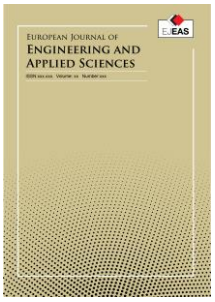
European Journal of
Engineering and Applied Sciences

ISSN: 2651-3412 **Journal homepage:** dergipark.gov.tr/EJEAS

Published by Çorlu Faculty of Engineering, Namık Kemal University



Original Research Article

Blend Films of Cellulose and Soy Protein Isolate Prepared from Gamma Irradiated Solutions

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Received: 04.12.2018 *Accepted: 31.12.2018*

Abstract: Blend solutions of cellulose and soy protein isolate were prepared by utilizing an amine/salt solvent system. The solutions were gamma irradiated before casting blend films that have more stable molecular network than the ones cast from non-irradiated solutions. Due to irradiation, the interactions between the two polymers altered and shifted that were analyzed by infrared spectroscopy. The thermal analysis showed small differences of thermal stability between the films formed from irradiated and non-irradiated solutions. Up to 10 kGy irradiation dose on the solutions, the resulting films exhibited higher elongation at break. Furthermore, their transparency lowered. Despite their transparency, the films absorbed less water indicating the effect of gamma irradiation on the molecular structure.

Keywords: *Blend film, Cellulose, Gamma irradiation, Soy protein.*

Selüloz ve Soya Proteininin Gama Işınlanmış Solüsyonlarından Hazırlanan Karışım Filmler

Özet: Selüloz ve soya proteini izolatının harman çözeltileri, bir amin/tuz çözücü sistemi kullanılarak hazırlandı. Gama ışınıyla ışınlanan çözeltilerden harman filmlerin oluşturulmuş ve ışınlanmamış filmlerden daha kararlı moleküler ağa sahip oldukları gözlenmiştir. Işınlama bağlı olarak, kızılötesi spektroskopi ile analiz edilen iki polimer arasındaki etkileşimler değişti ve yayıldı. Termal analiz, ışınlanmış ve ışınlanmamış çözeltilerden oluşturulan filmler arasında küçük termal kararlılık farkları gösterdi. 10 kGy ışınlama dozu uygulanan solüsyonlardan elde edilen filmler kopmada daha yüksek uzama göstermiştir. Dahası, filmlerin şeffaflıkları azaldığı gözlendi. Şeffaflıklarındaki değişime rağmen, filmler, gama ışımasının moleküler yapı üzerindeki etkisi dolayısıyla daha az su emdiler.

Anahtar kelimeler: *Gama ışınımı, Karışım filmler, Selüloz, Soya proteini.*

Geliş: 04.12.2018 *Kabul: 31.12.2018*

1. Introduction

The two common methods to process blended polysaccharides and proteins into films and membranes are solution casting and thermal processing. Since the focus in this research is to produce highly miscible blends with strong interactions between cellulose and proteins, the latter is applied by employing a cosolvent, ethylene diamine/potassium thiocyanate (ED/KSCN). Because it is very effective and practical, the commonly adopted method to make films is to manually spread dilute film solutions (typically 5-10 wt% concentration) of biopolymers on an appropriate platform. Then, depending on the solvent, either coagulation before drying or directly drying under controlled conditions takes place. With technologic equipment, wide and long films with

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a given thickness can be mechanically produced [1].

The quality of blend films is governed by the composition of film forming solution, casting conditions including post and/or pretreatments, and drying method. For instance, the composition of biopolymers in solution strongly affects the cellulose-protein interaction and their miscibility and thus the properties of resulting film [1]. In solution casting by coagulation, gel sheet formed by spreading the solution must have a fixed casting thickness. The composition and temperature of coagulant must be the same for every gel sheet. Drying conditions, such as the substrate on which the films dry, drying time, ambient temperature, airflow, must be constant in order to obtain reproducible results.

Tailoring films by beginning from a blend of proteins, polysaccharides and/or lipids is a useful approach to take advantage of the functional properties of each component. In blend films, proteins significantly contribute to tensile properties by functioning as binder for structural integrity [1]. However, proteinaceous films could be poor water resistance and thus lose their integrity upon exposing to water. In particular, globular proteins and water-soluble proteins including collagen hydrolysate and gelatin are the proteins showing such a weakness. Modification of these proteins by chemical, enzymatic and physical methods has been applied to enhance primarily their stability in water as well as their tensile strength.

The chemical modification has included crosslinking agents from aldehyde family (Figure 1), especially formaldehyde (also mentioned for the plastics of casein and soy protein), glutaraldehyde and glyoxal as the common ones. The enzymatic modification has mainly focused on an enzyme called transglutaminase for its ability to crosslink proteins. The enzyme and glyoxal chemical treatments were effective in improving the stability of proteins in water but not the tensile properties. Although formaldehyde and glutaraldehyde were found to be efficient for enhancing such properties, their toxicity must pose the problem of the aldehyde residues remaining in final applications, i.e. food [2] and biomedical [3]. In contrast to these treatments, gamma (γ) irradiation could be an alternative method for crosslinking proteins [4].

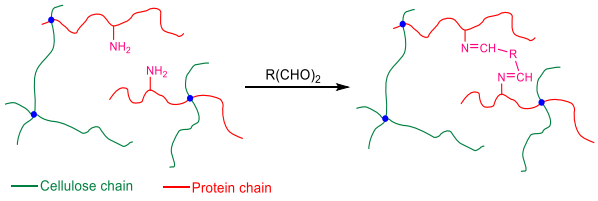

Figure 1. Illustrated crosslinking of protein network to form network structure via dialdehyde mediated interchain crosslinking (redrawn and modified) [1]

Yidan Zhu [5], in Dr. Kotek's research group, continued the research of Douglass to improve the functional properties of cellulose/soy protein concentrate (SPC) films. Even though expected results from crosslinking the films were achieved in terms of the stability of the films in water and the mechanical properties, the appearance and size of the films were severely affected.

1.1. Gamma (γ) Irradiation

Gamma-irradiation alters the conformation of proteins, oxidize the amino acids, disrupt the covalent bonds and generate protein free radicals. Crosslinking, aggregation, fragmentation, and oxidation by oxygen radicals formed in the radiolysis of water are confirmed chemical changes in irradiated proteins. In a film forming solution, the irradiation activates OH^- and O_2^- radicals that could react with molecules of proteins. As a result, covalent cross-linkages are formed in the solution causing functional and structural changes in proteinic films [6-9].

Exposure to radiation was a useful way of creating crosslinks in edible films from soy, casein and whey proteins. Their protein structure was moderately altered to a more stable and more ordered form [10]. Ouattara et al. [11] reported that gamma-irradiation increased the concentration of milk proteins with high molecular weight in the film forming solution. The authors also suggested two hypotheses to explain the effect of the irradiation: (1) more amino acids from proteins with different physicochemical properties participate in intermolecular interactions (2) covalent crosslinks in and between the polymer chains are formed in the film forming solution.

Lee et al. studied the effect of γ-irradiation on SPI. Irradiation doses up to 50 kGy were applied to the film forming solution of SPI. The water vapor permeability of resulting films decreased about 10% at 4 kGy dose and the tensile strength was doubled at 50 kGy [9]. Xu et al. [12] added starch solution into SPI solution both of which were modified with similar γ-irradiation doses. The water absorption capacity of the resulting blend films decreased almost linearly with increasing irradiation doses. It was concluded that the viscosity of blend solution did not decrease more above 30 kGy due to crosslinking of the proteins/starch and aggregation of the polypeptide chains. This resulted in an increase in water resistance and tensile strength and a surface that was more glossy and smoother.

The effect of gamma-irradiation on cellulose is of interest in our research since the blends that will be produced are all cellulose-based. The irradiation on the polymer generates cellulosic substrates by degradation [13]. While high doses degraded cellulose thereby decreasing the DP and the crystallinity, crosslinking was observed at low doses of radiation (10< kGy) [14]. At any extent, this could highly affect the structure and the reactivity and thus the physical and chemical properties [15]. As a result, properties, such as low elasticity, low wet strength, low adhesive capacity to some materials, and low shape stability, can be improved [14].

The objective of this study is to prepare blend films from cellulose and globular proteins, particularly soy protein, by utilizing gamma irradiation to stabilize the resulting films. The treatment at various doses is applied to the film forming solution that contains the both polymers at the same composition. The physicochemical properties of the films will be analyzed and compared in order to understand the effect of the gamma radiation. The blend films that are produced by solution casting method can be used for food packaging and biomedical applications.

2. Materials

The amine/salt system is composed of 65 wt% ethylenediamine (ED) and 35 wt% potassium thiocyanate (KSCN), both are reagent grade and purchased from Sigma-Aldrich. KSCN was first dried overnight in a vacuum oven at 60°C to remove excess moisture. A known weight of that was slowly added into the corresponding weight of ED while mixing. Then, the mixture of ED and KSCN were stirred by slightly heating until the salt was completely dissolved. Finally, the solvent was left to cool and to equilibrate.

Cellulose was received from Buckeye Technologies Inc. as sheets of dissolving wood pulp that was an acetate grade, pressed, refined and bleached. The sheets were shredded in a grinder forming cellulose crumbs. The obtained cellulose was coded as Buckeye VFC cellulose by the company and it has a DP of ca. 400. Soy protein isolate (PRO-FAM 955®) containing 93.8% dry basis protein was provided by ADM Specialty Products-Oilseeds. Prior to dissolution, the cellulose and the soy protein isolate (SPI) were dried overnight in a vacuum oven at 60°C to eliminate undesired moisture.

Methanol used as a coagulant was ACS reagent grade and provided by BDH Industries Ltd. The tools for film casting were polyester transparent PET films, Teflon films, a glass casting board, and a stainless steel bar with 5 – 50 mil casting thickness that were all obtained from Byk-Gardner.

3. Preparation of Film Forming Solution

3.6 g of ground cellulose, 2.4 g of SPI, and 94 g of ED/KSCN were all added into a three-necked round bottom flask to obtain a solution with 40% protein content. The center neck of the flask held a glass stirring rod with a Teflon© blade inside the flask. The other ending of the glass rod was attached to an electric motor for stirring. One of the side necks was attached to a water-cooled condenser with nitrogen flowing inside to prevent the evaporation of ED from the solvent. The other side neck was simply plugged with a glass stopper.

The flask was kept in an oil bath at 90°C and the cellulose-SPI-ED/KSCN mixture was stirred for 2 – 3 hrs until complete dissolution. The obtained blend solution was allowed to cool down to room temperature, and then heated back to the same temperature to transfer into a glass jar. A total of four jars of solution were prepared for exposing to gamma radiation.

The jars were placed in the ^{60}CO gamma radiation chamber of Nuclear Engineering Department in North Carolina State University. The blend solution in four jars was irradiated at the doses from 5 – 20 kGy with a 5 kGy increment. The dose rate was 0.71 kGy/day and the irradiation was performed at ambient conditions.

4. Film Casting

Each jar with the irradiated solution was heated to ensure a steady flow. A little irradiated solution was poured onto a PET film that was held on a glass casting board. Then, the solution was dragged by using the casting bar with 25 mil casting thickness to obtain gel sheets, a form of film prior to coagulation. These sheets handled with the PET films were immediately transferred into a methanol bath. The coagulation of the sheets could be observed within several seconds turning into wet films.

The wet films were kept in the first coagulation bath for at least 15 min. Subsequently, they were soaked into a fresh coagulation bath for no less than 20 min. This coagulation step was repeated 3 times total to remove all residues of the ED/KSCN solvent. After the last coagulation bath, the films were stacked and separated by Teflon films between two flat rectangular glasses. A brick was placed on top applying an even pressure to obtain uniform films. This stack of films with the applied pressure were left to dry in a vacuum oven for 24 hrs at ambient temperature. Two additional days of drying between 40 and 50°C was also applied producing completely dry and uniform films. The resulting films were coded as CS0, CS5, CS10, CS15, CS20. The numbers in the codes represent the radiation doses to which the film forming solutions were exposed.

5. Characterization Techniques

Fourier transform infrared spectroscopy (FTIR) of all films was measured with a Thermo Electron Nexus 470 FTIR with OMNIC software analysis. FTIR spectrum of each film was obtained between 4000 and 500 cm^{-1} wavenumbers in absorbance mode over 64 scans at the resolution of 4 cm^{-1}. The obtained raw data was plotted using OriginPro 9.1, an analysis and graphing software.

For accurate tensile data, we opted to test 5 films from each composition. To condition the films, they were left overnight in the testing facility. The thickness of each film to be tested was measured 10 times with the Thwing-Albert Electronic Thickness Tester. Following this, each individual film was cut into 4 strips with the same width (1/2 inch) and taped on both ends 5 cm apart. This produced 20 strips for each composition, resulting in a total of 100 strips to be tested.

The tensile testing was carried out using the MTS Q-test Tensile Testing Machine and performed in accordance with ASTM D882: Tensile Testing of Thin Plastic Sheeting. Testing used the 250 lb. load cell, 50 mm gauge length, and 10 mm/min initial speed.

Thermo-gravimetric analysis (TGA) was utilized to study the changes of mass of the investigated polymer systems against increasing temperature. It was performed on the Perkin Elmer TGA under a dynamic nitrogen atmosphere at a heating rate of 20°C/min from 20°C to 550°C using ca. 5 mg samples of each material. Post analysis was performed using the raw data on Origin 8.5, a data analysis and graphing software.

Light transmission test was performed on a Varian Cary 3 UV-Vis Spectrophotometer between the wavelength of 360 and 700 nm. A small area of each film was secured onto the wall of the sample holder. Transparency was assessed from the light transmitted through the films relative to air.

Water absorption capacity of the films were examined to determine the amount of water absorbed over a 24 h period. Each film was pre-weighed, soaked in deionized water for 24 h, and then weighed again to calculate the increase of mass in percentage.

6. Results and Discussion

6.1. Chemical structure

The blend films from irradiated solutions exhibited similar absorption peaks to the control one. Figure 2 represents the FTIR spectra of all films in the wavenumber range of 3750–2750 cm^{-1} and 1750–750 cm^{-1}. The stretching vibration of hydroxyl groups (OH) at 3370 cm^{-1} was predominant for all films. The peaks at 1649 and 1543 cm^{-1} for the control one indicated on the spectrum are amide I (C=O stretching) and amide II (N–H bending) bands, respectively. Although there is no shift occurred for the amide I peak, its intensity gradually decreased by increasing irradiation dose. In addition, the amide II band shifted to higher wavenumber due to the gamma irradiation. Both changes of amide peaks are a result of the reorganization of the interaction between the two polymers, the blend solution of which were exposed to the irradiation.

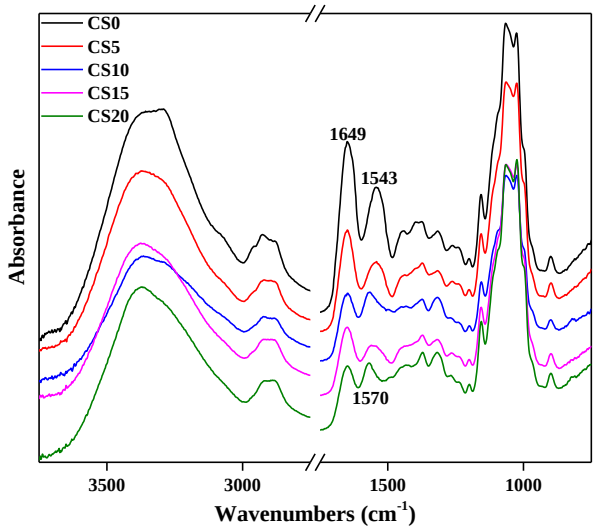

Figure 2. FTIR spectra of all blend films

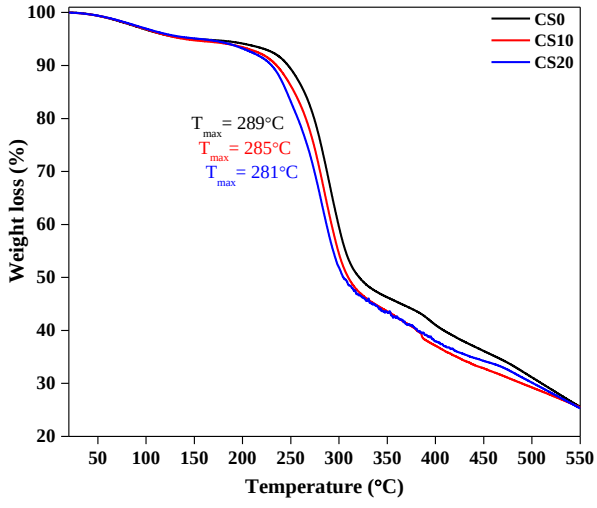

Figure 3. TGA thermograms of the films from irradiated control solutions.

6.2. Thermal properties

Figure 3 shows the percent weight changes of the irradiated blend films by increasing temperature. The maximum degradation temperature of non-irradiated films somewhat decreased by increasing irradiation dose. Although it was not significant, the thermal stability of the films was influenced by the film forming solution exposed to gamma radiation. Unlike chemical crosslinking, this result is expected from γ-irradiation, which causes polymer degradation.

6.3. Tensile properties

The thicknesses of blend films were not significantly different from each other as the same casting thickness was applied to every film forming solution. The tensile properties of blend films shown in Table 1 had diverse changes for each parameter upon increasing the irradiation dose.

Table 1. The tensile properties of all films

Films	Modulus (GPa)	Tensile strength (MPa)	Elongation (%)	Thickness (mm)
CS0	2.86 ± 0.23	55.7 ± 7.8	14.5 ± 8.5	0.028 ± 0.001
CS5	2.91 ± 0.27	56.2 ± 6.9	20.6 ± 7.2	0.028 ± 0.002
CS10	2.46 ± 0.26	56.4 ± 6.9	25.9 ± 7.4	0.027 ± 0.003
CS15	2.93 ± 0.23	49.8 ± 5.9	11.9 ± 7.5	0.027 ± 0.002
CS20	2.12 ± 0.14	34.2 ± 3.9	13.7 ± 7.1	0.033 ± 0.001

The effect of gamma irradiation dose on tensile strength and the elongation at break is demonstrated by Figure 4, which is a plot of the data from Table 1. As indicated in the figure, the tensile strength showed a very subtle increase whereas the percent elongation almost doubled at 10 kGy dose. According to Pruzinec et al. [14], crosslinking was observed below such dose for cellulose thus improving the elasticity of the film network. The protein in the same network also participated in the crosslinking. As already mentioned, the irradiation yields polymer radicals in proteins that can associate with two hydrogen atoms. It is likely that alkyl radicals in the irradiated blend solution reacted with one another through hydrogen-bonding crosslinks. They could also interact with the free radicals of the end of polymer chain to form terminal-crosslinks [12]. Hypothetically, more amino acids participating in intermolecular interactions [11] with cellulose contributed to the increase in both the tensile strength and the percent elongation. Furthermore, above 10 kGy doses, the films lost significant strength and elasticity as expected due to the degradation of cellulose.

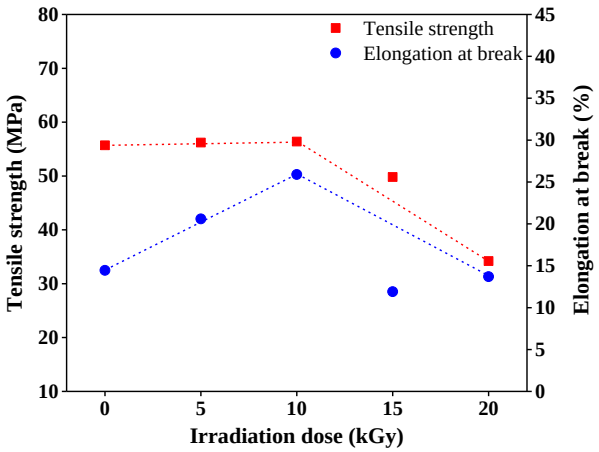

Figure 2. Effect of γ-radiation dose on tensile strength and elongation of the blend films with 40% SPI

6.4. Transparency

The light transmission provides information about the transparency of the films in the visible light spectrum from

400–700 nm wavelength. As plotted in Figure 5**,** the transmission decreases with a decreasing wavelength.

The control film had around 87% light transmission at 700 nm and remained about the same through 500 nm. On the other hand, the gamma irradiation treated samples were clearly showed a lower light transmission for the same region. Therefore, the visibly observed transparency change was also confirmed by the light transmission indicating the effect of irradiation.

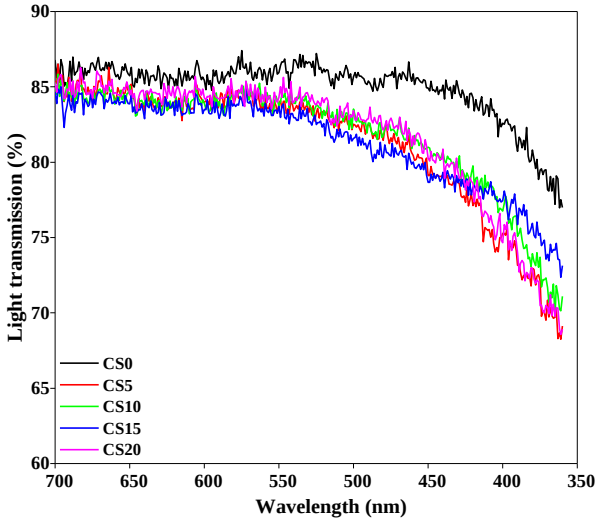

Figure 5. Effect of γ–irradiation on the light transmission of the blend films

6.5. Water absorption

Figure 6 illustrates the changes in water absorption capacity of the films by increasing irradiation dose. The control film absorbed about 62% water that dropped to 46% after exposing to 10 kGy dose of irradiation indicating certain degree of crosslinking between the cellulose and soy protein isolate. Increasing the irradiation dose above that point did not show significant influence in the capacity.

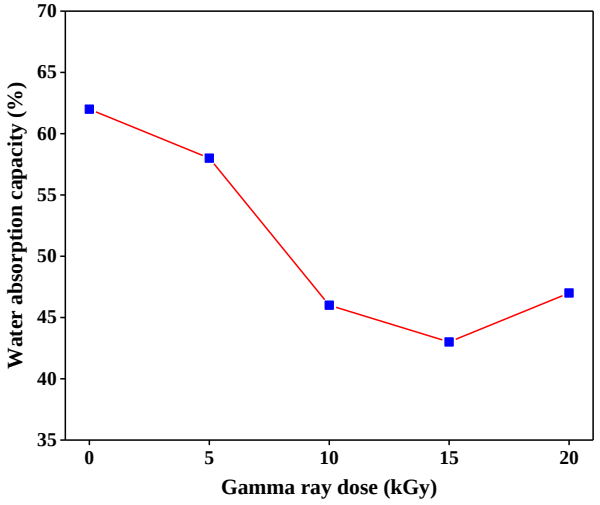

Figure 6. Influence of γ–irradiation on the water absorption capacity of the blend films

The chemical crosslinking with glutaraldehyde also resulted in reduced water absorption capacity for cellulose/soy protein concentrate films [5]. Furthermore, Xu et al. [12] reported gradual decrease in the water absorption by increasing irradiation dose for SPI-based films.

7. Conclusions

Uniform and strong cellulose/soy protein isolate films were produced using the ED/KSCN solvent system. Gamma irradiation was applied to the film forming solutions to stabilize the molecular network structure of the blend films. The interaction between cellulose and soy protein, exhibited by FTIR spectra, was rearranged after exposure to the irradiation. The TGA analysis revealed insignificant changes in the thermal stability. The irradiation up to 10 kGy led to higher elongation at break in the resulting films. Moreover, the transparency of the films somewhat decreased. However, the water absorption capacity significantly decreased.

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