POLİTEKNİK DERGİSİ

JOURNAL of POLYTECHNIC

ISSN: 1302-0900 (PRINT), ISSN: 2147-9429 (ONLINE)
URL: http://dergipark.org.tr/politeknik

Development of chitosan and polycaprolactone based trilayer biocomposite films for food packaging applications

Gıda ambalajlama uygulamaları için kitosan ve polikaprolakton esaslı üç katmanlı biyo-kompozit filmlerin geliştirilmesi

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Bu makaleye şu şekilde atıfta bulunabilirsiniz(To cite to this article): Sogut E. ve Seydim A.C., "Development of chitosan and polycaprolactone based trilayer biocomposite films for food packaging applications", ***Politeknik Dergisi***, 24(1): 263-273, (2021).

Erişim linki (To link to this article): http://dergipark.org.tr/politeknik/archive

DOI: 10.2339/politeknik.628222

Development of Chitosan and Polycaprolactone Based Trilayer Biocomposite Films for Food Packaging Applications

Highlights

- *Chitosan (CH) films including nanocellulose and grape seed extract were obtained by casting*
- *CH-based trilayer film was prepared by compression with two polycaprolactone sheets*
- *Resultant trilayer films provided active properties with adequate physico-mechanical properties*

Graphical Abstract

The bioactive chitosan film incorporated with nanocellulose and grape seed extract was inserted between 2 layers of polycaprolactone films to obtain trilayer films.

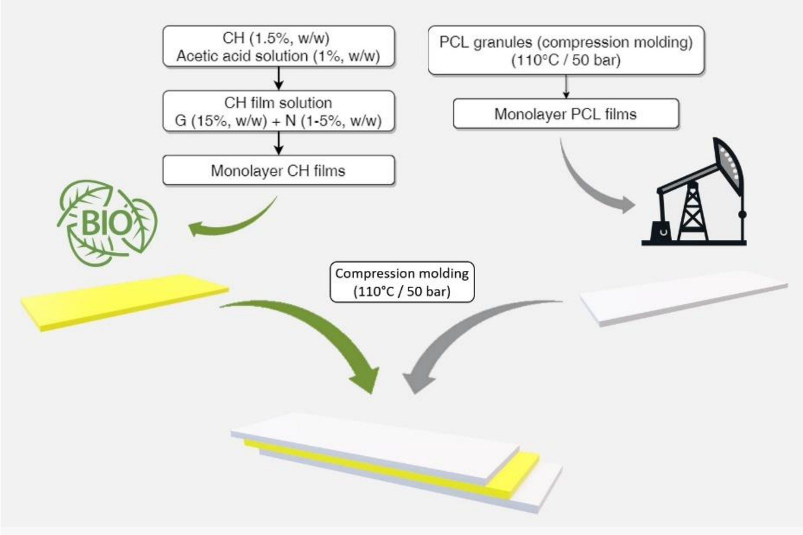

Figure. Combination of bio-based polymer layers with synthetic polymers by compression molding process to prepare composite trilayer films

Aim

The aim of this study was to prepare a trilayer active film for food packaging applications.

Design & Methodology

Chitosan (CH) films were obtained by casting method and sandwiched between 2 layers of polycaprolactone (PCL), which were formed by compression molding, to form trilayers. CH films were also incorporated with grape seed extract (G) (15%, w/w) and/or nanocellulose (N) (1-5%, w/w). The tensile properties, transmittance, opacity, water vapor permeability (WVP), antimicrobial activity, and release behavior of trilayers were determined.

Originality

CH films with active properties could have a potential to be used as active layers along with conventional packaging films that could reduce the amount of film used.

Findings

The elastic modulus (EM) of trilayer films were not significantly affected by the N content, while higher N concentrations resulted in higher tensile strength (TS) values. The incorporation of G led to higher elongation values and resulted in lower EM and TS values. Film samples, including N, presented lower WVP values, whereas higher WVP and water solubility values were obtained with G inclusion ($p<0.05$). L and transmittance values increased with the increasing N content while the opacity values decreased ($p<0.05$). Furthermore, films added G showed significantly higher a* and b* values. The addition of N caused slower release of G from CH films through the selected food simulants. The obtained trilayer films also inhibited selected main pathogenic bacteria.*

Conclusion

The fabrication of PCL and CH films in the trilayer form enhanced the properties of CH and made these films more appropriate for food packaging.

Declaration of Ethical Standards

The authors of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

Politeknik Dergisi, 2021; 24(1) : 263-273 *Journal of Polytechnic, 2021; 24 (1): 263-273*

Gıda Ambalajlama Uygulamaları için Kitosan ve Polikaprolakton Esaslı Üç Katmanlı Biyo-Kompozit Filmlerin Geliştirilmesi

Araştırma Makalesi / Research Article

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(Geliş/Received : 02.10.2019 ; Kabul/Accepted : 18.03.2020)

ÖZ

Kitosan (CH) filmler, dökme yöntemi ile elde edilmiş ve üç katmanlı filmler oluşturmak için pres ile oluşturulan iki polikaprolakton (PCL) film arasında tekrar preslenmiştir. CH filmlere ayrıca üzüm çekirdeği ekstaktı (G) (% 15, ağırlıkça) ve/veya nanoselüloz (N) (% 1-5, ağırlıkça) eklenmiştir. Üç katmanlı filmlerin mekanik özellikleri, transmittans, opaklık, su buharı geçirgenliği (WVP), antimikrobiyal aktivite ve salım davranışları belirlenmiştir. Üç katmanlı filmlerin elastik modülü (EM), N içeriğinden önemli ölçüde etkilenmezken, yüksek N konsantrasyonları, daha yüksek çekme dayanımı (TS) değerleriyle sonuçlanmıştır. G'nin filmlere ilave edilmesi daha yüksek elastikiyet değerlerinin elde edilmesini sağlamıştır ve daha düşük EM ve TS değerleriyle sonuçlanmıştır. N içeren film örnekleri daha düşük WVP değerleri gösterirken, G ilavesi ile birlikte daha yüksek WVP ve suda çözünürlük değerleri elde edilmiştir ($p<0.05$). L^* ve transmittans değerleri artan N içeriği ile artarken, opaklık değerleri azalmıştır ($p<0.05$). Ayrıca, G eklenen filmler önemli derecede yüksek a^* ve b^* değerleri göstermiştir. N ilavesi, G'nin CH filmlerden, seçilen gıda simülasyonlarına daha yavaş salınımına neden olmuştur. Elde edilen üç katmanlı filmler ayrıca seçilen ana patojenik bakterileri de inhibe etmiştir. PCL ve CH bazlı filmlerin üç katmanlı olarak üretilmesi, CH özelliklerini geliştirmiş ve bu filmleri gıda ambalajları için daha uygun hale getirmiştir.

Anahtar Kelimeler: Kitosan, polikaprolakton, nanoselüloz, üzüm çekirdeği ekstraktı.

Development of Chitosan and Polycaprolactone Based Trilayer Biocomposite Films for Food Packaging Applications

ABSTRACT

Chitosan (CH) films were obtained by casting method and sandwiched between 2 layers of polycaprolactone (PCL), which were formed by compression molding, to form trilayers. CH films were also incorporated with grape seed extract (G) (15%, w/w) and/or nanocellulose (N) (1-5%, w/w). The tensile properties, transmittance, opacity, water vapor permeability (WVP), antimicrobial activity, and release behavior of trilayers were determined. The elastic modulus (EM) of trilayer films were not significantly affected by the N content, while higher N concentrations resulted in higher tensile strength (TS) values. The incorporation of G led to higher elongation values and resulted in lower EM and TS values. Film samples, including N, presented lower WVP values, whereas higher WVP and water solubility values were obtained with G inclusion ($p<0.05$). L^* and transmittance values increased with the increasing N content while the opacity values decreased ($p<0.05$). Furthermore, films added G showed significantly higher a^* and b^* values. The addition of N caused slower release of G from CH films through the selected food simulants. The obtained trilayer films also inhibited selected main pathogenic bacteria. The fabrication of PCL and CH films in the trilayer form enhanced the properties of CH and made these films more appropriate for food packaging.

Keywords: Chitosan, polycaprolactone, nanocellulose, grape seed extract.

1. INTRODUCTION

Recently, packaging materials made of biopolymers are preferred because of environmental problems arise from plastic pollution as well as increasing demands for high-quality foods. Petroleum-based plastics are mostly used due to better thermomechanical and barrier performances [1]. Nevertheless, the usage of conventional synthetic plastics causes serious problems such as global warming, increasing energy demand, destruction of rainforests [2]. Therefore, both researchers and manufacturers focus on producing environment-friendly, natural products, and materials from renewable sources.

Chitosan (CH) has great potential among bio-based polymers as it has intrinsic antimicrobial activity and relatively better properties such as lower water affinity (soluble in acidic aqueous medium), higher mechanical strength, polycationic nature, more compatibility with many organs (for medical applications), suitable for modification, being physically and biologically active [3]. CH is a linear cationic (1, 4)-2-amino-2-deoxy-d-

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glucan, allowing electrostatic interactions with other compounds due to its cationic property [4]. CH has been studied extensively by adding antimicrobials [5], nanofillers [6], and antioxidant agents [7, 8]. However, the use of those edible or biodegradable materials has some drawbacks, such as being sensitive to moisture, having a weak barrier, and thermomechanical properties [9]. The disadvantages of bio-based polymers have been delayed by combining with other polymers [10] or have enhanced with suitable reinforcement agents like montmorillonite [11], nanocellulose [12], metal oxides [13]. Nanocellulose (N) has been used as a reinforcing agent by creating a percolated network within the polymer, leading to enhanced mechanical and barrier properties [14]. Previous studies reported increased water resistance [15], tensile strength [16], and stability of active agents [17] when nanocellulose used as a reinforcement agent.

Blending biopolymers with biodegradable polyesters have been widely studied to enhance the barrier performance or increase the tensile strength [18]. However, the low miscibility between hydrophilic biopolymer and a more hydrophobic polyester resulted in phase separation and reduced impact properties [19]. Even though, blending a hydrophilic polymer with a polyester led to a decrease in water affinity while maintaining its biodegradable nature; the resultant blend film undergoes different morphologies due to the phase separation and miscibility problems. Similarly, Averous et al. [20] reported a phase separation in thermoplastic starch and polycaprolactone blend films, which polycaprolactone layer located on the surface probably due to the different viscosities leading the migration of lower viscous polymer towards to surface. Therefore, using multilayers instead of blending is the other strategy to easily enhance the biopolymers by combining the better properties of different materials [21]. In this way, biopolymers, sensitive to water, would not migrate to the food matrix when used as internal layers in a multilayer structure, which has external layers that act as a barrier. Among biodegradable polyesters, polycaprolactone (PCL) is used as an additive due to its compatibility with a wide variety of materials and biodegradable properties, even though being a synthetic polymer. PCL is a semi-crystalline polymer, which is fabricated via ring-opening polymerization with a catalyst [22]. It has promising properties such as biodegradability, low viscosity, low melting point, water resistance, and excellent mechanical properties, and is suitable for food packaging [23]. Sharmin et al. [24] fabricated CH and PCL based trilayer films and reported an increase in mechanical properties when compared to single layers, as evidenced by morphological analysis results. Similarly, Fabra et al. [25] fabricated a multilayer film composed of PCL and thermoplastic starch by electrospinning and found enhanced barrier properties. The water vapor and gas permeability of PCL, CH, and cellophane based multilayer films were also improved by Shi et al. [26].

In recent years, food products require new packaging techniques to increase food quality and security while maintaining the organoleptic characteristics of food products [27]. The addition of valuable compounds like antimicrobials into food packaging materials with a controlled medium is a better solution than the direct use of preservatives within the food matrix [28]. Among these solutions, antimicrobial packaging films have been found effective in suppressing the growth of many microorganisms existing in foods [29]. Plant extracts such as grape seed extract include catechins, phenolic acids, which have both antioxidant and antimicrobial properties [30]. Thus, grape seed extract has been extensively studied by incorporation into bio-based materials such as chitosan [7, 31], starch [32], and gelatin [33] to improve the oxidative stability of foods and the antimicrobial effectiveness of packaging materials. Multilayer formulations were also studied by bringing active properties to external or internal layers. For instance, Alix et al. [19] produced starch and PCL based multilayers, including CH as an antimicrobial agent, and reported a decrease in the number of bacteria on the film. On the other hand, Fabra et al. [25] incorporated β-carotene into PCL layers and coated starch film with these layers to obtain antioxidant multilayer films.

The consumers' demands on high-quality foods with increased shelf life have caused changes in food packaging technology toward mechanically resistant and biodegradable materials with active properties. In this study, it was aimed to fabricate active trilayer films composed of PCL/CH/PCL, including nanocellulose and grape seed extract. CH includes high amounts of hydroxyl and amino groups, which provide several possibilities for interaction with other components and grafting active agents. Therefore, CH was used as the middle layer carrying the other ingredients (G and/or N) to allow surface interaction between PCL layers. The tensile properties, water vapor permeability, release behavior, optical and antimicrobial performances of prepared films were then analyzed.

2. MATERIAL and METHOD

2.1. Materials

Grape seeds were kindly supplied by a local winery in Denizli/Turkey and extracted with distilled water. The extract was freeze-dried (BW-100F, Bluewave Industry Co., Ltd. Shanghai, China), and the grape seed phenolics were obtained in a powder form (G). Chitosan (CH) (ChitoClear® CG1600, viscosity>1200 cP, >75% deacetylated, high molecular weight>320 kDa) was obtained by Primex (ChitoClear®, Siglufjordur, Iceland). Nanocellulose (N) (crystal length in 100-150 nm, crystal diameter in 9-14 nm and zeta potential of -35 mV with a crystallinity index of 80%) was supplied by Blue Goose Biorefineries Inc. (BGB ULTRA™, Canada) in 8.0% (w/w) suspension. Polycaprolactone (PCL, CAPA 6800) was purchased from Perstorp Holding AB (Sweden). Glycerol, acetic acid, ethanol, and other chemicals (analytical grade) were supplied from Sigma Aldrich (St. Louis, Missouri, USA).

2.2. Fabrication of Film Samples

CH film solution at 1.5% (w/w) was obtained by using aqueous acetic acid (1%, w/w). Glycerol was utilized as a plasticizer (internal plasticizer) at 0.25% (w/w). The neat CH films are very brittle and rigid; thus, a biocompatible plasticizer like glycerol was included to obtain desirable mechanical strength without changing the structure of CH. Therefore, glycerol was used to form hydrogen bonds between adjacent chains by decreasing the acetamide groups' mobility and hindering the recrystallization. G at 15% and N at 1, 2, 5% of concentration was then added to CH film solutions. The CH solutions were homogenized for 10 min with a homogenizer. The solutions were then filtered under vacuum and degassed. CH film solutions (50 g) was cast onto the plates (Ø=15 cm) followed by drying at 25°C. In preliminary studies, CH films were prepared with varying G concentration (2-20%, w/w, based on dry CH powder). The results indicated weak active properties (antimicrobial, etc.) for lower concentrations and the loss of structural integrity of CH film for higher concentrations. The similar trials were carried out for optimizing the N content inside the CH films. Higher N concentrations than 5% did not further positively affect the barrier performance and mechanical strength of CH films due to a possible aggregation of N through the film matrix. Therefore, the N concentrations of 1 and 5% was set as the limits that maintain the integrity of the film samples with an improved structure having antioxidant activity.

The compression-molding technique was used to obtain PCL films. Briefly, PCL granules were placed between two steel sheets (Teflon coated) and pre-heated at 110°C for 2 min (Qualitest Molding Press, USA). Pre-heated layers were heated at 110 °C/50 bar for 2 min and then cooled at 25 °C for 2 min.

CH films were sandwiched between two PCL film layers and then pressed at the same condition selected for PCL film production to prepare trilayer films. The amount of pressed PCL granules was adjusted to control the thickness of PCL film layers as well as controlling the thickness of trilayer films. Control film was coded as PCL/CH/PCL. G included films were denoted with PCL/CHG/PCL while N included films were coded as 1N, 2N, and 5N for 1%, 2%, and 5% of N inclusion, respectively. The obtained films were kept at desiccators having 50% relative humidity (RH) at 25°C for one week as a conditioning step. The thickness of film samples was taken from six random positions with a digital micrometer (Digimatic Micrometer Quantu-Mike IP65, Mitutoyo, Japan).

2.3. Mechanical Properties of Film Samples

Mechanical properties were determined by the ASTM standard method D882 [34] by using the strain-stress curves. The tensile strength (TS), elastic modulus (EM), and elongation (ε, %) at break values of films were determined with a universal testing machine (Lloyd LR5, AMETEK, Inc, West Sussex, UK).

2.4. Water Resistance Properties of Film Samples

The ASTM standard gravimetric method (E96-95) was used to measure the water vapor permeability (WVP) values of film samples [35]. Briefly, film samples were mounted onto permeability cups, including bi-distilled water (100% RH), and the weight of cups was taken periodically at 53% RH and 25°C.

The water uptake (WU) values of film samples (2×2 cm) were determined after the immersion of film samples into water at 25°C for 1 h. To calculate the uptake values, the initial weight of films was taken after keeping films at 105°C for 1.5 h, and the rate of change in the weight of samples was measured as the water uptake values.

Water solubility (WS) values of film samples (2×2 cm) were measured by determining the total dry content of film samples after immersion in distilled water for 1 h. The initial dry content of film samples (105°C/24 h) after and before immersed in water (25°C/24 h) were determined separately to find the soluble matter in water.

2.5. Optical Properties

The opacity of film samples was determined by dividing the absorption spectrum obtained between 400-800 nm to film thickness (AU nm/mm). The absorbance spectrum of film samples (1×4 cm) was measured via spectrophotometer (Shimadzu, UV-1601, Tokyo, Japan). The percent transmittance of film samples was taken at 450 nm wavelength with a UV–vis spectrophotometer (Shimadzu, UV-1601, Tokyo, Japan). The CIE L^*, a^*, and b^* color values were measured with Minolta Chroma Meter (CR-400, Konica Minolta, Inc., Japan) using a white standard calibration plate (Y=92.7, x=0.3160, y=0.3321) as a background.

2.6. Release Rate of Active Compounds

Two food simulants, simulant A (10% v/v ethanol) and simulant B (3% v/v acetic acid), were tested for the release behavior of G from trilayer films [36]. Film samples (50 mg) were exposed to simulant (10 mL) and stirred during the experiment. The release rates were determined by taking the absorbance at different time intervals. The measurements were performed by using the corresponding calibration curve. The release kinetics were estimated with the Korsmeyer-Peppas [37] model.

$$M_t/M_\infty = kt^n \quad (1)$$

where $Mt/M\infty$ is the fraction of active compound released at time t, k is the rate constant and n is the diffusion exponent.

2.7. Antimicrobial activity of film samples

Escherichia coli (ATCC 26922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Listeria monocytogenes* (ATCC 19115) were the test organisms chosen for antimicrobial performance analysis. The selected cultures were grown in brain heart infusion (BHI) broth for 18-24 h at 37 °C.

BHI solid media was then inoculated with a suitable dilution of grown cultures (10^5-10^6 CFU/mL). Film samples (Ø=1.5 cm) were placed onto Petri dishes, including selected bacteria to perform the zone of inhibition. The plates were incubated at 37 °C for 24 h and then examined for antimicrobial activity.

2.8. Morphology

The cross-section images of trilayer films were obtained with a polarized light microscope (Carl Zeiss, Primotech, Jena-Germany) using an imaging application (Matscope).

2.9. Statistical Analysis

The statistical analysis was performed using an analysis of variance (ANOVA) and Tukey's multiple comparison tests. The differences between samples were compared at a 95% confidence level with Minitab 17 software (Minitab Inc., Brandon, UK). Three observations were carried out for each film with three replicates.

Table 1. Elastic modulus (EM), tensile strength (TS), and elasticity (ε, %) values of film samples

Film samples	EM (GPa)	TS (MPa)	ε (%)
PCL/CH/PCL	2.20±0.12^{a}	14.02±0.34^{a}	9.67±0.08^{b}
PCL/CHG/PCL	2.15±0.11^{a}	8.63±0.65^{b}	14.69±1.20^{a}
PCL/CH1N/PCL	2.79±0.10^{a}	16.46±2.90^{a}	10.66±2.39^{b}
PCL/CHG1N/PCL	2.64±0.47^{a}	10.10±2.49^{b}	16.59±2.92^{a}
PCL/CH2N/PCL	2.92±0.14^{a}	16.07±3.98^{a}	12.00±2.36^{ab}
PCL/CHG2N/PCL	2.70±0.10^{a}	11.68±6.21^{b}	15.51±1.66^{a}
PCL/CH5N/PCL	2.63±0.01^{a}	12.13±2.89^{ab}	10.48±1.06^{b}
PCL/CHG5N/PCL	2.58±0.03^{a}	10.47±1.23^{b}	16.10±3.57^{a}

$^{a-b}$ Different letters in the same column indicate significant differences between samples ($p < 0.05$)

3. RESULTS AND DISCUSSION

3.1. Mechanical Properties of Film Samples

The mechanical properties of film samples are presented in Table 1.

It was aimed to enhance the resistance of CH films against deformation when a stress is applied, with the reinforcement of N and using PCL layers. Generally, film samples incorporated with G showed lower TS ($p<0.05$) and EM values ($p>0.05$) and higher ε values ($p<0.05$). The incorporation of G, highly hydrophilic, into a polymeric matrix might improve the chain mobility by forming continuous and cohesive matrices leading to higher flexibility [38]. The expected behavior from a composite is to observe a decrease in elasticity with an increase in strength [1]. Although there is an increase in tensile properties, the inclusion of N did not significantly alter the EM and TS values when compared with PCL/CH/PCL films. The increase in N content up to 2% increased the TS and EM values. In comparison, 5% of N negatively affected the mechanical properties, which might be due to the aggregation, resulted from reaching very high N concentrations [15]. The increase in tensile properties might be due to the efficient reinforcement behavior of N at the nanofiller and polymer interface [1]. No further improvements in TS and EM were observed after 2% N inclusion as an indication of saturation in the strength of trilayer films. On the other hand, G did not show any synergistic effect when used with N, which might be due to its hydrophilic nature leading to a possible poor interface adhesion between CH and PCL. These results are in agreement with Pereda et al. [39] who studied CH and olive oil-based films reinforced with cellulose nanocrystals. Similar results were observed by Sharmin et al. [24] for PCL and CH based trilayer films, including silane monomer. The authors reported an increment in TS and EM values while indicating a reduction in elongation, probably due to the increased stiffness by the cross-linking effect of silane. Besides, Khan et al. [1] observed an increment in the viscoelasticity of methylcellulose films enhanced with two layers of PCL films when methylcellulose concentration increased.

3.2. Water Resistance Properties of Film Samples

The water resistance properties of films are shown in Table 2.

In our preliminary studies [16], the WVP values of neat CH films reduced when N used as a filler. The hydrophilic nature of CH results from the free hydroxyl and amino groups that are highly attractive for water molecules [24]. The interaction of hydroxyl groups from CH and N might lead to lower WVP values. A similar effect was also observed in trilayer films when CH was incorporated with N. The addition of N reduced the WVP values ($p<0.05$) while the inclusion of G increased the WVP values ($p<0.05$). The decrease in WVP with the reinforcement of N might be due to the tortuous structure created with the help of the high aspect ratio of N [1]. N incorporation might also strengthen the interaction, such as physical and intermolecular, between polymer chains,

Table 2. Thickness, water vapor permeability (WVP), water solubility, and water uptake values of film samples

Film samples	***Thickness (mm)***	***WVP (g-mm/kPa-h-m^2)***	***WS (%)***	***WU (%)***
PCL/CH/PCL	0.18±0.01^{b}	0.40±0.01^{b}	14.87±0.23^{b}	47.37±5.32^{b}
PCL/CHG/PCL	0.19±0.02^{b}	0.94±0.02^{ab}	17.83±2.95^{ab}	45.76±1.19^{b}
PCL/CH1N/PCL	0.21±0.02^{a}	0.33±0.04^{c}	20.85±2.08^{ab}	58.04±2.82^{b}
PCL/CHG1N/PCL	0.25±0.02^{a}	1.04±0.32^{a}	23.03±1.08^{ab}	66.62±8.01^{ab}
PCL/CH2N/PCL	0.22±0.02^{a}	0.31±0.01^{c}	23.12±4.74^{ab}	96.50±2.22^{a}
PCL/CHG2N/PCL	0.22±0.01^{a}	0.48±0.02^{b}	25.69±0.46^{a}	89.05±1.07^{a}
PCL/CH5N/PCL	0.23±0.04^{a}	0.32±0.05^{c}	22.14±1.65^{ab}	71.61±2.08^{ab}
PCL/CHG5N/PCL	0.22±0.07^{a}	0.97±0.06^{ab}	24.92±2.44^{ab}	71.02±3.12^{ab}

$^{a-b}$ *Different letters in the same column indicate significant differences between samples (p <0.05)*

causing resistance to water molecules. The strongly hydrophilic nature of grape seed extract owing to the hydrogen bonds might cause an increase in WVP [40]. Khan et al. [1] reported similar results for PCL composite films when highly hydrophilic methylcellulose added to the formulation.

On the other hand, Khan et al. [14, 22] and Takala et al. [21] also showed enhanced barrier properties for trilayer films made of PCL as external layers and methylcellulose as an internal layer. The water solubility of film samples increased when both N and G, and N or G were added to the formulation when compared to PCL/CH/PCL films. Likewise, the incorporation of G with N higher than 2% caused a rise in water uptake values. The increase in water uptake might be due to the hydrophilicity of components, added to formulations, presented more free hydrophilic positions for hydrogen bonding of water molecules [31]. Besides, the relatively low WVP values and decreased water affinity cannot be only related to the addition of N, PCL layers are also responsible for enhanced water resistance. The external layers, PCL, protected whole film structure from water penetration; thus, WVP and WS decreased while WU increased. CH film matrix is highly soluble in water; however, PCL degrades slowly, which makes the obtained trilayer films excellent barrier candidates to be used in an aqueous medium. Similarly, Liu et al. [41] and Sun et al. [42] indicated an increase in WS values of CH film when incorporated with protocatechuic acid and young apple polyphenols. Moreover, Shi et al. [26] showed an increased water resistance ability for trilayer films made of starch (internal layer) and PCL (external layers) when compared to single film properties.

Table 3. Optical properties of film samples

	T (%)	**Opacity (AU nm/mm) x10^{-3}**	***L****	***a****	***b****
PCL/CH/PCL	0.80±0.01^{a}	3.96±0.25^{a}	90.34±1.47^{b}	-0.57±0.29^{b}	17.12±2.47^{c}
PCL/CHG/PCL	0.70±0.03^{a}	3.93±0.34^{a}	90.23±0.85^{b}	-0.68±0.24^{b}	23.87±2.22^{b}
PCL/CH1N/PCL	0.80±0.08^{a}	3.41±0.03^{a}	91.30±5.01^{b}	-1.74±0.07^{c}	11.75±0.72^{d}
PCL/CHG1N/PCL	0.50±0.01^{b}	3.21±0.23^{a}	91.06±0.26^{b}	-1.50±0.04^{c}	25.16±0.74^{b}
PCL/CH2N/PCL	0.85±0.07^{a}	3.46±0.31^{a}	93.97±0.40^{a}	-1.78±0.16^{c}	13.44±1.32^{d}
PCL/CHG2N/PCL	0.55±0.07^{b}	3.44±0.04^{a}	90.93±0.77^{b}	-0.83±0.34^{b}	22.58±2.17^{b}
PCL/CH5N/PCL	0.82±0.07^{a}	3.39±0.42^{a}	94.14±0.15^{a}	-1.63±0.03^{c}	11.40±0.26^{d}
PCL/CHG5N/PCL	0.54±0.01^{b}	3.57±0.81^{a}	88.38±0.34^{b}	0.76±0.28^{a}	29.09±0.80^{a}

$^{a-b}$ *Different letters in the same column indicate significant differences between samples (p <0.05)*

3.3. Optical Properties of Film Samples

The optical properties of film samples are presented in Table 3.

The inclusion of N and G did not significantly change the opacity of trilayer films, while the transmittance values of film samples incorporated with both G and N were the lowest (p<0.05). Moreover, as the N content increased, the transmittance values (T) slightly increased (p>0.05). Those obtained significantly lower transmittance values for G included films might be due to the heat application leading a less oriented chain plan [43]. Velickova et al. [44] found similar results for the optical properties of CH and beeswax-based multilayer and composite films. Besides, Quiles-Carrillo et al. [45] found a reduction in transmittance of multilayer polylactic acid films, including internal layers with gallic acid, probably due to the thermal post-treatment at 150°C. Zhu et al. [46] also observed significantly lower transmittance values for trilayer films of polylactic acid and gliadin. Film samples have high lightness values (>90), whereas only PCL/CHG/PCL and PCL/CHG5N/PCL films displayed lower lightness values when compared to PCL/CH/PCL film due to the more impenetrable matrix ($p>0.05$) (Table 3). The color values of film samples were probably affected by the oxidation of phenolic compounds from grape seed by heat application, as already observed by Sogut and Seydim [15]. The highest effect of oxidation was observed in PCL/CHG5N/PCL due to the highest a^* values. Even though stable opacity values were observed, presenting no significant differences between samples, film samples were susceptible to color changes with a rapid variation in a^* and b^* values. The a^* values started to decline with the increase in N content, except 5% of N inclusion, and with the addition of G, while an opposite trend was observed for b^* values ($p<0.05$). The color results showed that film samples, including G, maintained in the yellow region (lower a^* values), and that of films with only N was maintained in the green-yellow area. The b^* values of G included trilayers were significantly higher, which might be due to the heat-induced oxidation of phenolics. Similar effects have been observed by Fabra et al. [25] for multilayer films made of PCL and corn starch, including β-carotene.

3.4. Release studies

A simple semi-empirical equation can be used to understand the release behavior of an agent from a polymeric matrix. For a thin film, the first 60% of release at any time can be explained by multiplying the square root of time with a constant for Fickian diffusion. However, the other case will limit the application when the release rate is not dependent on time (zero-order). Thus, the release behavior can be explained by combining Fickian and non-Fickian mechanisms, as in the case of the Korsmeyer-Peppas model (Equation 1).

Table 4. Korsmeyer-Peppas constants for food simulant A and simulant B

	Simulant	n	k	R^2
PCL/CHG/PCL	A	0.18 ± 0.02^{b}	0.46 ± 0.01^{d}	0.79
	B	0.18 ± 0.01^{z}	0.57 ± 0.01^{z}	0.93
PCL/CHG1N/PCL	A	0.23 ± 0.01^{a}	0.60 ± 0.01^{b}	0.73
	B	0.19 ± 0.03^{y}	0.63 ± 0.01^{x}	0.81
PCL/CHG2N/PCL	A	0.16 ± 0.01^{a}	0.53 ± 0.01^{c}	0.92
	B	0.27 ± 0.01^{x}	0.64 ± 0.01^{k}	0.84
PCL/CHG5N/PCL	A	0.14 ± 0.01^{d}	0.76 ± 0.02^{a}	0.87
	B	0.29 ± 0.01^{y}	0.59 ± 0.03^{y}	0.95

$^{a\text{-}d,\ x\text{-}k}$ *Different letters in the same column indicate significant differences between samples for simulant A, and simulant B, respectively ($p<0.05$)*

Many of the release mechanisms in the film samples fall between Fickian and non-Fickian cases. Korsmeyer-Peppas model is used for both Fickian and non-Fickian behavior. Thus, release kinetics were measured for film samples, including G, by using the Korsmeyer-Peppas model. The release kinetics of the selected model estimated for simulant A and B and are shown in Table 4.

Films presented lower n values than 0.5 as an indication of non-Fickian behavior [47]. Films, including 5% of N, showed the lowest n and highest k values ($p<0.05$). Generally, the increase in N concentration caused a reduction in n and an increment in k values ($p<0.05$). The small n values indicated that obtained film samples have a partial solubility in simulant A and B. Grape seed phenolics inside the film matrix might partially diffuse from the trilayer films confirming a longer and incomplete release (not reached equilibrium). This mechanism is explained by quasi-Fickian diffusion related to the partial diffusion of active agents from the swollen film matrix because of structural rearrangements [48].

As shown in Figure 1, the N concentration significantly affected the release behavior of film samples, presenting a lower release rate with increasing N concentrations. The slower release rates obtained for higher N concentrations have an advantage in active packaging applications; especially for food products by extending their actions due to the interaction between G included layer and N. Thus, the strategy of using trilayer films to carry the valuable compounds while allowing slower release to the food surface have a potential for increasing food safety, as evidenced by Boumail et al. [10]. The release rate of G in food simulant B was faster than simulant A, which might be due to the highly soluble nature of CH in acidic solutions. Talon et al. [49] also found an increase in the release of polyphenols from CH film in simulant B. Quiles-Carrillo et al. [45] produced multilayer films of polylactic acid by electrospinning with varying deposition time and varying external layer thickness. The authors indicated a highly confined release for gallic acid due to the presence of top polylactic acid layers. Similar to our study, the external layers acted as a barrier to the release of gallic acid

Similarly, Zhu et al. [46] found a slower release of thymol from polylactic acid and gliadin based trilayer films, which might be due to the barrier effect of the polylactic acid layer.

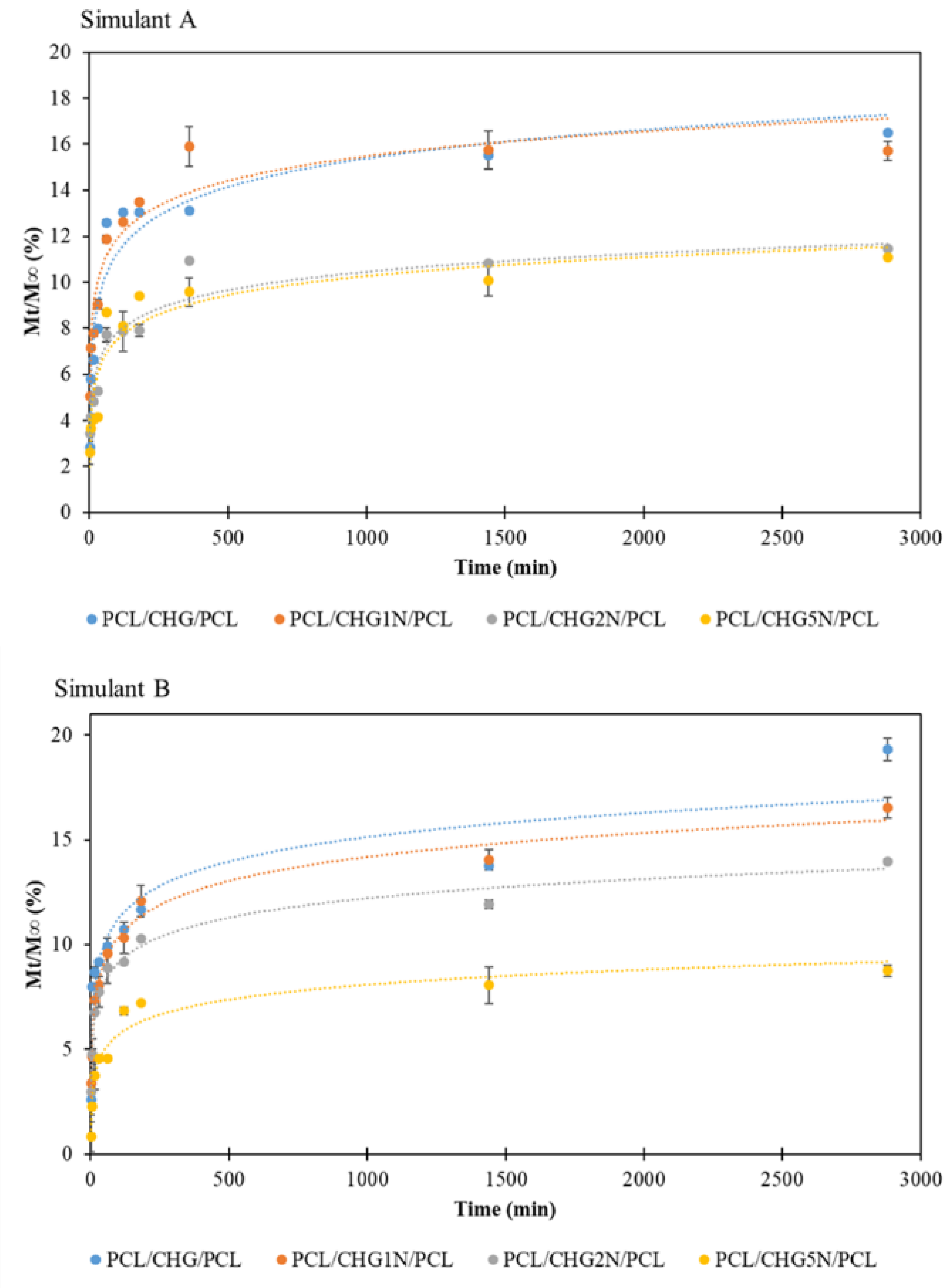

Figure 1. Release behavior of film samples through simulant A, and B.

3.5. Antimicrobial Activity of Film Samples

The zone of inhibition values of film samples is presented in Table 5.

Table 5. The zone of inhibition values (cm) of film samples

	E. coli	*L. monocytogenes*	*S. aureus*	*P. aeruginosa*
PCL/CH/PCL	*	*	*	*
PCL/CHG/PCL	** (1.8)	*	** (2)	** (2)
PCL/CH1N/PCL	*	*	*	*
PCL/CHG1N/PCL	** (1.8)	*	** (1.9)	** (1.9)
PCL/CH2N/PCL	*	*	*	*
PCL/CHG2N/PCL	** (2.2)	** (2)	** (1.9)	** (1.7)
PCL/CH5N/PCL	*	*	*	*
PCL/CHG5N/PCL	** (2.1)	** (2)	** (2)	** (2.1)

* For those films, a clear zone was not found around the film samples; however, any growth was not observed under these film samples

** For those films a clear zone was found around the film samples and the values in bracket express the inhibition zones around the film samples

CH and grape seed phenolics are also known by their antimicrobial effects [3, 30]. In the inhibition zone tests, only G included films showed zones around films because CH cannot diffuse on the agar surface. The common pathogens, responsible for food spoilage, were selected to determine the effectiveness of film samples. The typical pictures of G included trilayers against *E. coli* are presented in Figure 2.

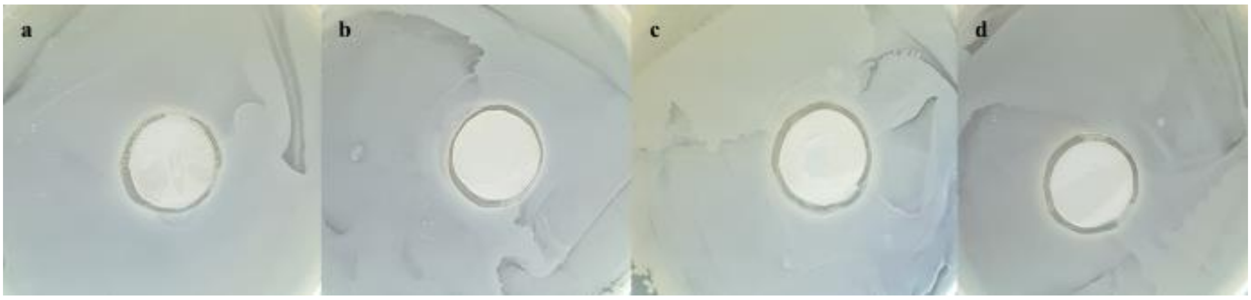

Figure 2. Antibacterial effect of G included trilayer films against *E. coli* (trilayer films including G (a), G1N (b), G2N (c), and G5N (d)).

Generally, G included trilayer films presented higher antimicrobial activity against selected bacteria, which might be due to phenolic compounds having both antimicrobial and antioxidant properties [50]. Furthermore, higher inhibition zones were detected against *E. coli* and *P. aeruginosa*, while *L. monocytogenes* was found as the most resistant bacteria. Oppositely, higher inhibition zones for *S. aureus* than *E. coli* were shown by Zhu et al. [46] for polylactic acid and gliadin based trilayer films, including thymol. Besides, no significant differences were observed between PCL/CHG2N/PCL and PCL/CHG5N/PCL films suggesting that both 2% and 5% of N inclusion with grape seed phenolics had a similar inhibitory effect. The surface polarity of Gram (-) bacteria, contains primarily of lipopolysaccharides, including phosphate and pyrophosphate groups leading to higher negative charges than Gram (+) bacteria. Thus, the more efficient activity of these films against Gram (-) bacteria than Gram (+) bacteria can be explained by the higher hydrophilicity of Gram (-) bacteria, making them most sensitive to CH.

Boumail et al. [10] observed similar behaviors for trilayer antimicrobial films made of methylcellulose and PCL. Jin and Zhang [51] also reported more notable activity against Gram (-) bacteria for polylactic acid films, including nisin. Furthermore, Alix et al. [19] observed an antimicrobial activity for PCL and starch-based trilayer films, including CH against *Pseudomonas fluorescens*. The authors stated that CH powder was more effective when used inside starch (internal layer) when compared to the formulations, including CH inside the PCL layer (external layer).

3.6. Morphology

Optical microscopic images were taken for morphological inspection of the cross-section of trilayers. The effect of G and N in at the interface of the internal layer was examined. The images of PCL/CH2N/PCL (a) and PCL/CHG2N/PCL (b) films are presented in Figure 3 (the images of other film samples have similar observations).

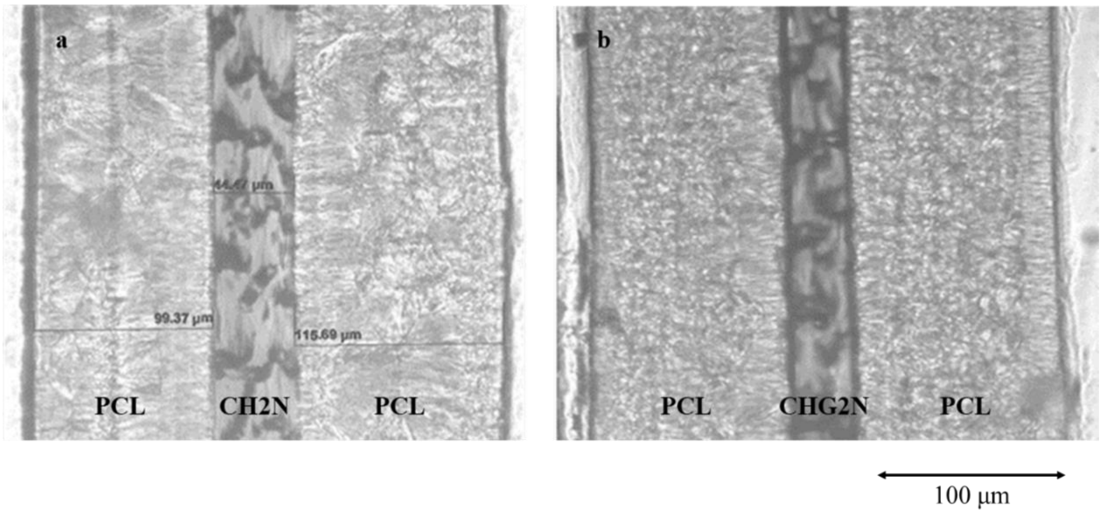

Figure 3. Cross-section images of PCL/CH2N/PCL (a) and PCL/CHG2N/PCL (b) films

PCL/2N/PCL film sample represented a smooth surface at the interface, indicating a better adhesion between layers. However, G included film showed heterogeneous areas with small cavities at the interface, which resulted in less adhesion between layers. The addition of grape seed phenolics into CH films might produce a rougher surface that affects the adhesion between layers when the heat was applied. Boumail et al. [10] reported less smooth and serrated shapes for methylcellulose and PCL based multilayer films when incorporated with bioactive agents. Moreover, Quiles-Carrillo et al. [45] observed that the electrospun interlayers of polylactic acid-containing gallic acid had little porosity. The authors stated that multilayers still had fibrilar morphology in the internal layers even after thermal post-treatment, which might be due to the high thickness of the inner layer, leading an insufficient heat transmission.

6. CONCLUSION

The addition of grape seed phenolics and nanocellulose significantly affected the physical properties and antimicrobial activity of films. Grape seed phenolics promoted an increase in WVP, WS, and WU values due to its highly hydrophilic nature. G included CH films presented higher opacity, and lower L^* values. Nanocellulose improved the tensile properties of film samples while causing a decrease in the elasticity of film samples. Trilayer films showed antimicrobial activity against selected bacteria. Thus, these films offer the possibility to control food deterioration, such as spoilage or oxidation, by providing a slower release of bioactive compounds. The results indicated that the further increment in nanocellulose concentration adversely affected the mechanical and barrier properties of film samples. Thus, lower concentrations than 5% of nanocellulose would be more suitable for the formation of CH based films. In this study, trilayer films were fabricated by a simple method to enhance food safety with increased shelf life. However, further tests should be carried out on the effects of trilayer packaging for food products and food products inoculated with pathogenic microorganisms during storage.

ACKNOWLEDGEMENT

The authors acknowledge the financial support provided by Suleyman Demirel University Scientific Research Projects Coordination Unit (Projects OYP-5273-DR-12 and OYP-4914-D2-17). The authors are also thankful to Assoc. Prof. Dr. Ali DURMUŞ for his valuable contributions.

DECLARATION OF ETHICAL STANDARDS

The author(s) of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

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