfaces. The addition of glycerol might have changed the physical properties of CH, such as the orientation of molecules, especially in the upward surface (Ferreira et al., 2009). However, no direct conclusion could be drawn from surface analysis for the physical state of polymers. PCL film solutions had the lowest contact angle values; thus, PCL film solutions could be more suitable to coat CH film surface to form bilayer films. Similarly, Kurek et al. (2014) observed contact angle values lower than 90° for the CH film surface. Ferreira et al. (2016) also reported an increase in contact angle upon the addition of glycerol into chitosan-based bilayers.

Moisture content and barrier properties of film samples

Table 1 shows the moisture content, thickness, WVP, and OP values of film samples.

Table 1. Thickness (µm), moisture content (%), water vapor permeability (WVP), and oxygen permeability (OP) values of film samples

Film samples	Thickness (µm)	Moisture (%)	WVP (g mm/(kPa h m^{2}))	OPx10^{13} (cm^{3}/(m s Pa))
CH	42±2^{e}	11.93±0.41^{c}	3.96±0.36^{b}	3.05±0.21^{b}
CH-gly	49±4^{e}	23.45±0.98^{a}	8.68±2.80^{a}	3.14±0.06^{b}
PCL	108±2^{b}	0.56±0.21^{d}	0.18±0.01^{c}	>D.L.*
COC	153±6^{a}	9.37±0.20^{c}	0.05±0.01^{c}	>D.L.*
CH/PCL	67±2^{d}	7.97±0.85^{c}	0.67±0.10^{c}	3.38±0.13^{b}
CH/COC	88±4^{c}	10.70±0.54^{c}	0.15±0.09^{c}	4.40±0.20^{a}
CH-gly/PCL	70±1^{d}	19.41±0.71^{b}	0.87±0.07^{c}	3.57±0.24^{b}
CH-gly/COC	69±5^{d}	18.87±0.17^{b}	0.24±0.02^{c}	4.46±0.15^{a}

Any two means in the same column followed by the same letter were not significantly different (P >0.05) by Tukey's multiple range test.

*D.L.:0.1-300 cc/(m^{2} day) (>D.L., out of sensitivity limit)

Even though the same amount of PCL and COC solutions were used to coat the surface of CH films, the thickness values of bilayer films varied. The differences in the thickness of the coatings might be attributed to the differences in homogeneity between film solutions and uncontrolled evaporation of the second layer. Similar behavior was observed by Ortega-Toro et al. (2015) when fabricating bilayer films of starch and PCL. The CH-gly film showed the highest moisture content, while PCL film had the lowest value (P <0.05). Generally, CH-gly based films presented higher moisture contents (P <0.05), which could be due to a higher affinity of glycerol to water molecules. Besides, the moisture content of CH films was higher than PCL and COC monolayers (P <0.05), since CH has more hydration layers than PCL and COC, leading to highly retained water molecules (Kurek et al., 2014). Suyatma et al. (2005) also found higher moisture content in CH films when plasticized with 40% of glycerol (based on CH content). Bilayer films showed lower moisture content when compared to CH monolayers, contrary to the results obtained by Ortega-Toro et al. (2015) for starch-PCL bilayer films, which might be due to the differences between bilayer formation type.

The barrier properties of a food packaging material are fundamental however, WVP of CH films is high, which making them inappropriate for several applications. CH-gly films showed higher WVP values than that of CH films because of the plasticization effect of glycerol. The negative effect of glycerol is attributed to the significant interaction (H-bonding) between glycerol and water. Glycerol might also soften the structure leading to chain mobility and an increase in water molecule transfer through the film matrix (Bertuzzi et al., 2007). These values are also consistent with moisture contents of films, which are higher in films having higher moisture contents; since higher water content resulted in higher molecular mobility and permeability values. Similar results were reported by other researchers (Xu et al. 2005; Bourtoom and Chinnan, 2008; Bonilla et al., 2013) for CH films. PCL and COC films are hydrophobic materials known as impermeable to water vapor; thus, the application of PCL and COC positively affected the WVP of coated CH films. Bilayer films presented significantly lower WVP values when compared to CH and CH-gly films (P <0.05), and these values were similar to PCL and COC monolayer films (P >0.05). Similarly, Sogut and Seydim (2018b) observed lower WVP values for bilayer films when compared to their relative monolayer films. However, Priyadarshi et al. (2018) observed a reduction in moisture content and WVP values of glycerol plasticized CH films. The differences in the results might be due to differences in film preparation steps, such as using different weak acids for protonation of CH.

OP values of CH-gly films were higher than CH films, which might be related to the higher moisture content leading to an increase in mobility and solubility of gas molecules (Kurek et al., 2014). Besides, the oxygen barrier properties of bilayers were slightly decreased, exhibiting higher OP values. There were no significant differences between PCL based bilayer films and CH monolayers; however, COC based bilayer films showed significantly higher OP values. Among the same bilayer films, glycerol, including films, also had higher OP values (P >0.05). The coating of CH surface with a film-forming solution including chloroform might have caused a deformation on the surface of the whole film, and rapid evaporation of chloroform might have produced larger voids on the surface of the second layer allowing the oxygen transfer easily. Similarly, Liu et al. (2017) reported higher oxygen permeability for polyvinyl alcohol (PVA)/CH bilayer films when PVA coated with higher CH contents. On the contrary, Valencia-Sullca et al. (2018) reported an improved OP for chitosan-starch bilayer films concerning monolayers.

Mechanical and optical properties of film samples

The TS and E values and strain-stress curve of plasticized and non- plasticized films are shown in Table 2 and Figure 4, respectively.

The highest TS values were observed in CH films, and the inclusion of glycerol reduced the TS of CH films (P <0.05). Besides, the coating of CH with PCL and COC led to a decrease in tensile strength (*P* <0.05) for all film samples. The weakened cohesion forces, and less compatibility and weak interfacial interaction between layers might have caused a decrease in rigidity. The application of chloroform-based film solutions might have increased the cavitation leading to the formation of cracks and damaged surfaces, as in the case of barrier properties. Thus, the tensile properties of bilayer films resulted in lower values.

Table 2. Mechanical and optical properties of film samples

Film samples	TS (MPa)	ε (%)	T_i** (%)	Gloss** (60°)
CH	48.5±5.6^{a}	12.7±4.6^{cd}	78.91±0.27^{bc}	29±4^{b}
CH-gly	25.7±5.5^{b}	40.5±4.5^{ab}	78.44±2.07^{bc}	9±1^{c}
PCL*	-	-	76.75±0.93^{bc}	7±1^{cd}
COC	16.9±3.3^{c}	3.8±0.6^{d}	82.77±1.43^{a}	43±1^{a}
CH/PCL	37.0±2.0^{b}	20.0±2.0^{c}	79.59±0.23^{ab}	7±1^{cd}
CH/COC	29.0±4.0^{b}	29.0±1.0^{bc}	76.56±0.36^{bc}	7±2^{cd}
CH-gly/PCL	18.8±3.8^{c}	35.2±4.2^{b}	77.38±0.09^{bc}	5±1^{d}
CH-gly/COC	18.9±2.3^{c}	46.6±3.1^{a}	75.83±3.07^{c}	8±1^{c}

Any two means in the same column followed by the same letter were not significantly different (*P* >0.05) by Tukey's multiple range test.

*PCL film did not break at determined conditions (because of high deformability)

**Bilayer face was used for the experiments

On the other hand, each layer might have shown different deformation behaviors during tests, due to the lack of compatibility between layers. However, the addition of glycerol and coating application improved the flexibility of CH films (*P* <0.05). The highest elasticity was observed in CH-gly/COC films, followed by CH-gly films (*P* <0.05). Film samples, including more water, showed higher elongation at break values. Non-plasticized CH was more brittle than CH-gly films, which might be due to the stronger cohesive energy (Sothornvit and Krochta, 2005). Glycerol might have reduced the interaction between polymer chains, causing more deformation without breaking during stretching tests (Kurek et al., 2014). Similar to these results, Priyadarshi et al. (2018) also reported a decrease in tensile strength and an increase in elasticity for glycerol plasticized CH films. Moreover, Ferreira et al. (2016) and Sharmin et al. (2012) have found similar tensile properties for FucoPol and CH based bilayer films and CH-PCL based trilayer films, respectively.

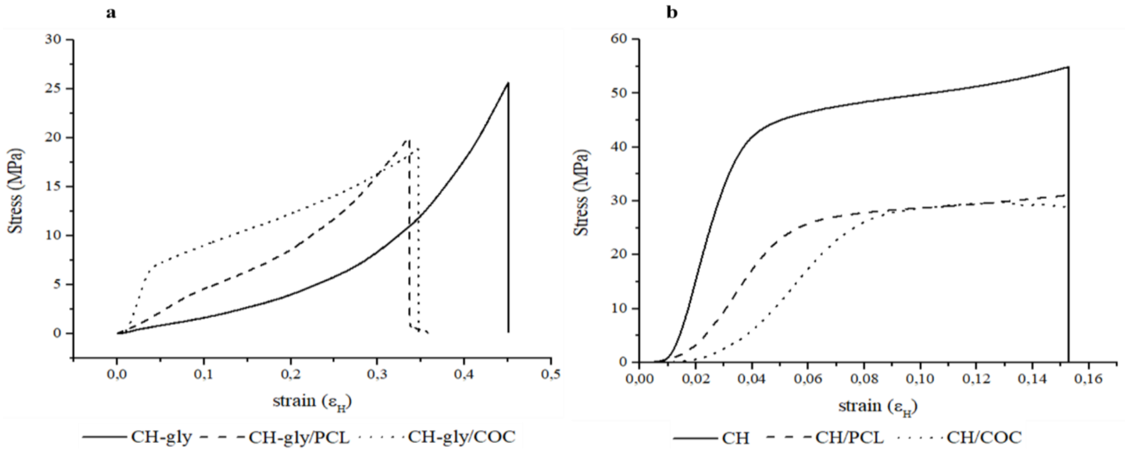

Figure 4. Strain-stress curves of film samples (a=plasticized films, b=non-plasticized films)

Table 2 presents the internal transmittance (T_i) and gloss values of film samples. The highest Ti value was observed in COC monolayer films ($P < 0.05$), whereas the addition of glycerol and application of the second layer resulted in lower T_i values. CH and COC monolayer films showed a more transparent structure ($P < 0.05$), whereas CH-gly, PCL, and bilayer films showed more opaque structure. However, films had transmittance values around 80%, which can be considered as transparent films. Valencia-Sullca et al. (2018) also found a decrease in transmittance for bilayer films composed of cassava starch-CH bilayer films.

Gloss values were consistent with T_i results and significantly influenced by the presence of glycerol and application of the second layer. The highest gloss value was observed in the COC monolayer, followed by CH film ($P < 0.05$). Generally, bilayer films and CH-gly film showed significantly lower gloss values indicating that the application of the second layer and glycerol promotes a rougher surface, thus decreasing gloss. Similar gloss values were observed by Bonilla et al. (2011) for CH film samples.

CONCLUSION

Results indicated a possibility for the production of CH based PCL and COC bilayer films with enhanced physicomechanical properties. On a microscopic scale, better adhesion was observed for CH and PCL layer, and lower contact angle values were obtained. On the contrary, contact angle values of COC solutions shifted to a higher value, and phase separation was observed during SEM analysis. In general, bilayer films prepared with CH and PCL showed better potential applications than CH based COC bilayer films. The application PCL and COC as a second layer and the inclusion of glycerol as a plasticizer implied undesirable changes in the tensile properties of film samples. These applications made CH films more stretchable but less resistant to fracture with similar optical properties. Generally, PCL based bilayer films showed better properties when compared with COC based bilayer films. In conclusion, the fabrication of a bilayer film, including a synthetic polymer layer on the surface of a bio-based polymer increased the possible use of CH for food packaging applications, which can be an alternative to commonly used synthetic multilayer films.

Conflict of interest

The authors declare that there is no conflict of interest.

Author Contributions

Ece Sogut: Formal analysis, Conceptualization, Investigation, Methodology, Writing - original draft, Writing - review & editing. Atif Can Seydim: Writing- Reviewing and Editing.

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