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SHELLAC RESIN EFFECT ON THE PROPERTIES OF ZEIN FILM
EFEKAT ŠELAK SMOLE NA OSOBINE ZEINSKOG FILMA

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ABSTRACT

In this paper, zein-based films with the addition of shellac were synthesized by lamination on the existing dry zein film (L samples) and by adding shellac alcohol solution during the synthesis of zein film in a ratio of 50-50 by casting process (M samples). Zein films without the addition of shellac were designated as control samples. Physico-chemical (thickness, moisture content and solubility), mechanical (tensile strength and elongation at break) and barrier characteristics (water vapor permeability) were examined for all samples. The resulting films were transparent, glossy, light yellow (control) to ocher (samples with shellac), flexible, and non-sticky. The results showed significantly higher values of elongation at the break of the samples with shellac (11.49% for M and 18.99% for L) compared to the control (7.14%). Significantly lower water vapor permeability values were found, 10.04 g/(m^2·h) for L, and 18.41 g/(m^2·h) for M, compared to the control pure zein film (40.33 g/(m^2·h)).

Keywords: *shellac resin, zein film, properties.*

REZIME

Značaj biopolimernih materijala, u kontekstu redukcije otpada, zaostalog nakon upotrebe konvencionalne ambalaže, uz upotrebu obnovljivih sirovina za njihovo dobijanje opravdava intenzivna istraživanja prirodne, razgradive ambalaže. Zein je hidrofoban protein koji se nalazi u kukuruzu i u ovom radu sintetisani su i okarakterisani filmovi na bazi zeina. Velika mana biopolimernih filmova je njihova propustljivost vodene pare i gasova. Visoka hidrofobnost lipidnih komponenata u vidu voska, ulja, smola, koja ih čini nerastvorljivim u vodi, a rastvorljivim u tipičnim organskim rastvaračima, objašnjava zašto su voskovi najefikasnije prepreke za transfer vodene pare. Zbog toga je zeinskim filmovima dodata šelak smola. Šelak je dodat na dva načina: (1) laminacijom na postojeći suv zeinski film (L uzorci), (2) dodatkom šelak alkoholnog rastvora prilikom sinteze zeinskog filma u odnosu 50-50 postupkom razlivanja (M uzorci). Zeinski filmovi bez dodatka šelaka su označeni kao kontrolni uzorci. Svim grupama uzoraka ispitane su fizičko-hemijske (debljina, sadržaj vlage), mehaničke (zatezna jačina i izduženje pri kidanju) i barijerne karakteristike (propustljivost vodene pare). Dobijeni filmovi su transparentni, sjajni, svetlo žute (kontrola) do oker boje (uzorci sa šelakom), fleksibilni, nelepljivi. Rezultati su pokazali značajno veće vrednosti izduženja pri kidanju kod uzoraka kojima je dodat šelak, 11.49% za M i 18.99% za L uzorke u poređenju sa kontrolom (7.14%). Ove vrednosti su veće kod laminiranih uzoraka. Takođe, konstatovane su značajno manje vrednosti propustljivosti vodene pare 10,04 g/(m^2·h) kod L uzoraka, 18.41 g/(m^2·h) kod M uzoraka, u odnosu na kontrolni zeinski film (40.33 g/(m^2·h)).

Ključne reči: *šelak smola, zein film, svojstva.*

INTRODUCTION

Zein is an environmentally friendly material, recognized as safe for food applications (*Weissmueller et al., 2016*). It is generated as a co-product when corn grains are processed for food, feed, agriculture products, and fuel (*Reddy and Yang, 2011*). Among biopolymers, zein possesses a unique hydrophobic character. Practically zein with hydrophobic and hydrophilic groups in its structure has a market amphilphilic character. It is an ideal alternative candidate to synthetic polymers.

Due to its renewable qualities, non-toxicity, low water vapor permeability, grease/oil proof, biodegradability, and biocompatibility, biopolymer zein has garnered a lot of interest from the scientific community (*Ibrahim et al., 2019*). Zein's capacity for polymerization enables the production of membranes and films that can serve a variety of functions in place of synthetic plastics. Several zein-derived compounds are being used in food, medicine, cosmetics, coating agents, and adhesives (*Jaski et al., 2022*). Extrusion, spin casting, and solvent casting are all simple methods for making zein films. Zein produces biodegradable films with good tensile and water-barrier qualities when it is either dissolved in aqueous ethanol or aqueous acetone (*Kasai, 2018*). Solvent casting, similar to the tablet-coating process, requires that zein be dissolved in a hydrated organic solvent, followed by drying at room temperature or under specified conditions, which generally involves the development of hydrophobic, hydrogen and limited disulfide bonds between zein chains. It has been suggested that the high strength and low gas permeability of the film, as well as the high hydrophobicity of zein, compared to biopolymer films from other sources, are due to the capacity of zein molecules to combine with one another and create a meshwork structure during the film casting process (*Yong et al., 2015*).

Zein derives desirable properties that make it a promising ingredient in the formulation of biodegradable films, bioplastics, fibers, and the delivery of bioactive compounds for various applications (*Giteru et al., 2021*). Zein derivatives in novel applications preserve perishable products, including fruits, vegetables, dairy, meat and nuts. Zein coatings carrying bioactive compounds such as phenolic acids were also shown to improve the nutritional quality of food products and even add sensory characteristics such as color and shine, perceived as desirable by consumers (*Jaski et al., 2022*)

In order to create new or improved functional properties, scientists often modify food proteins using various treatments (physical, chemical or enzymatic) that change its conformation and structure, and consequently its physicochemical and functional properties (*Glusac and Fishman, 2021*). An appropriate polymer film for coating application generally requires both ideal mechanical and barrier properties, such as elongation, tensile strength, and water and gas permeability

(*Yong et al., 2015*). To improve the mechanical and barrier properties of zein films, with the aim of approaching commercial polymer materials in terms of properties, they can be laminated/blended with other materials (biopolymers, essential oils, resins, waxes, etc.). Commonly, these are lipid components that are added to the film-forming solution, which improve functional film properties by increasing the hydrophobicity of the film.

Shellac is a physically enhanced version of lac resin, an animal-derived natural biopolymer obtained from small insects that feed on the sap of particular host trees. In its purest form, shellac is a polyester macromolecule made of polyhydroxy aliphatic and sesquiterpene acid inter- and intra-esters (*Thombare et al., 2022*). A refined lac product called shellac has been granted GRAS approval by the USFDA and EU with the E number E904 for use as a food additive (*Srivastava and Thombare, 2017*). Due to its special qualities, it has a wide range of uses in the pharmaceutical and food industries (*Jo et al., 2014; Chitravathi et al., 2014; Stummer et al., 2010*).

Shellac has excellent film-forming and binding properties that, combined with its bio-compatible nature, make it an excellent choice as a coating agent. Coating various fruits and vegetables with water-resistant material has been found effective in extending their post-harvest shelf life. Because of the increasing awareness about natural products and evidence of health issues with synthetic waxes used for coating, shellac or shellac-based coating has been gaining popularity (*Du et al., 2019*).

Shellac is a hard amorphous resin that contains small amounts of wax, yellow coloring matter, and odiferous matter. The color of shellac ranges from amazingly light blonde to greatly dim brown, with numerous assortments of brown, yellow, orange, and ruddy in between. The color is impacted by the sap of the tree from which the lac is gathered and the time of collection (*Yuan et al., 2021*). Shellac is insoluble in water, glycerin, hydrocarbon solvents, and esters but solvent in alcohol and natural acids (*Ghoshal et al., 2009*).

Shellac is a degradable film-forming agent with good barrier properties to achieve food protection. However, the poor mechanical strength of shellac films greatly limits their application. Fortunately, shellac modification or the preparation of shellac-based composite films addresses these shortcomings (*Soradech et al., 2013*). Alternatively, shellac could be applied as a layer on other kinds of biodegradable film or as part of composite film, thereby enhancing the performance of these films.

The aim of this paper was to produce zein-shellac films by lamination and by making a composite film. Further, the aim was to characterize the properties of synthesized biopolymer films based on zein and shellac obtained by lamination of one layer of biopolymer on another (L) and composite films obtained by mixing filmogenic solutions (M). Plain zein film was used as a control (C) in order to compare obtained results and propose a more suitable film preparation method.

MATERIAL AND METHOD

Control film, labelled as C, was obtained by Šuput et al. (2017) method. Corn zein (Acros Organic, Belgium) was dissolved (10% w/v in 85% ethanol) and 0.5 g PEG 400/g zein was added and stirred until complete dissolution. Film forming solution was heated in a water bath at 80°C, poured into Petri dishes (10 g per dish) and dried at room temperature.

Shellac was dissolved (10% w/v in 85% ethanol) overnight at a magnetic stirrer and as dissolved, it was ready to be applied. The same amount of zein and shellac solution was mixed and poured into Petri dishes and dried at room temperature. In this way a composite film was obtained, labelled as M. Laminated film, labelled as L, was obtained when zein film was formed (same as control) and when it was dried the same amount of shellac solution was poured (laminated) over it and eventually dried.

Tensile strength (TS) and elongation to break (EB) were measured on the Instron Universal Testing Instrument Model No 4301 (Instron Engineering, Canton, Massachusetts, USA), according to the standard method EN ISO 527-3:1995. Film samples were cut into rectangular strips (15x80 mm). The initial grip separation was set at 50 mm, and the crosshead speed was set at 50 mm/min. TS and EB measurements for each sample were repeated eight times.

Moisture content was determined according to the Rhim et al. (2002) method. Film samples (1x1cm^2) were weighed and dried in an air-circulating oven at 105 °C for 24 h. Moisture content (MC) was determined as the percentage of weight reduction during film drying, expressed on the total weight of the film (1):

$$MC(\%) = 100 \cdot \frac{(m_2-m_1)-(m_3-m_1)}{m_2-m_1} \quad (1)$$

where: m_1- mass of measuring vessel, m_2- mass of film samples with measuring vessel prior drying, m_3- the mass of dried film samples with measuring vessel.

Right after determining the moisture content, the film samples (1x1cm^2), dried to the constant mass, were used to determine the film solubility according to the method of Popović (2013). Film samples were immersed in deionized water at room temperature for 30 min, with stirring. After 30 min, excess water was decanted and samples were dried in the oven, for 60 min, and weighed (m_4). The solubility in water of the examined films was calculated (2):

$$\text{Solubility}(\%) = 100 \cdot \frac{(m_3-m_1)-(m_4-m_1)}{m_3-m_1} \quad (2)$$

where: m_1- the mass of measuring vessel, m_2- the mass of film samples with the measuring vessel prior to drying, m_3- the mass of dried film samples with the measuring vessel, m4- the mass of dried film samples with the measuring vessel after immersion and drying.

Water vapor barrier properties of films were determined gravimetrically according to the standard method ISO 2528:1995. Three replicates of each sample were tested simultaneously.

Statistical analysis was carried out with the software package StatSoft Statistica ver. 10.0. All data were presented as mean values with their standard deviation indicated (mean ± SD). Variance analysis (ANOVA) was performed, with a confidence interval of 95 % ($p < 0.05$). Means were compared by the Tukey test.

The mean values' color plot diagram for all observed variables was developed by R software v.4.0.3 (64-bit version). The corrplot instruction was applied, with the "color" method, as a graphical tool to represent the correlation between the tested responses of observed samples.

RESULTS AND DISCUSSION

Based on the visual inspection of the obtained films, it was first noticed that the control zein film was light yellow in color, while the films with added shellac were darker brown in color. (Figure 1). All films were shiny and transparent. The obtained films had good tactile characteristics: they were firm and flexible, not greasy or sticky. The M sample was distinguished according to the appearance of the surface: its surface was not compact and homogeneous, but rough.

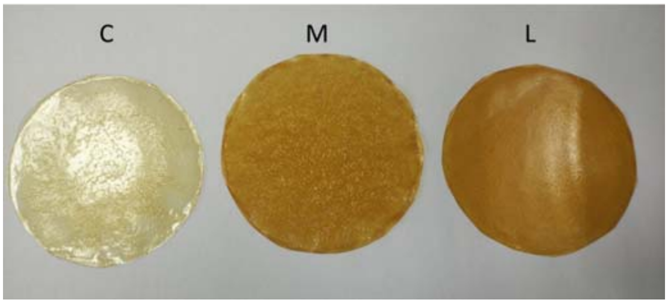

Fig. 1. Visual examination of zein film (C), composite zein/shellac film (M) and laminated zein/shellac film (L)

The results of mechanical, barrier and physico-chemical properties are shown in Table 1. The results of Table 1, show there is a statistically significant difference between the elongation at break (%), water vapor permeability (g/(m^2·h)) and solubility degree (%) for the observed film samples.

Based on the obtained results, it can be concluded that the addition of shellac has a favorable effect on the mechanical properties because, in the case of the composite film (M), an increase in tensile strength is observed, which means that the M are the strongest, which means that the highest force value is required for their cut. In the case of the L, the highest value of elongation at break was found, which indicates that these samples are the most elastic. The obtained results are in agreement with another study that also reported that incorporating zein into chitosan film could result in a rougher, more elastic and softer film structure with lower water vapor permeability compared with a single chitosan film (*Escamilla-Garcia et al., 2013*). In another study, Soradech et al. (2012) found that combining individual properties of shellac–gelatin composite films contributed to the improvement of many characteristics: the strength, flexibility, and coating efficiency of the films on a hydrophilic substrate, and provided good storage stability.

Table 1. Characterization of zein film, composite zein/shellac film and laminated zein/shellac film

Film sample	Tensile strength (MPa)	Elongation at break (%)	Water vapor permeability (g/(m^2·h))	Moisture content (%)	Solubility degree (%)
C	6.18±1.02^a	7.71±1.97^a	40.33±0.67^a	7.14±0.41^a	27.19±1.13^a
M	15.51±2.31^b	11.49±2.34^b	18.41±0.33^b	5.11±0.37^a	35.81±1.24^b
L	8.96±0.98^a	18.99±1.76^c	10.04±0.51^c	5.91±0.16^a	32.45±0.97^c

$^{a\text{-}c}$ Means in the same column with different superscripts are statistically different ($p \leq 0.05$)

The obtained results, presented in Table 1, showed that there was a statistically significant difference between the values of elongation at break (%), water vapor permeability (g/(m^2·h)) and solubility degree (%) for the observed film samples. The most significant influence of the added shellac was in terms of barrier properties with respect to water vapor. The water vapor permeability value of the control sample was 40.33 g/(m^2·h), while the M sample had 18.41 g/(m^2·h) water vapor permeability and the L sample was 10.04 g/(m^2·h). Lower values of water vapor permeability are considered desirable, so lamination as processing proved to be more effective regarding this issue than blending biopolymers. In the case of laminated films (multilayer barriers), the hydrocolloid component (zein) provides structural–mechanical support as well as a smooth surface, which facilitates the application of a lipid layer (shellac) with good integrity. This explains why laminated films have better mechanical and barrier efficiencies than composite films (*Phan The et al., 2008*). Similar results were obtained by Takahashi and colleagues who found that cross-linking carboxymethyl starch film with zein molecules could significantly decrease water vapor permeability (*Takahashi et al., 2002*).

Moisture content values were uniform for all tested film samples, ranging between 5.11 and 7.14. Since it is optimal if the moisture content is as low as possible, that means that shellac addition (in both ways) improved this characteristic. Film solubility is connected with the water diffusion, the ionization of the carboxyl and amino groups, the dissociation of hydrogen and ionic bonds and with relaxation of the polymer in the presence of moisture (*Mathew et al., 2006*).

The structures of shellac and zein are different: the polymer structure of shellac gives it a more amorphous and random structure, while the protein structure of zein gives it a more ordered and structured arrangement. The are many other samples when zein or shellac were combined via blending or lamination with other components which resulted in improved film properties. Du et al. fabricated konjac glucomannan–shellac composite films with improved hydrophilicity, good mechanical properties, and thermal stability, suggesting possible applications as biodegradable packaging (*Du et al., 2019*). Comparing pure soybean protein isolate films to composite films made by mixing shellac and soybean protein isolate, it was found that the latter had much better water resistance (*Zhang et al., 2020*). Recently, a novel natural composite film was created by combining gelatin, shellac, and carboxymethyl cellulose. The composite film has shown minimal porosity, a propensity for particle aggregation, high homogeneity, tensile strength, antimicrobial characteristics, elongation, air permeability, and thermal stability (*Mohamed et al., 2019*). When olive oil- or glycerol-plasticized zein films were laminated with glycerol-plasticized whey protein films, the ultimate tensile strength was significantly higher (about 2–3 times) and the barrier properties were improved (about 1.8–2 times) against water vapor compared to the single whey protein films (*Ghanbarzadeh and Oromiehi, 2008*). Films made of whey powder and sodium caseinate that were laminated with zein film experienced a related effect (*Yong Cho et al., 2002*). All literature data is based on the fact that composite films and laminated films could be expected to have better mechanical and barrier properties than biopolymers of which they are composed, which strongly depends on the characteristics and compatibility of components (*Abdullah et al., 2022; Soradech et al., 2012*). As for composite films with monolayer structure, added components (in this case shellac resin) are evenly distributed in the biopolymer matrix. However, their water resistance ability is much lower than the laminated films. In the case of laminated films, the biopolymer matrix offers smooth surfaces and structural-mechanical support, making it easier to apply the shellac layer. This is why laminated films have higher mechanical and barrier efficiency. Both mechanisms are effective methods for improving the mechanical and barrier properties of biopolymers.

The color correlation analysis was applied to investigate the connections between observed samples (Figure 2). A color correlation diagram was created to show the statistical significance of the correlation coefficients between the different variables and the responses. Positive correlations are represented by blue color, and negative correlations are represented by red color, while the circles' size indicates the correlation's strength (*Šuput et al., 2023*). The color correlation analysis was applied to investigate the connections between observed samples. A positive correlation between the tensile strength was noticed (r= 0.685, $p \leq 0.05$). On the other hand, a negative correlation between water vapor permeability and the elongation at break and water vapor permeability and solubility degree was noticed (r=-0.906 and r=-0.772, $p \leq 0.05$, respectively).

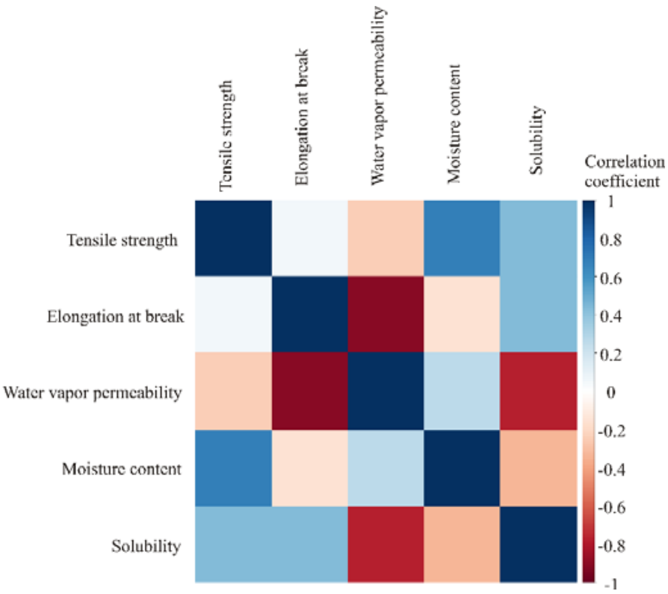

Fig. 2. Color correlation diagram between the observed variables.

CONCLUSION

In this work, it was proven that the zein control film has satisfactory mechanical and barrier characteristics, which were improved by the addition of shellac. Combining zein films with shellac through lamination or blending improved the moisture resistance, strength and flexibility of the films. The most significant contribution is the improvement of barrier properties. It has been proven that the value of water vapor permeability can be reduced by 2 times if the zein film is blended with shellac, or even 4 times reduced if the zein film is laminated with a layer of shellac. This investigation proved that they can be successfully combined to obtain new films with improved properties.

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