

# PULLULAN-CHITOSAN COATINGS ONTO POLYETHYLENE FOILS FOR THE DEVELOPMENT OF ACTIVE PACKAGING MATERIAL

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Polyethylene (PE) material in the form of foils as conventional packaging material was functionalised with polysaccharide pullulan and an additional layer of chitosan macromolecular solution. In this study, the best concentration of pullulan (10%, 20% or 30%) was determined with the objective of improving the barrier properties of conventional PE food packaging material. As additional layer, a chitosan macromolecular solution was adsorbed to the material in order to endow it with antimicrobial activity. In this way, a functional composite material was developed, which can find potential application as active packaging, to offer prolonged shelf-life to packaged food. The functionalized PE material was analysed from the physico-chemical point of view (gravimetric analysis, goniometry, FTIR spectroscopy, standard test for oxygen permeability, SEM analysis). Moreover, antimicrobial testing of the foils was done and the desorption of both polysaccharides from the PE surface was evaluated. The latter properties are essential when evaluating the practical value of active packaging.

**Keywords:** pullulan, chitosan, PE functionalization, active packaging

## INTRODUCTION

Over recent decades, much research on the development of innovative food packaging materials has been carried out, with a view to combating pathogens, reducing spoilage and waste, optimizing process efficiency, reducing the need for chemical preservatives, improving the functionality of foods and improving the nutritional and sensorial properties of food in response to the demands of different consumer niches and markets, as well as in terms of affordability.<sup>1</sup> However, few of these investigations were economically, eco and health friendly; thus, it is still a great challenge to study new concepts of active packaging material. Nowadays, advanced bioactive packaging materials are very popular. The active packaging concept means that natural polymers are considered to be used as bioactive functional substrates. Examples of natural biodegradable and bioactive substances are less employed antimicrobial polysaccharides and their derivatives.<sup>2</sup>

Early attempts to employ pullulan in the food packaging industry lagged behind its established use as a food additive (*e.g.*, thickening agent, binder, stabilizer), the first works dating back to the beginning of the '90s.<sup>3</sup> At that time, it was understood that great benefits would have arisen from certain peculiar properties of pullulan, such as its high water solubility and the barrier property against oxygen and carbon dioxide. At the beginning, water-soluble edible films of pullulan were proposed as edible pouches for premeasured portions that could be gradually dissolved in water or in hot food.<sup>3</sup> However, the first massive application of pullulan in food packaging can be considered its use as an edible film coating, *i.e.*, a relatively thin layer of pullulan material applied and formed directly on the surface of the food product, which can be eaten along with the product.<sup>4</sup> Many studies have shown the use of pullulan coatings to increase the shelf-life of fruits.<sup>5</sup> Pullulan coatings had good adhesive properties, high mechanical strength and did not react with food ingredients. In addition, pullulan coatings were colorless, tasteless and odorless, and had limited permeability to gases, such as oxygen and carbon dioxide.<sup>6</sup> Pullulan coatings have been used for extending the shelf-life of apples (completely and in pieces), strawberries, kiwifruit, carrots, blueberries, strawberries, peppers and Brussels sprouts.<sup>6–12</sup>

Pure and composite films based on tapioca starch and pullulan were investigated as to their stability during dry and humid storage (RH 23 and 85%). By using a proper composite ratio (5% starch and 2 or 5% pullulan), the film could be optimally prepared to exhibit both mechanical strength and storage stability against humidity.<sup>13</sup> Faris *et al.* discussed a new perspective antifog coating made of pullulan film.<sup>14</sup> The same authors presented the preparation and characterization of oxygen barrier pullulan sodium montmorillonite (Na<sup>+</sup>-MMT) nanocomposite coatings onto PE, which show excellent barrier performance even under high relative humidity conditions.<sup>15</sup> Pullulan has thus emerged as one of the most important biopolymers, with the greatest perspective, due to its great properties, as a coating for food packaging and thus, it is still challenging to apply it in any novel form for packaging systems. As regards the development of the active packaging concept, chitosan as an antimicrobial amino-biopolymer obtained from chitin is also very attractive. Its low production costs and biocompatibility make chitosan an interesting material for food processing, biomedical applications and water purification, just to mention a few.<sup>16–19</sup>

Chitosan has been recently approved as a food ingredient by the FDA; therefore, the use of chitosan for new product development and as natural antimicrobial agent is expected to become even more popular. In food products, chitosan offers a wide range of applications, *e.g.* for preservation of food from microbial deterioration, formation of edible biodegradable films, coagulation of proteins and lipids from wastewater, enhancing gelation in surimi and fishery products, as well as for clarification/deacidification of fruit juice.<sup>20–23</sup> Besides several direct applications in food products, chitosan also exhibits a potential for use as food supplement with anti-cholester-olemic, anti-ulcer, anti-uremic and anti-tumour effects.<sup>20–24</sup> Due to its great bioactive properties, chitosan may be an ideal potential substance for polyethylene coatings – in line with the active packaging concept.<sup>25–29</sup> Due to its bacteriostatic function, it prolongs the lag phase and, consequently, reduces the growth rate of microorganisms, thus extending the shelf-life of products and maintaining product quality and safety.<sup>30,31</sup>

Although there are several contributions focusing on the use of chitosan in different structural forms or on its combination with other antimicrobial/antioxidant agents for food preservation,<sup>30–33</sup> there has been little work done on the use of complementary synergistic formulations of chitosan and pullulan. The preparation and characterization of pullulan-chitosan and pullulan-carboxymethyl chitosan blended films was discussed by Jia Wu *et al.*<sup>34</sup> Our literature survey revealed that most of the research is limited to edible films and not to layer-by-layer coatings applied onto PE, as proposed in this work.

This paper aimed to determine the best concentration of pullulan, out of three levels (10%, 20% and 30%), for its use as coating to improve the barrier properties of polyethylene. A chitosan macromolecular solution (1%) was adsorbed as an additional layer onto polyethylene in order to impart antimicrobial activity. In this way, a functional composite material could be developed, which can find potential application in the active packaging area. The functionalized PE material was analyzed as follows: i) gravimetry: to determine the amount of coating applied on the polyethylene film, ii) goniometry: to measure the hydrophilic or hydrophobic character of the functionalized material surface; iii) FTIR spectroscopy: to study the elemental composition of the coating on the PE surface; iv) scanning electron microscopy (SEM): to analyze the morphology of the functionalized PE and to observe the coating structures; iv) standard test for oxygen permeability and; v) antimicrobial testing: to examine the inhibition properties against bacteria and fungi. In addition, desorption studies were performed (using total organic carbon TOC analysis).

## EXPERIMENTAL

### Materials

10%, 20% and 30% of aqueous pullulan solutions were prepared in a volume of 25 mL. All the solutions were made by the same procedure. The solute and the solvent (water) were mixed for 3 hours, between 40 °C and 50 °C, with a cover to avoid evaporation. In order to obtain better adhesion of pullulan onto the PE foils, glycerol was added to these solutions (3%, 6%, 9%, respectively). Moreover, in order to impart antimicrobial properties, a 1% chitosan acidic solution (pH 3.6 adjusted with concentrated lactic acid) was prepared.

Table 1  
Sample description

| Coatings onto PE                           |
|--|
| Reference PE material                      |
| 10% Pullulan + 3% Glycerol                 |
| 20% Pullulan + 6% Glycerol                 |
| 30% Pullulan + 9% Glycerol                 |
| (10% Pullulan + 3% Glycerol) + 1% Chitosan |
| (20% Pullulan + 6% Glycerol) + 1% Chitosan |
| (30% Pullulan + 9% Glycerol) + 1% Chitosan |

Conventional PE foils – polyethylene of normal quality, transparent (GSM 46.28 g/m<sup>2</sup>, thickness 50 µm, slippery 0.207), from Makoter D.O.O, (cleaned by ethanol (MW 46.07 g/mol), 99.8% (GC) from Honeywell, Sigma-Aldrich) were first coated by pullulan-glycerol solutions by roll-to-roll printing, using a Johannes Zimmer machine, Austria, type MDF-R-23. Then, the foils were dried at 40 °C for 20 min (in a Kambič drier) and a chitosan macromolecular solution was applied onto PE as a second layer by the same roll-to-roll printing technique. At the end, the foils were again dried, as mentioned above. Different coating procedures resulted in 7 differently treated PE samples, which are presented in Table 1.

## Methods

### *Gravimetric measurements of sample mass*

All the functionalized samples were weighed and their weight was recoded up to four decimal places for the purpose of comparing the values with those of untreated reference foils, which were previously cleaned, dried and cut to the size of the print. The final weight differences between the reference foils and the functionalized foils were calculated for absolute dry samples.

### *Goniometry*

The static contact angle (SCA) measurement gives information on the hydrophilic/hydrophobic nature of the material. The water contact angles of hydrophilic materials are below 90°, while they are above 90° for hydrophobic ones. A DataPhysics goniometer, Germany, with SCA 20 software, was used to measure the static contact angle of the PE samples at room temperature with Milli-Q water. A small drop (3 µL) of liquid (water) was carefully placed on the small flat surface of the PE foil pieces.

### *Surface composition of functionalized foils: ART FTIR spectroscopy*

The ART FTIR spectra were recorded on a Perkin Elmer Spectrum GX NIR FT-Raman spectrometer. The ATR accessory contained a diamond crystal. All the spectra (16 scans at 4 cm<sup>-1</sup> resolution, background and the sample spectra were obtained in the 4000-650 cm<sup>-1</sup> wavenumber range) were recorded at room temperature. The spectra of the samples were deconvoluted with smoothing filter and baseline corrected (automatically). For the FTIR measurements, the samples were cut to pieces and each sample was analyzed on 5 different spots to avoid possible error caused by non-homogeneity of the coating surface.

### *XPS analysis*

Spectra were recorded using a PHI TFA XPS instrument, Physical Electronics, USA, in order to assess the surface of the sample. The base pressure in the XPS analysis chamber was approximately  $6 \times 10^{-8}$  Pa. The samples were excited with X-rays over a 400-µm spot area with monochromatic Al K $\alpha$  1,2 radiation (1486.6 eV), operating at 200 W. Photoelectrons were detected with a hemispherical analyzer, positioned at an angle of 45° with respect to the normal to the sample surface. The energy resolution was about 0.6 eV. Spectra were recorded for at least two locations on each sample, using an analysis area of 400 µm. Surface elemental concentrations were calculated from the survey-scan spectra, using Multipak software.

### *Oxygen permeability*

The oxygen permeability was determined using an Oxygen Transmission Rate System PERME® OX2/230, Labthink Instruments Co., Ltd. P.R. China, according to standard ASTM D3985. OTR (oxygen transmission rate) values and coefficient values are the average results obtained after five measurements. All the specimens were conditioned at 23 °C and 50% relative humidity, 24 h prior to testing (flux = 10 mL/min). The thickness of PE was measured with a caliper in 5 different places.

### *SEM*

The morphology of PE foils, with and without coated formulation (reference PE), was inspected with scanning electron microscopy (SEM), using a FE-SEM SUPRA 35 VP (Carl Zeiss). A small piece of foil was placed onto the sample holder and attached to it with conductive carbon tape. All the samples were analyzed with accelerated voltage of 1 kV and 20  $\mu\text{m}$ -sized aperture, with variable working distance (4-5 mm).

#### Desorption test

Both the functionalized and reference PE foils were immersed into distilled water for 12 h. Then, the foils were removed from the desorption bath and the remaining solution was analyzed by the total organic carbon (TOC) quick standard test 0-99 06.14 TOC 600 – Macherey-Nagel. The determination of TOC was carried out in two steps: 1) disposal of the inorganic carbon (TIC), 2) decomposition of the organic carbon (TOC) and detection of the carbon dioxide formed by means of an indicator (range: 40-600 mg/L C, factor: 0410 (-), wavelength (HW = 5-12 nm): 585 nm, decomposition time: 2 h, decomposition temperature: 120 °C).

Both polysaccharides, pullulan and chitosan, possess C in their structure, thus, if desorption occurs, TOC is expected to increase in the desorption bath.

#### Antimicrobial test

For the antimicrobial test of the functionalized PE foils, a modified version of ISO 22196 (Plastics – Measurement of antibacterial activity on plastics surfaces) was followed.<sup>35</sup> This is currently the test protocol of choice for testing surfaces for antimicrobial effectiveness. Gram-positive bacteria (*Staphylococcus aureus* ŽMJ72) and fungi (*Aspergillus flavus* ŽMJ25), both from the culture collection of the Laboratory for Food Microbiology at the Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, were tested. After the determination of the number of viable cells, antimicrobial activity was calculated using Equation (1), and the percentage of reduction – using Equation (2).

$$R = U_t - A_t \quad (1)$$

$$\text{Reduction (\%)} = ((U_t - A_t)/U_t) \times 100 \quad (2)$$

where R is the antibacterial activity;  $U_t$  is the number of viable cells recovered from the untreated material after incubation;  $A_t$  is the number of viable cells recovered from the treated material after incubation.

## RESULTS AND DISCUSSION

The results of gravimetric measurements for PE foils are given in Table 2. It can be seen for all the samples that their mass increased, which somehow indicates that the application of the coatings was successful. It can also be remarked that, when chitosan was applied onto different pullulan-coated polyethylene samples, the mass increased to a higher extent, which proved the attachment of an additional chitosan layer. In most of the cases, additional chitosan adsorption increased the coated mass by around 100%.

The application of the pullulan solution coating onto the PE foil was also proved by FTIR spectroscopy. Figure 1 shows the following FTIR spectra: FTIR spectrum of pure pullulan (pink line, Fig. 1) and that of the PE foil (red line, Fig. 1) as references, as well as the spectra of the PE foil coated with the 10% and 20% pullulan solutions, with no addition of glycerol. In this way, only pullulan coating onto PE may be seen – for better distinguishing the effect of pure pullulan coatings. The FTIR spectrum of the PE foil (blue line, Fig. 1) shows characteristic signals at the following wavenumbers: 2912  $\text{cm}^{-1}$ , 2849  $\text{cm}^{-1}$ , 1472  $\text{cm}^{-1}$  and 718  $\text{cm}^{-1}$ , while the spectrum of pullulan (black line, Fig. 1) shows typical signals at 3309 and 988  $\text{cm}^{-1}$ , which correspond to the vibration of the OH group. The FTIR spectra of the PE foil coated with 10% (green line, Fig. 1) and 20% (red line, Fig. 1) solutions of pullulan indicate the typical signals of pullulan. According to FTIR analysis, we can conclude that the PE foils were successfully coated with the pullulan solution.

In Figure 2, the spectra of the reference PE material (blue line), pullulan (black line) and PE foil treated with 10%, 20% and 30% pullulan solutions and an appropriate amount of glycerol (green, red and pink line) are shown. The FTIR spectra of the PE foil coated with 10% (green line, Fig. 2), 20% (red line, Fig. 2) and 30% (pink line, Fig. 2) solutions of pullulan and an appropriate amount of glycerol reveal typical signals of pullulan. The signals at the wavelength of 3309  $\text{cm}^{-1}$  and 998  $\text{cm}^{-1}$  are more intensive for the PE foil coated with the 30% pullulan solution. According to FTIR analysis, we can conclude that the PE foils were coated with the pullulan solution and glycerol did not have an influence on the spectra of the treated foil.

Table 2  
Differences of mass before and after applying the pullulan coating (+ added glycerol) and further chitosan macromolecular solutions (two replications were made for each sample)

| Film         | Mass before (g) | Pullulan (%) | Glycerol added into pullulan solution (%) | Mass after (g) | Second layer of chitosan (1% solution) | Difference in mass; <i>i.e.</i> coating mass (g) |
|--------------|-----------------|--------------|---|----------------|--|--|
| Polyethylene | 3.0059          | 10           | 3   | 3.0283         | No                                     | 0.0224   |
| Polyethylene | 2.9369          | 10           | 3   | 2.9582         | No                                     | 0.0213   |
| Polyethylene | 3.7094          | 20           | 6   | 3.7911         | No                                     | 0.0817   |
| Polyethylene | 3.3964          | 20           | 6   | 3.4925         | No                                     | 0.0961   |
| Polyethylene | 2.6303          | 30           | 9   | 2.7278         | No                                     | 0.0975   |
| Polyethylene | 2.9049          | 30           | 9   | 2.992          | No                                     | 0.0871   |
| Polyethylene | 2.8415          | 10           | 3   | 2.8833         | Yes                                    | 0.0418   |
| Polyethylene | 2.8915          | 10           | 3   | 2.9489         | Yes                                    | 0.0574   |
| Polyethylene | 3.2382          | 20           | 6   | 3.3939         | Yes                                    | 0.1557   |
| Polyethylene | 3.2547          | 20           | 6   | 3.4109         | Yes                                    | 0.1562   |
| Polyethylene | 3.1606          | 30           | 9   | 3.2981         | Yes                                    | 0.1375   |
| Polyethylene | 2.7192          | 30           | 9   | 2.8015         | Yes                                    | 0.1823   |

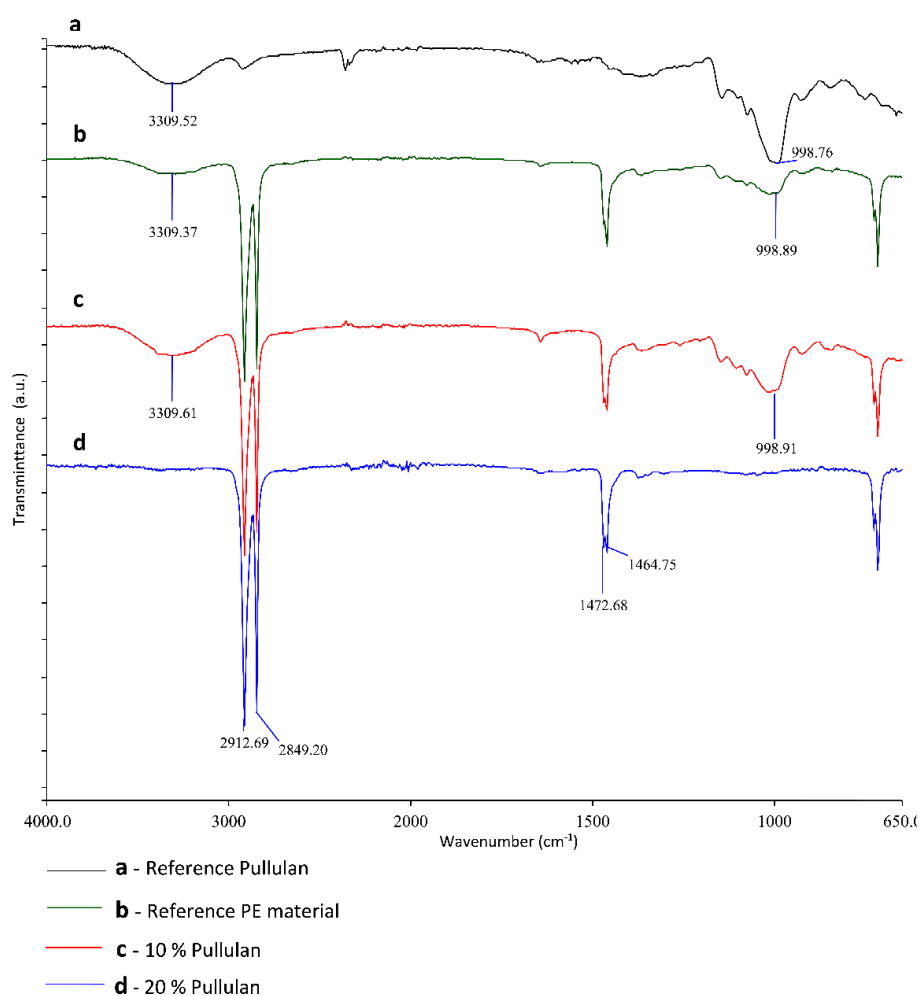


Figure 1: FTIR spectra of PE foil coated with pullulan solution

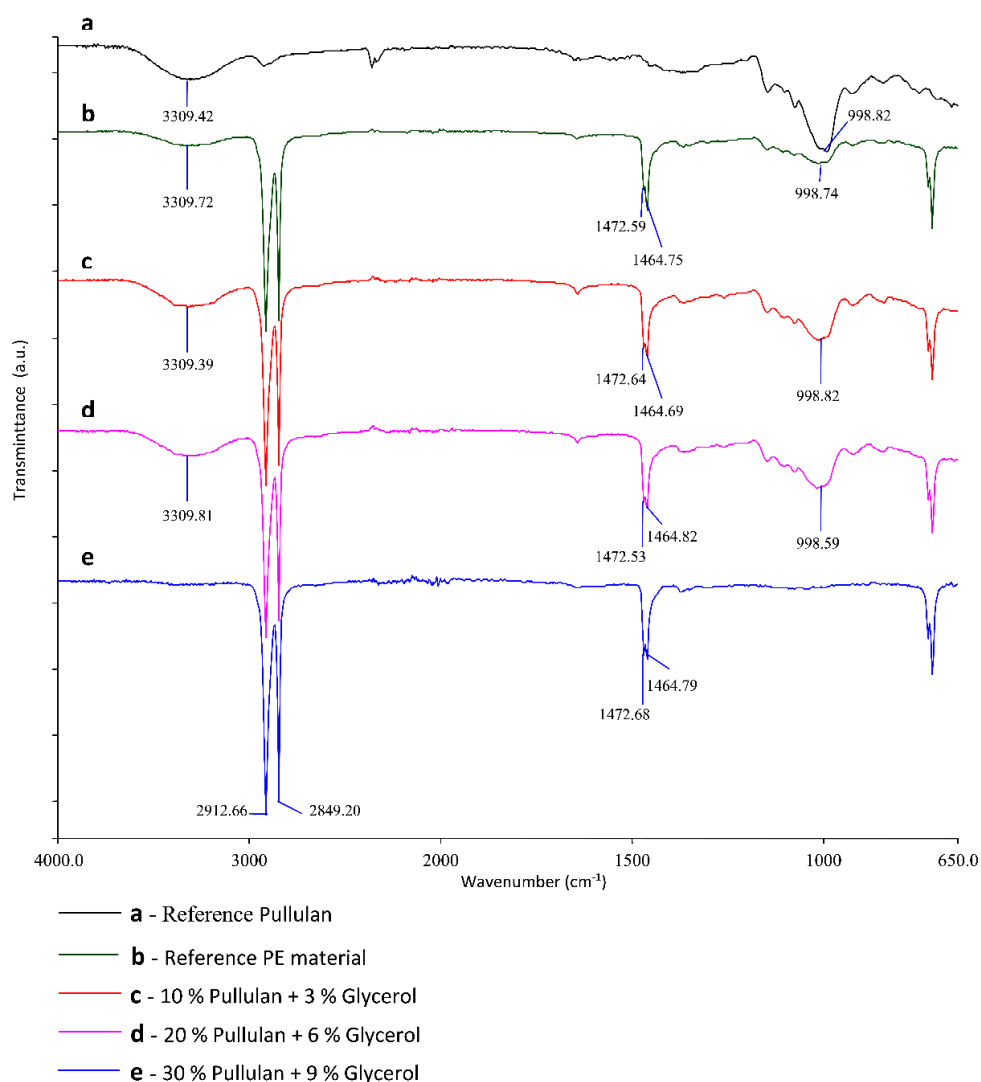


Figure 2: FTIR spectra of PE foil coated with pullulan solution and glycerol

Figure 3 presents the spectra of the reference PE material (blue line, Fig. 3), the pullulan reference (black line, Fig. 3) and chitosan reference (light green line, Fig. 3), as well as those for the PE foil treated with 10% (pink line, Fig. 3), 20% (green line, Fig. 3) and 30% (red line, Fig. 3) solutions of pullulan – with the addition of 3%, 6% and 9% glycerol, respectively, and further coated by 1% solution of chitosan.

The FTIR spectrum of the pure chitosan (light green line, Fig. 3) indicates typical peaks at 1649 and 1566 cm<sup>-1</sup>. These two wavenumbers are assigned to the carbonyl stretching vibration (amide I), and the N-H bending vibration (amide II) of a primary amino group, respectively.

FTIR spectra of the PE foil coated with 10% (pink line, Fig. 3), 20% (green line, Fig. 3) and 30% (red line, Fig. 3) solutions of pullulan with 3%, 6%, 9% glycerol, respectively, and further by 1% chitosan solution, exhibit the typical signals of pullulan (at 3309 and 998 cm<sup>-1</sup>) and chitosan (at 1649 and 1566 cm<sup>-1</sup>). The peaks at the wavelengths of 3309 cm<sup>-1</sup> and 998 cm<sup>-1</sup> are more intensive for the PE foil coated with the 30% pullulan solution. According to the FTIR analysis, we can conclude that the PE foils were coated with the pullulan and chitosan solutions. The higher the concentration, the more intensive are the peaks, indicating enhanced adsorption.

This finding was supported by the XPS results, where nitrogen content (in at.%) was determined. No nitrogen was detected on the reference PE. In all the samples coated by pullulan only, a small amount of nitrogen was detected (below 0.1%), which may be attributed to the presence of some impurities in commercial pullulan powder. The N amount was introduced onto the PE foil coated with 10%, 20% and 30% solution of pullulan with 3%, 6%, 9% glycerol, respectively, and further treated by 1% chitosan. For

the first sample, the amount of nitrogen was 1.7%, for the second – 2.2% and for the last one – 3.6%. These results clearly support the fact that an increasing amount of pullulan on the PE foils introduced more available functional groups for physical attachment of chitosan onto the pullulan-coated foils. This assumption is in accordance with the results of the desorption experiments described below.

In Table 3, the results for the contact angles determined by goniometry are listed. The reduction of the contact angle is of great importance for practical use of the developed material, since the hydrophilic surface of the foils would reduce the potential process of dew condensation on the foil (antifog efficiency) when in contact with food, which may worsen the packaging conditions and thus increase food contamination risks.<sup>36</sup>

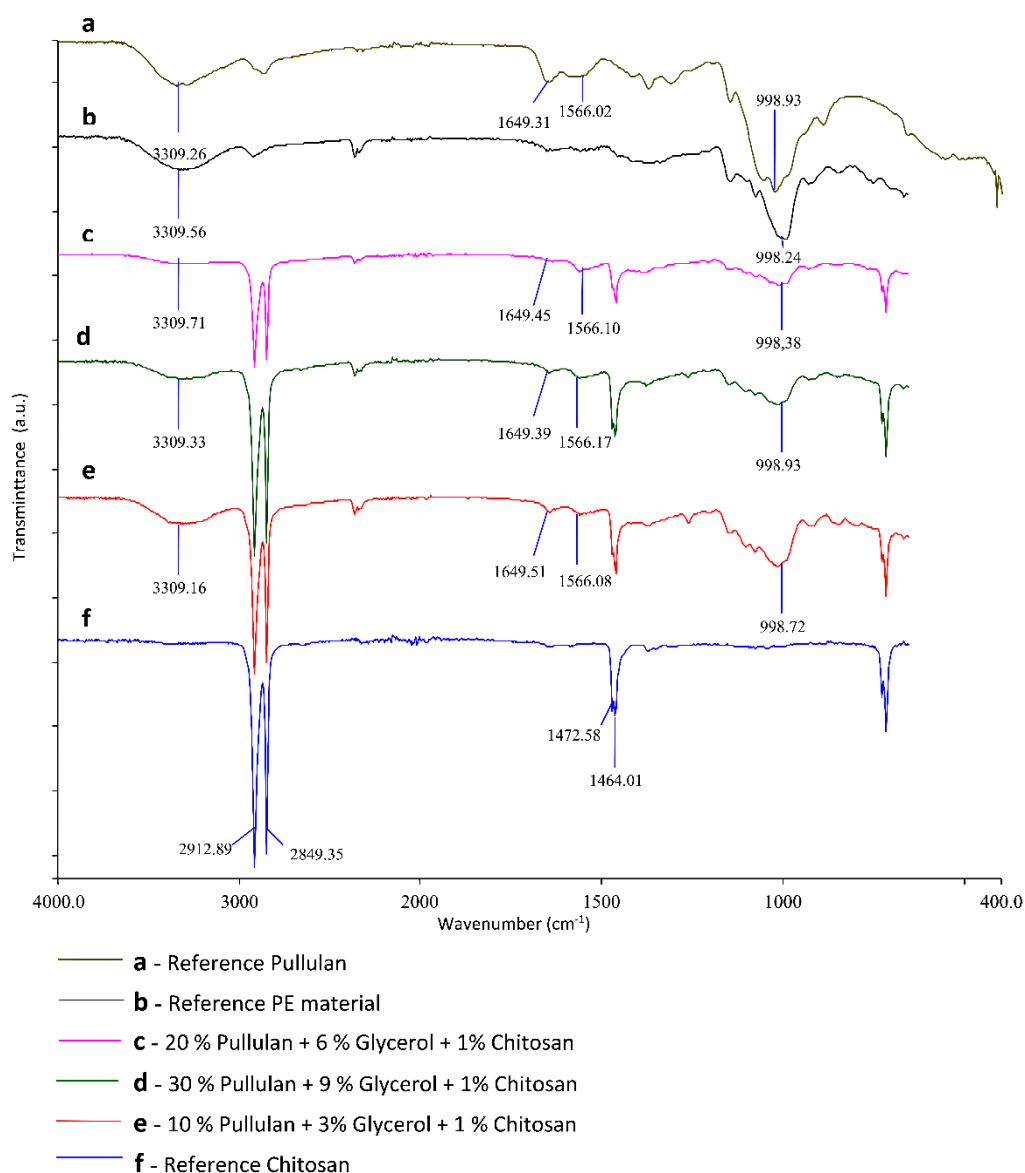


Figure 3: FTIR spectra of PE foil coated with pullulan solution, glycerol and 1% chitosan solution

Table 3  
Contact angles of samples

| Coted PE samples                           | Average CA (M) (°) |
|--|--------------------|
| Reference PE                               | 109.35             |
| 10% Pullulan + 3% Glycerol                 | 99.56              |
| 20% Pullulan + 6% Glycerol                 | 100.12             |
| 30% Pullulan+ 9% Glycerol                  | 92.95              |
| (10% Pullulan + 3% Glycerol) + 1% Chitosan | 104.30             |
| (20% Pullulan + 6% Glycerol) + 1% Chitosan | 105.87             |
| (30% Pullulan + 9% Glycerol) + 1% Chitosan | 104.85             |

Table 4  
Oxygen permeability test results

| Sample                                       | OTR<br>(cm <sup>3</sup> /m <sup>2</sup> d) | STDV<br>OTR | Coefficient<br>(cm <sup>3</sup> cm/cm <sup>2</sup> scmHg) | STDV<br>coefficient | Thickness<br>(mm) |
|--|--|-------------|---|---------------------|-------------------|
| Reference PE                                 | 3225.97                                    | 61.6        | 3.2334E-07  | 1.3707E-08          | 0.05              |
| 10% Pullulan +3%<br>Glycerol                 | 2119.7                                     | 49.0        | 2.1624E-07  | 6.66133E-09         | 0.05              |
| 20% Pullulan + 6%<br>Glycerol                | 2227.5                                     | 14.1        | 2.2842E-07  | 1.41067E-09         | 0.05              |
| 30% Pullulan + 9%<br>Glycerol                | 2228.6                                     | 26.0        | 2.28302E-07   | 2.39792E-09         | 0.05              |
| (10% Pullulan + 3%<br>Glycerol) +1% Chitosan | 2251.3                                     | 42.1        | 2.308E-07   | 4.32782E-09         | 0.05              |
| (20% Pullulan + 6%<br>Glycerol)+1% Chitosan  | 2596.8                                     | 70.6        | 2.6624E-07  | 7.2298E-09          | 0.05              |
| (30% Pullulan + 9%<br>Glycerol)+1% Chitosan  | 2419.4                                     | 15.2        | 2.48067E-07   | 1.51767E-09         | 0.05              |

The results obtained reveal that all the pullulan coatings onto PE reduced the contact angle, in comparison with the reference sample by around 10% or even more. The differences among pullulan concentrations are, however, very small and no significant influence on contact angle may be seen among them. The addition of chitosan, in general, led to a negligible increase of the contact angle, in comparison with the pullulan-polyethylene samples, *i.e.* the addition of chitosan as the second layer increases the contact angle by around 5%-10%, in comparison with the samples coated only with pullulan, but the contact angle is still lower than that of the reference PE. It is important that none of the polysaccharide coatings on PE increased the contact angle, which could cause problems during practical use, such as dew condensation, which may negatively influence the shelf-life of food by shortening it.

The results of the oxygen permeability test are listed in Table 4. As can be seen, the O<sub>2</sub> permeability decreased with increasing concentration of pullulan, so it does improve the oxygen barrier property. The highest decrease (35%) was obtained for the PE sample coated with the 10% pullulan solution. Nevertheless, the application of chitosan as a second layer does not influence the oxygen barrier property to a great extent, in comparison with the pure pullulan coatings, but still shows improved oxygen barrier properties in comparison with the reference PE. The morphology of the functionalized samples can be observed in the SEM images in Figure 4.

A clear indication of the pullulan presence, without/with the additional layer of chitosan, can be clearly distinguished on the SEM images of the PE foils (Fig. 4). It is noteworthy that all the images were taken at comparable magnification for easier comparison. Also, the SEM image for the reference PE foil is presented to clearly see the difference between the non-functionalized and the functionalized PE foils. As can be observed for the different formulations under study, the pullulan coating mainly formed spherical particles on the PE foils, most likely due to the difference in hydrophilicity: *i.e.* the pullulan tends to decrease its surface energy, forming spherical nano/micro-particles, lacking the ability to form thin-coated films. With increasing pullulan concentration, the 20% and 30% pullulan coatings (+glycerol) formed larger micro-particles, as opposed to the 10% pullulan coating, which mainly made smaller nanoparticles on the PE foils. With the addition of chitosan to the pullulan



(+glycerol) coating, its major influence could be clearly seen in the morphology of the 10% pullulan sample, by the presence of larger particles (which were not observed for the coating formed from 10% pullulan only on the PE foils). Also, increasing the concentration of chitosan did not have any significant effect on particle size and morphology of the coated PE.

The migration of both polymers, *i.e.* pullulan and chitosan, from the surface of the foils was followed by TOC. The results of TOC determinations in different desorption baths are presented in Table 5. It can be clearly seen from the obtained values that pullulan (together with glycerol) attached onto the foils individually and did not desorb from the foil surface. The highest amount of TOC for the desorbed pullulan was detected at the lowest concentration of pullulan as adsorbate (*i.e.* 10%).

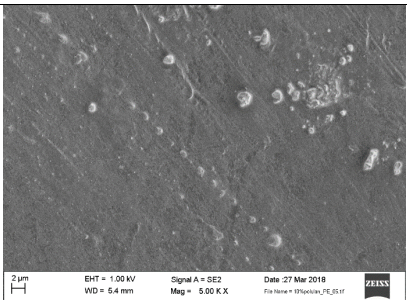
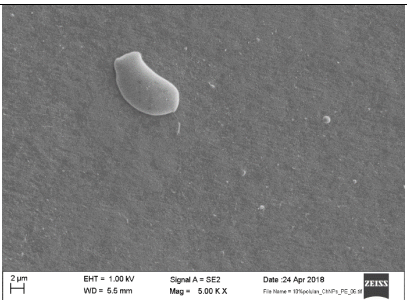
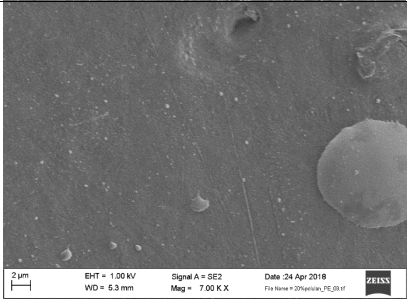
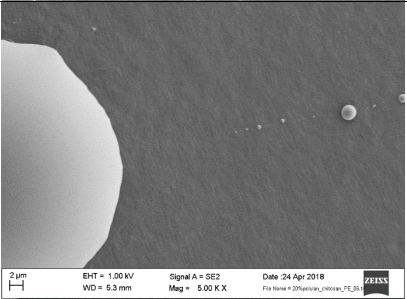

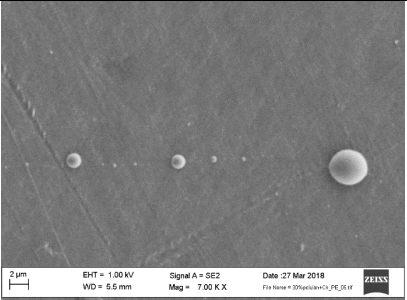

| Concentration<br>(mass<br>concentration) | Pullulan + appropriate amount of<br>glycerol   | Pullulan-glycerol + chitosan<br>(1 wt%)  |
|--|--|--|
| 10% + 3%                                 |     |    |
| 20% + 6%                                 |    |   |
| 30% + 9%                                 |   |  |
| PE foil: reference                       |  |  |

Figure 4: SEM analysis of functionalized foils

Table 5  
TOC results for different samples

| Desorption bath of sample                  | TOC       |
|--|-----------|
| 10% Pullulan + 3% Glycerol                 | >600 mg/L |
| 20% Pullulan + 6% Glycerol                 | 254 mg/L  |
| 30% Pullulan + 9% Glycerol                 | 226 mg/L  |
| (10% Pullulan + 3% Glycerol) + 1% Chitosan | <40 mg/L  |
| (20% Pullulan + 6% Glycerol) + 1% Chitosan | <40 mg/L  |
| (30% Pullulan + 9% Glycerol) + 1% Chitosan | 98 mg/L   |

Table 6  
Antibacterial activity against *S. aureus*

| Sample                                     | Cell number (log cfu/cm <sup>2</sup> ) | Reduction (%) |
|--|--|---------------|
| Control                                    | 4.75 ± 0.12                            | -             |
| 10% Pullulan + 3% Glycerol                 | 4.26 ± 0.21                            | 10.31 ± 4.41  |
| 20% Pullulan + 6% Glycerol                 | 4.64 ± 0.07                            | 0             |
| 30% Pullulan + 9% Glycerol                 | 4.01 ± 0.16                            | 12.92 ± 4.05  |
| (10% Pullulan + 3% Glycerol) + 1% Chitosan | 2.76 ± 0.52                            | 42.45 ± 10.28 |
| (20% Pullulan + 6% Glycerol) + 1% Chitosan | 4.32 ± 0.10                            | 9.01 ± 2.17   |
| (30% Pullulan + 9% Glycerol) + 1% Chitosan | 2.23 ± 0.81                            | 62.95 ± 17.12 |

Table 7  
Antibacterial activity against *A. flavus*

| Sample                                   | Cell number (log cfu/cm <sup>2</sup> ) | Reduction (%) |
|--|--|---------------|
| Control                                  | 2.52 ± 0.28                            | -             |
| 10% Pullulan + 3% Glycerol               | 2.34 ± 0.31                            | 0             |
| 10% Pullulan + 3% Glycerol + 1% Chitosan | 2.49 ± 0.15                            | 0             |
| 20% Pullulan + 6% Glycerol               | 2.24 ± 0.19                            | 10.98 ± 7.48  |
| 20% Pullulan + 6% Glycerol + 1% Chitosan | 2.36 ± 0.07                            | 6.02 ± 2.77   |
| 30% Pullulan + 9% Glycerol               | 2.29 ± 0.32                            | 0             |
| 30% Pullulan + 9% Glycerol + 1% Chitosan | 1.71 ± 0.16                            | 31.99 ± 6.48  |

These results may indicate that at lower concentration of pullulan, fewer OH groups are available, which means a reduced possibility of physical interactions to be formed between pullulan and polyethylene. This, in turn, leads to lower coating stability and higher desorption. In consequence, the total amount of organic carbon diluted into water is higher.

It can be also seen that chitosan acts as a good barrier for the desorption of pullulan. Obviously, when chitosan was applied as an additional layer onto the pullulan film, the physical interaction between both polysaccharides, as well as with the basic PE foils, was extended and thus the stability of the coating was improved. It seems that an additional layer of chitosan onto polyethylene decreased the total desorption amount. All the PE samples coated with pullulan and chitosan together have a lower TOC than the ones with no addition of chitosan. If desorption occurs to a great extent, then TOC should be significantly higher, as all pullulan, glycerol and chitosan possess C atoms in their backbones.

The antimicrobial properties of the functionalized PE foils against *S. aureus* are described in Table 6. Polyethylene shows no inhibition of *S. aureus*. It can be clearly seen that the attachment of pullulan onto the foils does not impart any significant antimicrobial effect. Polyethylene coated with 10% and 30% pullulan (+ 3.9% glycerol) exhibits some small reductions. The pullulan-polyethylene foils additionally coated with chitosan revealed an improvement of antimicrobial activity against *S. aureus*. The highest inhibition (63%) was recorded for sample (30% Pullulan + 9% Glycerol) + 1% Chitosan. Obviously, the highest amount of chitosan was attached onto the functionalized foils and thus more available amino groups induced an antimicrobial effect. It is known that a higher amount of protonated amino groups increases antimicrobial activity.<sup>37</sup>

The antimicrobial properties against *A. flavus* fungus are given in Table 7. Polyethylene shows no inhibition of *A. flavus*. As already known, fungi, due to their structure, are highly resistant to a wide

spectrum of currently available conventional antimicrobials.<sup>38</sup> It has been already discussed that the antimicrobial properties of chitosan are more antibacterial than antifungal.<sup>39</sup> From our results, it may be remarked that only the polyethylene foils coated with 30% of pullulan and further with a layer of 1% of chitosan macromolecular solution improved, to some extent (32% R), the antimicrobial activity against *A. flavus*.

Both antimicrobial tests showed that sample (30% Pullulan + 9% Glycerol) + 1% Chitosan, with possible further improvements (optimization), could find application in active packaging. It must be pointed out that the functionalization process is technologically and economically suitable to be scaled up to actual production, while some process design<sup>40,41</sup> and cost aspects must still be studied.<sup>42</sup>

## CONCLUSION

Gravimetric and FTIR results support successful attachment of pullulan and chitosan onto PE foils. This work has demonstrated that pullulan can improve barrier properties for oxygen permeability by itself, however, a supplementary layer of chitosan does not decrease oxygen permeability. Moreover, it is also important that the polysaccharide coatings onto PE did not increase the contact angle. An increased contact angle could cause problems in practical use: *i.e.* in contact with food, dew condensation may occur with highly hydrophobic materials, which could negatively influence food shelf-life. Desorption from PE increased with higher concentrations of pullulan, but decreased, while preserving high performance, when chitosan was used as a second layer. Both antimicrobial tests (against *S. aureus* and *A. flavus*) indicated that sample (30% Pullulan + 9% Glycerol) + 1% chitosan inhibited, to some extent, those two pathogens. To conclude, pullulan-chitosan adsorbates, as a layer-by-layer structure attached to PE foils, with some further modifications in adsorbate concentrations, chemical conditions, layer thickness *etc.*, could be definitely applicable as an advanced packaging material.

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## REFERENCES

- <sup>1</sup> N. Bumbudsanpharoke and S. Ko, *J. Food Sci.*, **80**, R910 (2015).
- <sup>2</sup> R. Kargl, T. Mohan and K. Stana-Kleinschek, in “Current Advances in Biopolymer Processing and Characterization”, edited by K. Martin, Nova Science Publishers Inc., 2017, vol. 1, pp. 1-13.
- <sup>3</sup> O. Pinar, F. Yangilar, P. Oğuzhan and F. Yangilar, *Afr. J. Food Sci.*, **4**, 57 (2013).
- <sup>4</sup> V. Trinetta, J. D. Floros and C. N. Cutter, *J. Food Saf.*, **30**, 366 (2010).
- <sup>5</sup> K. Kraśniewska, I. Ścibisz, M. Gniewosz, M. Mitek and K. Pobiega, *Materials*, **10**, 8 (2017).
- <sup>6</sup> S. Farris, I. U. Unalan, L. Introzzi, J. M. Fuentes-Alventosa and C. A. Cozzolino, *J. Appl. Polym. Sci.*, **131**, 1 (2014).
- <sup>7</sup> K. Kraśniewska, M. Gniewosz, A. Synowiec, J. L. Przybył and K. Bączek, *Postharvest Biol. Technol.*, **90**, 63 (2014).
- <sup>8</sup> M. Gniewosz, A. Synowiec, K. Kraśniewska, J. L. Przybył and K. Bączek, *Food Control*, **37**, 351 (2014).
- <sup>9</sup> A. Synowiec, M. Gniewosz, K. Kraśniewska, A. Chlebowska-Śmigiel and J. L. Przybył, *Molecules*, **19**, 12925 (2014).
- <sup>10</sup> M. Gniewosz, K. Kraśniewska, M. Woreta and O. Kosakowska, *J. Food Sci.*, **78**, 8 (2013).
- <sup>11</sup> K. Kraśniewska, M. Gniewosz, O. Kosakowska and A. Cis, *J. Food Prot.*, **79**, 493 (2016).
- <sup>12</sup> L. Li, J. Sun, H. Gao, Y. Shen and C. Li, *Int. J. Polym. Sci.*, **2017**, 8 (2017).
- <sup>13</sup> J. Y. Kim, Y. G. Choi, S. R. Byul Kim and S. T. Lim, *Food Hydrocoll.*, **41**, 140 (2014).
- <sup>14</sup> L. Introzzi, J. M. Fuentes-Alventosa, C. A. Cozzolino, S. Trabattoni and S. Tavazzi, *ACS Appl. Mater. Interfaces*, **4**, 3692 (2012).
- <sup>15</sup> L. Introzzi, T. O. J. Blomfeldt, S. Trabattoni, S. Tavazzi and N. Santo, *Langmuir*, **28**, 11206 (2012).
- <sup>16</sup> I. Leceta, P. Guerrero and K. De La Caba, *Carbohydr. Polym.*, **93**, 339 (2013).
- <sup>17</sup> M. Aider, *LWT-Food Sci. Technol.*, **43**, 837 (2010).
- <sup>18</sup> G. Yuan, X. Chen and D. Li, *Food Res. Int.*, **89**, 117 (2016).
- <sup>19</sup> F. N. Hafidani and N. Sadeghinia, *World Acad. Sci. Eng. Technol.*, **5**, 225 (2011).
- <sup>20</sup> M. K. Pati, D. Dash and M. K. Pati, *J. Sci. Eng. Res.*, **4**, 1 (2013).
- <sup>21</sup> T. A. Ahmed and B. M. Aljaeid, *Drug Des. Devel. Ther.*, **10**, 483 (2016).
- <sup>22</sup> Z. H. Murgic, A. Rescek, A. P. Sirocic, L. K. Krehula and Z. Katancic, in “Nanoparticles in Active Polymer

Food Packaging”, eBook, Smithers Rapra, UK, 2015, pp. 1-272.

<sup>23</sup> L. R. K. Aaron, L. Brody and E. P. Strupinsky, in “Active Packaging for Food Applications”, eBook, Boca Raton, Florida, 2001, pp. 131-168.

<sup>24</sup> S. Yildirim, B. Röcker, M. K. Pettersen, J. Nilsen-Nygaard and Z. Ayhan, *Compr. Rev. Food Sci. Food Saf.*, **17**, 165 (2018).

<sup>25</sup> E. Stoleru, S. B. Munteanu, R. P. Dumitriu, A. Coroaba and M. Droboță, *Iran. Polym. J. (English Ed.)*, **25**, 295 (2016).

<sup>26</sup> B. S. Munteanu, E. Pâslaru, L. Fras Zemljič, A. Sdrobis and G. M. Pricope, *Cellulose Chem. Technol.*, **48**, 565 (2014).

<sup>27</sup> T. Tkavc, I. Petrinic, T. Luxbacher, A. Vesel and T. Ristić, *Int. J. Adhes. Adhes.*, **48**, 168 (2014).

<sup>28</sup> U. Vrabčič Brodnjak and D. Muck, *Bulg. Chem. Commun.*, **49**, 85 (2017).

<sup>29</sup> T. de Moraes Crizel, A. de Oliveira Rios, V. D. Alves, N. Bandarra, M. Moldão-Martins *et al.*, *Food Hydrocoll.*, **74**, 139 (2018).

<sup>30</sup> P. K. Dutta, S. Tripathi, G. K. Mehrotra and J. Dutta, *Food Chem.*, **114**, 1173 (2009).

<sup>31</sup> V. Coma, A. Martial-Gros, S. Garreau, A. Copinet, F. Salin *et al.*, *J. Food Sci.*, **67**, 1162 (2002).

<sup>32</sup> B. Soni, B. Mahmoud, S. Chang, E. M. El-Giar and E. B. Hassan, *Food Pack. Shelf Life*, **17**, 73 (2018).

<sup>33</sup> E. Genskowsky, L. A. Puente, J. A. Pérez-Álvarez, J. Fernandez-Lopez and L. A. Muñoz, *LWT-Food Sci. Technol.*, **64**, 1057 (2015).

<sup>34</sup> J. Wu, F. Zhong, Y. Li, C. F. Shoemaker and W. Xia, *Food Hydrocoll.*, **30**, 82 (2013).

<sup>35</sup> International Standard - ISO 22196, (2007).

<sup>36</sup> P. Chevallier, S. Turgeon, C. Sarra-Bournet, R. Turcotte and G. Laroche, *ACS Appl. Mater. Interfaces*, **3**, 750 (2011).

<sup>37</sup> T. Ristić, S. Hribernik and L. Fras Zemljič, *Cellulose*, **22**, 3811 (2015).

<sup>38</sup> M. A. Jabra-Rizk, W. A. Falkler and T. F. Meiller, *Emerg. Infect. Dis.*, **10**, 14 (2004).

<sup>39</sup> T. Ristić, A. Zabret, L. Fras Zemljič and Z. Peršin, *Cellulose*, **24**, 739 (2017).

<sup>40</sup> M. Zapciu and S. Cavalieri, *Int. J. Simul. Model.*, **16**, 207 (2017).

<sup>41</sup> M. Straka, M. Malindzakova, P. Trebuna, A. Rosova and M. Pekarcikova, *Int. J. Simul. Model.*, **16**, 422 (2017).

<sup>42</sup> V. Todić, I. Cosic, R. Maksimovic, N. Tasic and N. Radakovic, *Int. J. Simul. Model.*, **16**, 108 (2017).