

BIOPOLYMER FILMS SYNTHESIS AND CHARACTERISATION SINTEZA I KARAKTERIZACIJA BIOPOLIMERNIH FILMOVA

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ABSTRACT

In this study, three biopolymer films were synthesized: zein (protein), gelatin (protein), and starch (polysaccharide) films and characterized by determining mechanical and physico-chemical properties. Gelatin films proved to be the strongest (tensile strength 84 N/15 mm), while the zein were most flexible (elongation at break 41.6 %). Moisture content was higher in starch films, which is a result of the hydrophilic nature of the polysaccharide films. Swelling degree was 22.5 % for zein, 90.9 % for gelatin, while the highest average value was recorded in starch film samples (840.6 %). Starch films had highest value of solubility degree (36.5 %), while zein film had 27.9 % and gelatin film had 11.85 %. Obtained characterization results are consequences of the different structure of the raw materials and synthesis routes. All undesirable characteristics could be improved by optimizing the composition of the film, as well as synthesis of composite/laminated films.

Key words: biopolymer films, zein, gelatin, starch, properties.

REZIME

U ovom radu sintetisana su tri biopolimerna filma različitog porekla, adekvatnim metodama sinteze. Sintetisani su zeinski i želatinski filmovi (proteinski), kao i skrobni film (polisaharidni). Skrobni i želatinski filmovi su dobijeni iz vodenog filmogenog rastvora, dok je zeinski film dobijen iz alkoholnog rastvora. Dobijeni filmovi su okarakterisani određivanjem mehaničkih i fizičko-hemijskih osobina: debljina, zatezna jačina i izduženje pri kidanju, sadržaj vlage, bubrenje i rastvaranje. Želatinski filmovi su se pokazali kao najčvršći (zatezna jačina 84 N/15mm), dok su zeinski bili najelastičniji (izduženje pri kidanju 41,6%). Izmereni sadržaj vlage je veći kod uzoraka skrobnih filmova u odnosu na zeinske i želatinske, što je posledica načina sinteze i hidrofилne prirode polisaharidnih filmova. Vrednost stepena bubrenja filma iznosila je 22,5% kod uzoraka zeinskog filma, a 90,9% kod uzoraka želatinskog filma, dok su najviše prosečne vrednosti zabeležene kod uzoraka skrobnog filma i iznosile su 840,6%. Skrobni filmovi su najrastvorljiviji u vodi (stepen rastvorljivosti 36,5%), dok je vrednost stepena rastvorljivosti kod zeinskih iznosila 27,9%, a kod želatinskih je bila najmanja i iznosila je 11,8%.

Na osnovu dobijenih rezultata konstatuju se različite osobine ispitivanih biopolimernih filmova, koje su posledica različite strukture polazne sirovine, kao i načina sinteze. Sve karakteristike filmova moguće je unaprediti optimizacijom sastava filma, kao i sintezom kompozitnih ili laminiranih filmova u zavisnosti od željene primene.

Ključne reči: biopolimerni filmovi, zein, želatin, skrob, osobine.

INTRODUCTION

Great efforts regarding food quality improvement and shelf-life extension concurrently with minimization of packaging waste and petroleum derivatives consumption have led to synthesis and application of biopolymer packaging materials (Lazić and Novaković 2010; Tharanathan, 2003). Biopolymers (IV generation of packaging) are self-destructive biodegradable packaging materials (Lazić and Gvozdrenović 2007) and come from renewable sources. Biopolymers are consisted of polymers, which could be decomposed to eventually obtain water, CO₂, CH₄ and biomass (McKee, 2012).

Preparation of biopolymer films includes biopolymers dissolution or dispersion in different solvents; solution evaporation and, finally, drying. This process (known as casting) is based on molecular weight reduction and polymer chains reorganization in the film matrix, and it has been used for the production of polysaccharide and protein biofilms (Embuscado and Huber 2009; Park et al., 2014). In order to be widely accepted, biopolymer edible films must have food-grade quality in accordance with current legislation.

Biopolymer films directly extracted from biomass are usually classified according to the predominant constituent material (Falguera et al., 2011). Main groups of chemical compounds that serve as sources for biopolymer films are proteins, polysaccharides and lipids (Šuput et al., 2013).

Hydrocolloids (proteins and polysaccharides) are molecules with expressed film forming characteristics and they are widely used to obtain biodegradable films.

Based on the origin, proteins are divided into plant proteins (gluten, zein, soy protein, pea) and animal proteins (casein, whey, collagen, gelatin, keratin). Physical and chemical properties of protein films are influenced by amino acid composition, electrostatic charge, structure changes due to pressure, heat, irradiation, mechanical damage, chemical and enzyme actions etc. (Krochta et al., 2002). Proteins are good film formers exhibiting excellent gas and lipid barrier properties (Popović et al., 2012). Compared with synthetic films, protein-based films exhibit poor water resistance and lower mechanical strength, susceptible to cracking due to the strong cohesive energy density of the polymer (Han, 2014). Yet, proteins are still generally superior to polysaccharides in their ability to form films with greater mechanical and barrier properties (Cuq et al., 1998). Protein interconnection by chemical, physical or enzyme treatments improves barrier and mechanical film properties (Senna et al., 2010; Bourtoom, 2009).

Polysaccharides and their derivatives (starch, cellulose, chitosan, alginates, pectin, carrageenan, etc.) are widely used in the production of biodegradable packaging materials. Polysaccharides are great materials for the film formation, as they show excellent mechanical and structural properties (Šuput et al., 2015; Han, 2014). Due to hydrophilic nature of these

molecules, the application of biopolymers based on polysaccharides is limited because they show poor barrier properties against water vapor (Falguera et al., 2011). Good barrier properties to gases and aromas, good mechanical properties and ability to carry active components are their major potential (Mikkonen et al., 2007).

The efficiency of lipid components in the biopolymer film preparation depends on the nature of lipids: structure, composition, hydrophobicity and interaction with other film components. Lipids form films which are opaque and relatively inflexible (Rhim and Shellhammer, 2005). The lipid biofilms are commonly used as coatings to protect food from moisture loss and to increase the gloss. Since lipids have water vapour permeability values, close to those of synthetic plastic films (Pérez-Gago and Rhim, 2014), they are usually combined with other biopolymers (hydrocolloids) as emulsion particles or multi-layer coatings in order to increase water vapour resistance (Mehyar et al., 2004). Hydrophobic substances used for biopolymer film synthesis include natural wax (beeswax, carnauba wax, candelilla wax); petroleum-based waxes (paraffin and polyethylene wax); petroleum, mineral and plant oils; fatty acids and resins (shellac and wood rosin) (Pérez-Gago and Rhim 2014; Rhim and Shellhammer, 2005). Films based on waxes have the greatest degree of resistance to moisture migration, but their application is limited because of its thickness, greasy surfaces, and possible taste on fat.

In this study, three different biopolymer films - protein and polysaccharide were synthesized and examined. The obtained films were characterized by determining the mechanical and physico-chemical properties with the aim of comparative characterization of important properties for their further application.

MATERIAL AND METHOD

Film synthesis

Zein film: Zein 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 water bath at 80 °C, poured into Petri dishes (8 g per dish) and dried at room temperature. Four films were prepared.

Gelatin film: Aqueous gelatin solution 10 % (w/w) was prepared and left for 30 minutes at room temperature to undergo gelation, and then dissolved in a water bath at 50 °C for about 20 min. Afterwards, 0.1 g glycerol/g gelatin was added and stirred. Film forming solution was poured into Petri dishes (20 g per dish) and dried at room temperature. Four films were prepared.

Starch film: Aqueous starch solution 1 % (w/w) was prepared and 130 % of sorbitol (based on the initial weight of the starch) and 0.5 % (based on the initial weight of the starch) of guar-xanthan modified mixture were added. Film forming solution was heated for 30 min in a water bath at 90 °C, poured into Petri dishes (50 g per dish) and dried at room temperature. Four films were prepared.

Methods

Film thickness was measured using a micrometer with sensitivity of 1 µm. Thickness measurements were carried out on each film at 8 different positions.

Mechanical properties

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 crosshead speed was set at 50 mm/min. TS and EB measurements for each sample were repeated eight times.

Moisture content is determined as a percentage of weight reduction during film drying, expressed on the total weight of the film:

$$MC (\%) = 100 [(m_2 - m_1) - (m_3 - m_1)] / (m_2 - m_1)$$

where,

m_1 - mass of measuring vessel, m_2 - mass of film samples with measuring vessel prior drying,
 m_3 - mass of dried film samples with measuring vessel

Film solubility

Dry film samples, after moisture content determination, 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:

$$\text{Solubility } (\%) = 100 [(m_3 - m_1) - (m_4 - m_1)] / (m_3 - m_1)$$

where,

m_1 - mass of measuring vessel, m_2 - mass of film samples with measuring vessel prior drying,
 m_3 - mass of dried film samples with measuring vessel, m_4 - mass of dried film samples with measuring vessel after immersion and drying

Film swelling

Film samples (1x2 cm) were weighed (m_1), and then dipped in deionized water at room temperature, for 2 min. Lastly, the samples were then removed from the water, the excess water was removed by a filter paper and samples were weighed (m_2). Swelling degree was calculated:

$$\text{Swelling } (\%) = 100 (m_2 - m_1) / m_1$$

Where,

m_1 - mass of film samples prior to dipping in deionized water,
 m_2 - mass of film samples after dipping in deionized water

Statistical analysis

Statistical analysis was carried out using OriginPro 8 (OriginLab Corporation, Northampton, MA, USA). All data were presented as mean value 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.

RESULTS AND DISCUSSION

Visual examination finds that all the obtained films were transparent, shiny, odorless and free of cracks or residual air bubbles (Figure 1). Zein films had the most pronounced dark yellow color, while starch films were completely transparent, without a hint of yellow. The strongest, but also the most non elastic, were gelatin films. It was confirmed that the casting was the simplest way to produce the films in laboratory conditions.



Fig. 1. Visual examination of various biopolymer films:
1. Zein 2. Gelatin 3. Starch

The results of mechanical properties examination are shown in Table 1. Film thickness varied in the range from 84.1 μm to 172.9 μm , for starch and gelatin film, respectively. Thickness values were significantly different ($p < 0.05$).

Table 1. Mechanical properties of various biopolymer films

Film sample	Thickness (μm)	Tensile strength (N/15mm)	Elongation at break (%)
Zein	131.4 ^b ±39.1	4.4 ^b ±1.5	41.6 ^a ±8.5
Gelatin	172.9 ^a ±27.1	84.0 ^a ±23.4	2.0 ^b ±2.1
Starch	84.1 ^c ±6.9	8.3 ^b ±2.8	1.6 ^b ±0.9

Basic indicators of mechanical properties of packaging materials are tensile strength (TS) and elongation at break (EB). The results, of mechanical properties of the tested films are shown in Table 1. Gelatin films had highest tensile strength (84 N/15 mm), while zein and starch films were not significantly different ($p > 0.05$). The most flexible samples were zein films, as evidenced by the elongation at break value (41.6 %), compared to significantly lower values for samples of gelatin and starch films (see Table 1). Compared with many synthetic polymers, tensile strength and elongation at break values of biopolymer films are quite low, but enough to make these films find application in the food industry (Souza et al., 2013, Park et al., 2014).

Moisture content was higher in the starch films samples (17.9 %) in relation to zein (9.1 %) and gelatin (10.2 %), which may be related to the synthesis route and the hydrophilic nature of the polysaccharide film.

Film solubility and swelling are connected with the water diffusion, the ionization of the carboxyl and amino groups, dissociation of hydrogen and ionic bonds and with relaxation of the polymer in the presence of moisture (Mathew et al., 2006). Solubility values for all examined biopolymer films are less than 40%, which is in agreement with earlier studies (Nguyen, 2012). The highest solubility value was recorded in case of starch film samples (36.5 %), and the lowest solubility value was recorded in case of gelatin film samples (11.8 %). Swelling is undesirable characteristic when choosing packaging materials for a specific purpose, such as packaging of food with high moisture content. In contact with water, polar water molecules are attracted to the hydrophilic surface of the film, and then penetrate into the matrix, binding to its polar groups (Ganji et al., 2010).

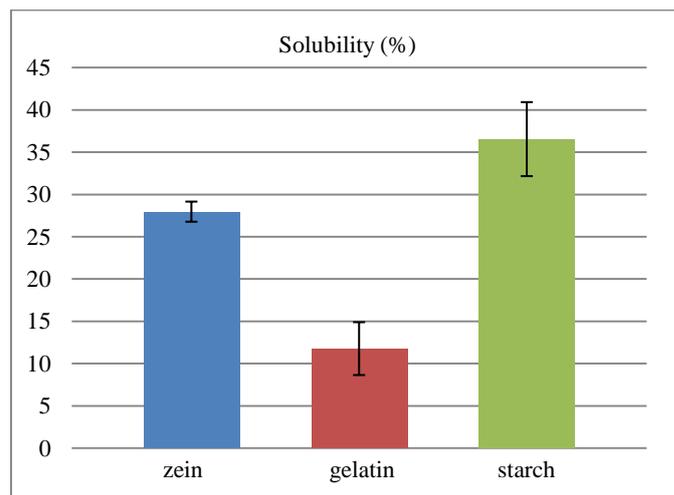


Fig. 2. Solubility of various biopolymer films

This leads to local dissolution of the hydroxyl group and the polymer swelling, releasing new groups as active binding sites (Bertuzzi et al, 2007; Ludwiczak and Muha, 2010). The values of investigated biopolymer films swelling degree are shown in Figure 3.

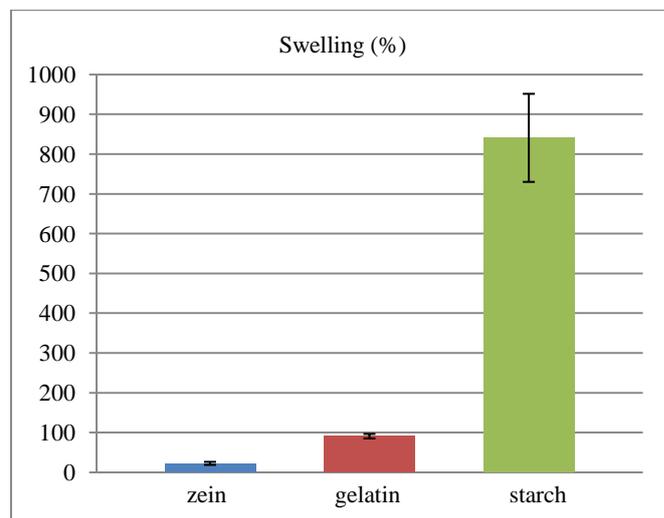


Fig. 3. Swelling degree of various biopolymer films

The lowest swelling degree value was measured in the samples of zein film and amounted to 22.5 %, while swelling degree for the gelatin film sample was 90.9 %. The highest average values were recorded in the sample of starch films and amounted up to 840.6 %. Such high values of the degree of swelling are the result of highly hydrophobic starch nature. Moisture content and the swelling degree were in correlation, which was in accordance with previous findings (Šuput et al., 2016; Fabra et al., 2010).

The solution of this problem can be the crosslinking or addition of some other polymer, or some lipid components, in order to lower the water solubility, as well as the swelling degree. Also, film aging may leads to moisture content reduction and solubility reduction due to increased networking (García et al., 2004; Bourtoom and Chinnan, 2009).

CONCLUSION

The purpose of this work was to present the possibility of using different laboratory synthesis techniques for the production of biopolymer films of various origins. The produced

films showed different characteristics in terms of their mechanical and physico-chemical properties. These results are consequences of different structure of the raw materials and synthesis routes. Based on visual examination it was found that all the obtained films were transparent, shiny, odorless and free of cracks or residual air bubbles. The strongest films were made of gelatine, while zein films were the most elastic, which correlated well with the results of mechanical properties. The highest moisture content and swelling degree, which were in correlation, were recorded for the starch film samples and lowest for the zein film samples. Solubility values for all examined biopolymer films were less than 40 %.

The obtained results are the characteristic related to the nature of examined biopolymers. As in synthetic polymers, also biopolymer materials, as monomaterials rarely could demonstrate optimal characteristics. However, all undesirable characteristics could be improved by optimizing the composition of the film, as well as synthesis of composite/laminated films. This work has been the basis for further development and improvement of biopolymer materials with great potential for application in the food industry.

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