MONO- AND BILAYER BIOPOLYMER FILMS: SYNTHESIS AND CHARACTERISATION

MONOSLOJNI I DVOSLOJNI BIOPOLIMERNI FILMOVI: SINTEZA I KARAKTERIZACIJA

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ABSTRACT

The purpose of this paper is to improve the properties of biopolymer protein monofilms and produce a novel bilayer biopolymer film on the basis of the monofilms analyzed. Biopolymer monolayer films, based on pumpkin oil cake (PuOC) and zein, and a bilayer film, based on PuOC and zein (PuOC/Zein), were produced as a result of the study. The visual, mechanical, physicochemical and structural properties of the films were evaluated. The results obtained showed that the PuOC film exhibited the highest elongation at break, followed by the bilayer film, whereas the zein film showed the lowest elongation at break. However, the zein film showed the highest tensile strength, followed by the PuOC film. The tensile strength of the bilayer PuOC/Zein film was almost 3 times lower than that of the PuOC film, and almost 4 times lower than that of the zein film. The physicochemical properties recorded indicate that the hydrophobic zein film is the least sensitive to moisture, affecting the moisture sensitivity of PuOC film by reducing the moisture content, swelling and total soluble matter of the bilayer PuOC/Zein film. Based on the FTIR spectra, it could be concluded that there are no significant differences between the two sides of the bilayer PuOC/Zein film. Both sides of the bilayer film examined indicated characteristic peaks for protein biopolymer films in the FTIR spectrum.

Key words: biopolymer films, pumpkin oil cake, zein, monolayer films, bilayer films, properties.

REZIME

Pogača uljane tikve goliće predstavlja nus proizvod koji se dobija nakon ekstrakcije hladno ceđenog ulja iz semena tikve. PuOC sadrži oko 63% proteina, 12% ugljenih-hidrata, 4,5% vlakana, 8,4% ulja i 13% drugih komponenata. Zbog ovakvog sastava, cela pogača PuOC predstavlja pogodan materijal za dobijanje biopolimernih filmova. Zein predstavlja jedan od najzastupljenijih proteina u kukuruzu. Jedna od najperspektivnijih primena zeina je upravo za dobijanje biorazgradivih filmova i ambalažnih materijala za pakovanje. Formira jake, sjajne, hidrofobne i veoma fleksibilne filmove. U radu je prikazana sinteza i karakterizacija biopolimernih monofilмова na bazi pogače uljane tikve goliće (PuOC) i zeina, kao i dvoslojnog filma na bazi PuOC i zeina (PuOC/Zein). Nakon sinteze, ispitane su osobine dobijenih filmova: vizuelna, mehanička, fizičko-hemijska i strukturna svojstva. Dobijeni rezultati su pokazali da najveće izduženje pri kidanju ima PuOC film, potom dvoslojni film, dok zeinski film ima najmanju vrednost izduženja pri kidanju. Međutim, zeinski film je pokazao najvišu zateznu jačinu, a potom PuOC film. Dvoslojni film je pokazao 3 puta manju vrednost zatezne jačine u poređenju sa PuOC filmom i, čak 4 puta manju vrednost zatezne jačine u poređenju sa zeinskim filmom. Fizičko-hemijske osobine su pokazale da je hidrofobni zeinski film najmanje osetljiv na vlagu, kao i da je uticao na osetljivost PuOC filma prema vlazi, smanjujući sadržaj vlage, bubrenje i ukupnu rastvorljivost dvoslojnog PuOC/Zein filma. Na osnovu dobijenog FTIR spektra, može se zaključiti da ne postoje značajne razlike između ispitivanih strana dvoslojnog PuOC/Zein filma. Kod obe strane dvoslojnog filma, pronađeni su karakteristični pikovi za biopolimerne proteinske filmove.

Ključne reči: biopolimeri, pogača uljane tikve goliće, zein, monofilmovi, dvoslojni filmovi, osobine.

INTRODUCTION

At present, synthetic polymer materials are leading materials in the packaging industry (Lazić and Popović, 2015). However, the intensive use of synthetic polymers has led to a major ecological issue of disposing and recycling synthetic packaging material waste, as well as the use of non-renewable resources. Biopolymer packaging materials have been studied for the past two decades as a possible alternative to synthetic polymers (Krkić et al., 2012; Šuput et al., 2014; Popović et al., 2015). Biopolymer materials can be obtained from renewable natural resources, and they decompose naturally in the environment under the influence of various external factors (Lazić et al., 2008; Lazić and Novaković, 2010). Biopolymers show a good film-forming ability and, as films, they feature satisfactory properties important for packaging application. Biopolymer films can be produced using a great variety of raw materials such as polysaccharides, proteins, lipids, with the addition of plasticizers and surfactants (Lazić et al., 2008; Lazić and

Popović, 2015). Protein-based films are the most attractive of all biopolymer-based films due to their impressive gas barrier properties (Ou et al., 2004). However, their application is limited by the high water-vapor permeability due to their hydrophilic nature and increased by adding plasticizing agents (Ye-Chong et al., 2014). Moreover, an advantage of protein-based biomaterials lies in their nature and structure, which provide a wide range of functional groups for interactions and modifications (Ke et al., 2012).

Nowadays, the production of packaging materials based on biopolymers obtained from agroindustrial waste is in expansion. Pumpkin oil cake (PuOC) is a by-product obtained from pumpkin seed using the cold-pressing oil extraction (Popović et al., 2015). PuOC contained the least-processed form of pumpkin seed protein and had 63% proteins, 12% carbohydrates, 4.5% crude fibers, 8.4% oils and 13% other components (Peričin et al., 2007). PuOC is a suitable material for producing bio-based films as the film thus obtained indicate good mechanical and gas barrier properties (Popović, et al., 2011).

Zein is a plant protein extracted from corn, consisting approximately of 45–50% of corn protein (Rishi and Munir, 2001). Zein is not soluble in water, but it is soluble in a 40–90% ethanol solution (Ye-Chong et al., 2014). It can form films using the casting method, which are glossy, hydrophobic, greaseproof, resistant to the growth of food-borne microorganisms, featuring excellent flexibility and compressibility, as well as a low water vapor permeability compared to most other agriculturally-based protein films (Embuscado and Huber, 2009; Ke et al., 2012). However, the solvent-casted zein film was rigid and brittle, and thus, plasticizers are needed to improve their flexibility (Ke et al., 2012). Zein has been commercially used as a coating for medical tablets, and has the potential to be used in biodegradable packaging (Embuscado and Huber, 2009).

The purpose of this paper is to improve the properties of biopolymer protein monofilms and produce a novel bilayer biopolymer film on the basis of the monofilms analyzed. Biopolymer mono-layer films based on pumpkin oil cake (PuOC) and zein, and a bi-layer film based on pumpkin oil cake and zein (PuOC/Zein) were produced as a result of the study. The visual, mechanical (elongation at break and tensile strength), physicochemical (moisture content, swelling and total soluble matter) and structural properties of the films obtained were determined.

MATERIAL AND METHOD

Materials

The ground hullless pumpkin (*Cucurbita pepo* L. c.v. Olinka) oil cake (PuOC) was collected from Agrojapra, DonjiAgič, Bosnia and Herzegovina, and stored at a temperature of 4°C. The following reagents were also used in the study: glycerol (85%, Mw 92,1 g/mol, Zorka Pharma Šabac), zein (Sigma-Aldrich Chemical Co. St. Louise, USA), polyethylene glycol (PEG) (Sigma-Aldrich Chemical, Belgium), ethanol (96%, Zorka Pharma Šabac) and NaOH (NPK-Inženjering Beograd).

Preparation of monolayer films

The film-forming suspension of PuOC (10 %, w/w) in deionized water was produced with the addition of 30 % glycerol (v/w, per weight of PuOC). After adjusting pH = 12 of the film-forming suspension (using 0.2 M NaOH), the suspension was incubated at 90 °C for 20 min, and then filtrated through a nylon filter. The solutions were casted onto Teflon-coated Petri dishes, and the films were dried for 2 days at room conditions (23 ± 2 °C, 50 ± 5 % RH).

The film-forming solution of zein (10% w/v) was prepared by suspending zein in an 85% ethanol solution with the addition of PEG 400 (50% w/w, per weight of zein), and incubating the solution at 80 °C for 5 min. The solutions were casted onto Teflon-coated Petri dishes and the films were dried for 24 h at room conditions (23 ± 2 °C, 50 ± 5 % RH).

Preparation of bi-layer films

The bilayer films were prepared using the PuOC films, which were casted onto Teflon-coated Petri dishes. After the films were dried, the zein solution was casted on the dried PuOC films, and left until it dried at room conditions (23 ± 2 °C, 50 ± 5 % RH).

Methods

Thickness

The film thickness was measured using a micrometer (Digico 1, Tesa, Swiss Made, Renens, Switzerland), with a sensitivity of 0.001 mm. The research results were expressed as the mean of ten measurements on each film, from which an average was obtained with a standard deviation (SD).

Mechanical properties

The tensile strength and elongation at break of the films were measured using an Instron Universal Testing Instrument Model No. 4301 (Instron Engineering Corp., Canton, MA), according to the ASTM standard method D882-01. Rectangular film strips (15x80 mm) were used. The initial grip separation was set at 50 mm, and the crosshead speed was set at 50 mm/min. The tensile properties testing for each type of the film were repeated at least three times, from which an average was obtained with a standard deviation (SD).

Physicochemical properties

The moisture content, swelling and total soluble matter of the films was determined according to Hromiš et al., (2015). At least three measurements were performed, and the average values were calculated, with the standard deviation (SD), on the basis of the following equations (Eq. 1-3):

$$\text{Moisture content (\%)} = \frac{100 (m_2 - m_1)}{m_1} \quad (1)$$

where m_1 is the mass of the film samples before drying, whereas m_2 is the mass of the dried film samples.

$$\text{Total soluble matter (\%)} = \frac{100 (m_3 - m_4)}{m_3} \quad (2)$$

where m_3 is the mass of the dried film samples, whereas m_4 is the mass of the dried film samples after immersion and drying.

$$\text{Swelling (\%)} = \frac{100 (m_6 - m_5)}{m_5} \quad (3)$$

where m_5 is the mass of the film samples before dipping in deionized water, whereas m_6 is the mass of the film samples after dipping in deionized water.

Structural properties

The structural properties of the bilayer PuOC/Zein films obtained were tested using the Fourier transform infrared spectroscopy (FTIR, Nicolet sS10). The FTIR analysis of the film samples was carried out in the wave number range from 4000 to 400 cm^{-1} , at a resolution of 4 cm^{-1} , using the IR spectrophotometer, Nicolet IS10, Thermo Scientific (Massachusetts, USA) and attenuation total reflection (ATR) extension. Each sample was scanned 32 times, whereas background shots were taken before the analysis of each sample. The Omnic 8.1 software (Thermo Fisher Scientific, MA, USA) was used to operate the FTIR spectrometer, collect and present all the data. Both sides of the bilayer film (namely PuOC and zein side) were recorded.

RESULTS AND DISCUSSION

All the films based on PuOC were greenish, elastic and flexible, with a mild pumpkin aroma. The zein films were yellowish, transparent and flexible, with mild corn aroma. The bilayer films based on PuOC and zein were more brittle than the monofilms. The following thicknesses of the films were obtained: 125 ± 3 (μm) for the monofilm based on PuOC, 101 ± 5 (μm) for the monofilm based on zein and 295 ± 10 (μm) for the bilayer film based on PuOC/Zein.

The mechanical properties of the mono- and bilayer films obtained are presented in Figure 1. The indicative mechanical properties of films are tensile strength (TS, MPa) (pulling force per film cross-sectional area required to break the film) and elongation at break (E, %) (degree to which film can stretch before breaking) (Gennadios, 2002). The results obtained

showed that the highest elongation at break was determined for the PuOC film (50 %), followed by the bilayer film (38 %), whereas the zein film showed the lowest value in this respect (11 %) (Figure 1a). However, the zein film showed the highest value of tensile strength (7.4 MPa), whereas the PuOC film followed with a slightly lower value (5.9 MPa). However, the tensile strength of the bilayer PuOC/Zein was almost 3 times lower than that of the PuOC film, and almost 4 times lower than that of the zein film (2.1 MPa) (Figure 1b).

Fig. 1. Mechanical properties of the mono- (PuOC, zein) and bilayer (PuOC/Zein) films - (a) elongation at break (E) and (b) tensile strength (TS)

The tested physicochemical properties of the mono- and bilayer films obtained are presented in Figure 2. The physicochemical properties of films are moisture content, swelling and total soluble matter, and those properties indicate the sensitivity of films to moisture and water. In the packaging industry, a film readily soluble in water is usually desirable, such as a readily soluble pouch containing food ingredients. However, when packaging materials are expected to provide water resistance and improve food integrity, water soluble materials are undesirable (Gennadios, 2002). The results obtained showed that the least sensitive film was the zein film, followed by the bilayer film, whereas the PuOC film was the most sensitive to water and moisture. In order to improve the sensitivity of biopolymer films to moisture and water, protein films are mostly combined with polysaccharides, or different lipids. Furthermore, the synthesis of composite or laminated films based on different biopolymers with desirable properties can lower the sensitivity. According to the results obtained, the hydrophobic layer of the zein film decreased the sensitivity of the PuOC film to moisture and water. Therefore, the bilayer PuOC/Zein film indicated

lower values of the moisture content, swelling and total soluble matter than the PuOC film (19.5 %, 31% and 3.9% for PuOC/Zein film and 25%, 44 % and 43.5 % for PuOC film, respectively) (Figure 2).

Fig. 2. Physicochemical properties of the mono- (PuOC, zein) and bilayer (PuOC/Zein) films - (a) moisture content, (b) swelling and (c) total soluble matter

Figure 3 and 4 display the FTIR spectra of the PuOC and zein sides, respectively, as well as of the bilayer PuOC/Zein film. Both sides of the bilayer film examined indicated characteristic peaks for protein biopolymer films, which was reported in numerous previous studies (Secundo and Guerrieri et al., 2005; Robertson et al., 2006; Soares et al., 2009; Song et al., 2009):

- Interval at 3000-3700 cm^{-1} corresponds to the stretching vibration of O-H and N-H group;
- Interval at 2800-3000 cm^{-1} corresponds to the C-H stretching vibration;
- Interval at 1629 i 1525 cm^{-1} corresponds to the C=O stretching (amide I) and N-H bending (amide II);
- Interval at 1220-1295 cm^{-1} corresponds to the amide III band.

Based on the FTIR spectra obtained, it could be concluded that there are no significant differences between the two sides of the bilayer film. Zein is a protein by origin, whereas the PuOC fraction is a composite structure. This means that it is composed of different compounds such as proteins, polysaccharides, lipids, etc. Upon analysing the FTIR spectra obtained, it could be concluded that PuOC has a dominant protein structure in the film form (after pH and temperature adjustments). Moreover, the absorbance values of the PuOC side are higher than those of the

zein side of the bilayer film (Figure 5), which is directly related to a greater number of absorbing bonds.

CONCLUSION

The results obtained show that biopolymer monofilms based on PuOC and zein, as well as bilayer films based on PuOC/Zein, exhibit mechanical properties that are typical of biopolymer films. Materials with adequate mechanical properties can be prepared for different biopolymer-based films, or their combinations to form laminated films.

Due to its hydrophilic nature, the PuOC film exhibited a high sensitivity to moisture and water. However, this property was improved by laminating the PuOC film with a second hydrophobic layer of the zein film.

Based on the FTIR spectra obtained, it could be concluded that there are no significant differences between the two sides of the bilayer PuOC/Zein film.

Fig. 3. FTIR spectrum of the PuOC side of the bilayer PuOC/Zein film

Fig. 4. FTIR spectrum of the zein side of the bilayer PuOC/Zein film

Fig. 5. Absorbance differences between the PuOC and zein sides at - (a) (2800-3600) cm^{-1} and (b) (800-1200) cm^{-1}

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