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EXPERIMENTAL RESEARCH ON BIOPOLYMERS OBTAINING BASED ON CHITOSAN, GELATIN AND PECTIN

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Abstract

Biopolymers are polymers obtained from biological origins and used for industrial applications in food packaging. A biopolymer should be non-toxic, non-carcinogenic, biodegradable and also adequately available for their widespread applications.
The aim of this study was to obtain packaging materials for food, as films prepared in the laboratory, based on natural polymers obtained from renewable resources:
- chitosan/gelatine and chitosan/gelatine/n-clay respectively;
- chitosan/pectin and chitosan/pectin/n-clay respectively;
- chitosan/gelatine/pectin and chitosan/gelatine/pectin/n-clay respectively. The nano-clay was added 3% to the mixing mass. In order to obtain films with a good flexibility, 5% glycerol was added.
These polymeric films have been characterized in terms of transmittance/transmission by UV/VIS spectroscopy and chemical structure by ATR-FTIR technique.
It was found that the optical properties of these films were slightly affected by the nano-clay concentration in their composition. As for the films based on chitosan - gelatine, chitosan-pectin, which contain nano-clay, this has increased the thickness of the films, giving them mechanical strength, but also opacity.
Analysis of the film microstructure obtained by FTIR exhibited a good compatibility among the gelatine-chitosan blend and showed that this matrix allowed a uniform distribution of the actives throughout the network.
The results obtained from the ATR-FTIR spectra confirmed the existence of specific chemical groups in the analysed polymers (-OH, -NH2, -NH, CH2, CH- groups), according to the data reported in the literature.

Key words**:** *chitosan, gelatine, pectin, polymeric films, nano-clay.*

INTRODUCTION

In recent decades, there has been a sharp increase in the use of films based on natural polymers in packaging for the food industry as a replacement for synthetic plastic films, driven by a societal desire for sustainable food packaging, which protects food products from external contamination and delays their deterioration by extending the shelf life and maintaining quality (Bonnaillie et al., 2014).
In addition to consumer requirements and to replace plastic packaging, a large variety of low-cost natural resource biopolymers have emerged (Ahvenainen et al., 2003). The biopolymers used mainly for edible films in food packaging are proteins, polysaccharides and lipids (Yao et al., 2017).
Films made from chitosan and gelatine, according to studies are increasingly provided for biomedical and food applications, as well as for packaging (Cordoba et al., 2016).
Chitosan is a polysaccharide derived from chitin, being its N-deacetyl form. This polymer has attracted the attention of researchers, due to its antimicrobial properties, being considered a natural preservative (Miteluț et al., 2015; Popescu et. al., 2018;). Chitosan acts on the cell of pathogenic and degrading microorganisms, interacting with the cell membrane, thus causing functional and structural changes, changing the permeability of the cell wall (Bonilla & Sobral, 2016).The intrinsic properties of chitosan make it ideal for fabricating films, and chitosan based films promise great potential as active packaging materials due to its antimicrobial

activity, non-toxicity and low permeability to oxygen (Kanatt et al., 2012).

Gelatine is a protein produced by partial acid/base hydrolysis of collagen. Gelatine can be extracted from various sources: pig skin, fish skin and bovine bone (Alexandre et al., 2016). Gelatine is a water-soluble protein substance, prepared by processes that involve the destruction of the tertiary, secondary and to some extent the primary structure of native collagen (Fernandez-Diaz et al., 2001), specifically by partial hydrolysis of collagen derived from the skin, white connective tissue, and animal bones (Morrison et al., 1999). Gelatine is a digestible protein that contains all the essential amino acids except tryptophan (Benbettaïeb et al., 2014). The composition of amino acids, especially with regard to proline and hydroxyproline, may vary from species to species, as a result of exposure to a wide range of environmental conditions (Ladislaus et al., 2007).

Pectins are a class of complex polysaccharides found in the cell walls of higher plants, where they act as a moisturizing agent and a cementing material for the cellulose network. They are typically produced during the initial stages of primary cell wall growth and account for about one third of the cell wall of the dry matter of dicotyledonous plants and of monocotyledonous plants. The main exceptions are the cell walls of the *Graminae* family, which may contain pectin with normal structure, but in very small quantities.

Nano-clay is one of the most commonly used nanomaterials in biopolymer film technology Nano-clay is characterized by a moderate negative surface charge, which has a perfect crystalline structure, consisting of a two-dimensional layer having a central octahedral sheet of aluminium oxide and magnesium oxide bonded with two external silica tetrahedra (Majdzadeh-Ardakani et al., 2010; Slavutsky et al., 2014).

Nano-clay can be used to control the release of antimicrobial compounds from the film into the packaged product and also to control the interior atmosphere of the package, depending on the improvement of gas and water vapor permeability (Fabra et al., 2009).

MATERIALS AND METHODS

MATERIALS USED IN THE EXPERIMENTS TO OBTAINING FILMS

Materials used in this experimental work were:
- chitosan with average molecular weight, nano-clay and glycerol provided from Center for Research and Eco-Metallurgical Expertise, University Politehnica of Bucharest, Romania;
- gelatine and pectin purchased from the local market (Bucharest, Romania).

METHODS USED TO OBTAIN FILMS

In order to obtain various films, experiments were realized in Center for Research and Eco-Metallurgical Expertise, University Politehnica of Bucharest, Romania.

The following films have been developed: chitosan-gelatine (C/G), chitosan-gelatine-nano-clay(C/G/N-clay), chitosan-pectin (C/P), chitosan-pectin-nano-clay (C/P/N-clay), chitosan-gelatine-pectin (C/G/P), chitosan-gelatine-pectin-nano-clay (C/G/P/N-clay).

Different experimental variants of the three individual polymers were tried, as well as of the mixture between them to obtain a polymer film with a smooth and homogeneous structure.

To prepare film forming solutions, gelatine was dissolved in distilled water at room temperature, while 1.5% chitosan (Average Molecular Weight) was dissolved in 1% acetic acid solution, by stirring on the magnetic plate, at temperature of 120 °C and 400 rpm.

Pectin was used both dissolved in 2.5% distilled water and in powder form. The nano-clay was dispersed in glycerol with distilled water.

After obtaining the individual solutions, they were homogenized in different proportions to obtain the films, according to Table 1. Ultrasonic bath was used, then the homogeneous solutions were placed in square plastic plates (25 ml/plate) (Figure 1) and allowed to dry at room temperature for 48 hours.

Figure 1. Homogeneous solutions placed in square plastic plates

Table 1. Different concentrations of films

Sample	Chitosan 1.5% (g/sol)	Gelatine 10% (g/sol)	Pectin 2.5% (g/sol)	Glycerol (g)	Nano-Clay (g)
1	47.5	47.5	-	5	
2	43.5	43.5	-	5	8
3	47.5	-	47.5 (g/sol)	5	-
4	43.5	-	47.5 (g/sol)	5	8
5	46.25	46.25	2.5 (g)	5	-
6	45.25	45.25	2.5 (g)	5	8

RESULTS AND DISCUSSIONS

ANALYSIS OF FILM TRANSMISSION BY UV-VIS SPECTROMETRY

The obtained films (Figures 2-7) were analysed in the UV-VIS field using the Helyos spectrophotometer to determine their transparency.

Portions of each film obtained were placed in the Thermo Electron Corporation Helyos Spectrophotometer Testing Cell. Their transparency was determined by measuring the absorbance at a wavelength of 800 nm and was calculated by the equation:

$$\text{Transparency} = \frac{A800}{x} = -logT800/x$$

Where: The T800 is transmitting at 800 nm, A is the absorption at 800 nm and x is the film thickness (mm).

To indicate transparency, films must be homogeneous, there should be no phase separation between different components. If the compatibility between the different constituents of the films is not good, then the light transmission is low or the opacity is high due to the reflection or light scattering at the two-phase interface. Films have excellent barrier properties in the UV region, regardless of the cross-section content.

As it can be seen in Figure 8 the most transparent film is the one based on CS-G, having the lowest transparency value, A800/x = 0.39, followed by Cs-G-P with 0.45. The opaquest films are the ones made of Cs-G-P-N/ clay and Cs-G-N/clay with the transparency value A800/x = 1.79, respectively A800/x = 1.70.

FOURIER TRANSFORMED INFRARED SPECTROSCOPY

The structure of the films (Figures 9-14) was determined using, characterized by the Infrared (IR) spectroscopy by ATR spectra, that was carried out using a Perkin Elmer One spectrometer (Spectrum 2000 FTIR-ATR).

FTIR spectroscopy was used to characterize the interactions between chitosan, gelatine, pectin and nano clay. FT-IR spectra were recorded in the wavelength range 700 cm^{-1}-4000 cm^{-1}. For each film, two repetitions were performed. FTIR spectra obtained for each sample showed for all films, carbonyl (C-O) stretches at 1799 cm^{-1} and 2514 cm^{-1}, N-H bending at 1549 cm^{-1}, C-N stretching at 1239 cm^{-1} and C-H, amide II, amide III, and O-H stretching between 2931 cm^{-1} - 3300 cm^{-1} (Pereda et al., 2011). According to researchers Chen et al. (2016) the observed peaks at wavenumbers of 2854 cm^{-1} and 2924 cm^{-1} can be assigned to antisymmetric and symmetric -CH2 stretching vibrations, while peaks in the range 3200 cm^{-1} - 3600 cm^{-1} can be assigned to different -OH stretching vibrations corresponding to inter- and intra-molecular H-bonds (Chen et al., 2016).

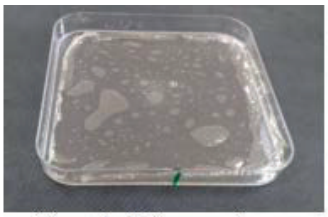

Figure 2. Chitosan and gelatin film (CS/G)

Figure 3.Chitosan and gelatin nano-clay film (CS/G/N-clay)

Figure 4.Chitosan and pectin film (CS/P)

Figure 5. Chitosan, pectin and nano-clay film (CS/P/N-clay)

Figure 6.Chitosan, gelatin and pectin film (CS/G/P/)

Figure 7.Chitosan, gelatin, pectin and nano-clay (CS/G/P/ N-clay)

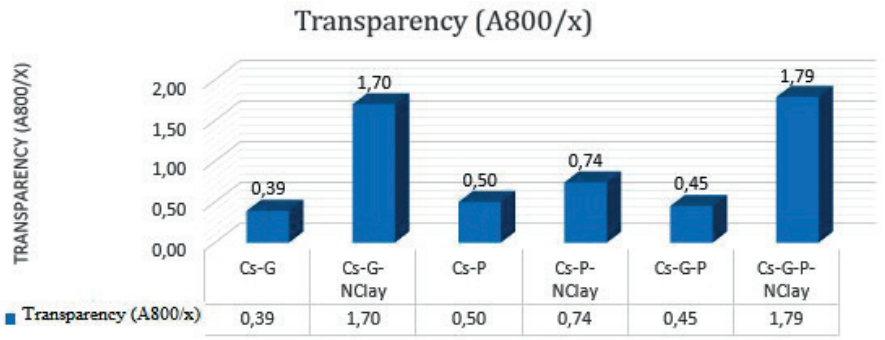

Figure 8. Transparency of the films

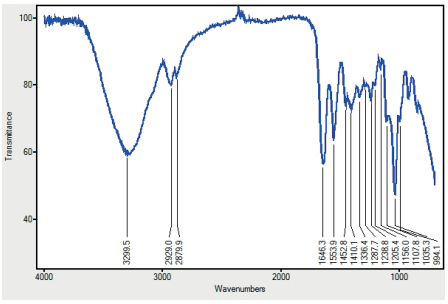

Figure 9. FTIR Spectra of films based on CS/G

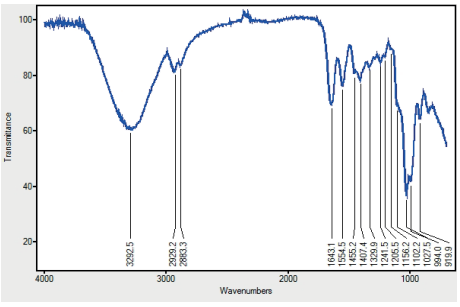

Figure 10. FTIR Spectra of films based on CS/G/N-Clay

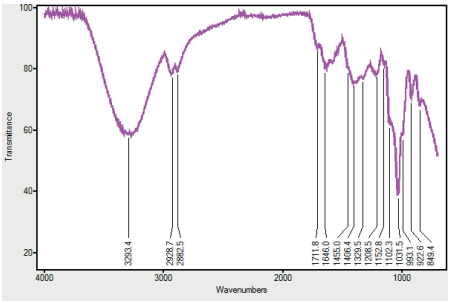

Figure 11. FTIR Spectra of films based on CS/P

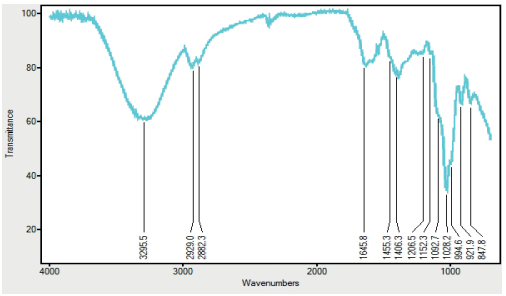

Figure 12. FTIR Spectra of films based on CS/P/N-Clay

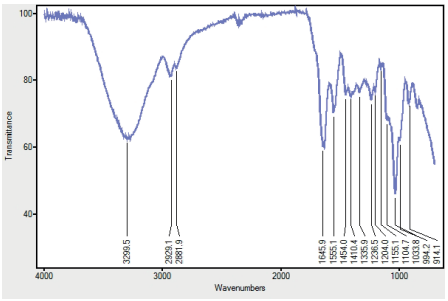

Figure 13. FTIR Spectra of films based on CS/G/P

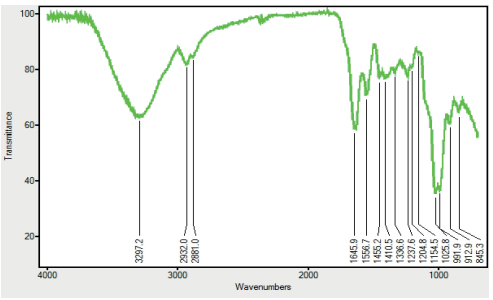

Figure 14. FTIR Spectra of films based on CS/G/P/N-Clay

The attribution of the absorption bands is in accordance with the literature study (Marcos-Fernández et al., 2006). Chitosan shows the absorption band of the C-C valence bond at 850-853 cm^{-1}, C-H at 2790 cm^{-1} -2881 cm^{-1} and the C-H (CH2) bond at 2931 cm^{-1} -2933 cm^{-1}. The absorption band specific for the N-H bond is found at 1316-1410 cm^{-1} and is due to the small amount of chitin present. Also, the valence vibration absorption band of the C-O bond at 1043 cm^{-1} and 1021 cm^{-1} is highlighted (Marcos-Fernández et al., 2006).

According to the literature, the chitosan-specific absorption vibrations are related to the following frequencies: 923 cm^{-1} -1033 cm^{-1} due to the valence vibration of the C–O bond in the secondary OH groups in the pyranose ring, 1152 cm^{-1} due to the valence vibration of the C bond. –O of the glycosidic bond, 1264 cm^{-1} due to the bending vibration of the N – H amide group, 1650-1559 cm^{-1} due to the valence vibration of the C = O bond of the amide I and the bending vibration of the N – H bond amide II, 2870 cm^{-1} attributed to the valence vibration of the CH bond in the pyranose ring, 2928 cm^{-1} attributed to the valence vibration of the CH2 group in the CH_2-OH bond and 3323 cm^{-1} attributed to the valence vibration of the NH2 group in the primary amines and the valence vibration of the OH group in the pyranose ring (Pasela et al., 2019).

Due to its unique amino acid sequence and numerous functional groups (groups), gelatine is a polymer containing free unprotected ε-amino groups (-NH2) derived from lysine or hydroxylisine (Dolete et al., 2019).

According to the data from the specialized literature (Hossana et al., 2014), the gelatine has three specific maxima, respectively at 1640 cm^{-1} due to the tensile vibration of the C = O bond specific to the amide I, at 1535 cm^{-1} the vibrations of the tension of the CN and deformation bond are highlighted of the NH bond of amide II, and at 1243 cm^{-1} -1260 cm^{-1} the vibrations of stretching of the CN bond and of bending of the NH bond specific to the amide III of gelatine appear. Also, in the specialized literature there are also mentioned the tensile vibrations of the bands - OH and NH located around 3200 cm^{-1}, the symmetrical stretching vibrations of the specific carboxylated salt bonds at about 1402 cm^{-1} and of the ester bond at 1090 cm^{-1}.

In the case of pectin, the FTIR spectrum shows two intense absorption bands, at 1748 cm^{-1} and 1637 cm^{-1} attributed to the valence vibration of the C = O bond in the ester and the bending vibration of the H_2O group which overlaps with the asymmetric stretch vibration of the carboxylate anion (COO–) (Bonilla & Sobral, 2016.).

The nano-clay shows the following specific absorption bands: at 1424 cm^{-1}, 876 and 712 cm^{-1} attributed to the valence vibration of the carbonate CO bond and weak absorption peaks at 1799 cm^{-1} and 2514 cm^{-1}, which are bands overlapping.

The absorption spectrum characteristic of nano-clay consists in highlighting the absorption band in the range 3300 cm^{-1}-3500 cm^{-1}, attributed to the valence vibration of the OH bond in the water molecule, the bands from 1685 cm^{-1} and 1622 cm^{-1} (the bending vibration of the O bond –H –O bending) (Kiros e al., 2013).

CONCLUSIONS

Films from natural polymers such as chitosan, gelatine and pectin were developed, in which nano-clay and glycerol were added, to obtain food packaging materials (Arvanitoyannis, 2002).

The combination of chitosan-gelatine, chitosan-pectin, chitosan-gelatine-pectin allows the obtaining of well shaped, homogeneous and semi-transparent films. In order to obtain flexible films, 5% glycerol and 3% nano-clay was added to the mixing mass.

The obtained films were characterized using UV/VIS spectroscopy to determine their degree of opacity and by ATR-FT-IR technique to characterize the interaction between the used polymers.

It was found that the optical properties of these films were slightly affected by the nano-clay concentration in their composition. As for the films based on chitosan-gelatine, chitosan-pectin, which contain nano-clay, the increased thickness of the films gave them mechanical strength, but also opacity.

Based on the results of this study, the developed materials could be suitable to be used as biodegradable based packaging materials in food industry.

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