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Characterization of films based on chitosan lactate and its blends with oxidized starch and gelatin

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ABSTRACT

Minimal inhibitory concentration (MIC) of chitosan lactate (CHL) was tested against bacteria and phytopathogenic fungi. Then, the structural, physicochemical and antimicrobial properties of films based on CHL, oxidized potato starch (OPS), and gelatin (GEL) were investigated. With the exception of *Rhizopus nigricans*, CHL was effective against the target organisms. Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) were more sensitive to CHL than Gram-negative bacteria (*Pectobacterium carotovorum* and *Escherichia coli*). Cryo-SEM images showed total miscibility between the polymers in the blends and the ATR-FTIR spectra revealed that there was an interaction among the polymeric components. Pure CHL films displayed the highest moisture content (25.51%), water vapor permeability ($48.78\ g\ mm\ m^{-2}\ d^{-1}\ kPa^{-1}$), and the lowest tensile and puncture strength (2.00 and 1.45 MPa, respectively) among the studied films. CHL50/GEL50 films had lower permeability, higher mechanical strength, and lower elongation compared to CHL50/OPS50 films. Films obtained from CHL and CHL50/GEL50 were completely water-soluble and did not show sorbitol recrystallization. The incorporation of CHL into OPS and GEL films did not affect their transparency and improved UV-blocking capacity. CHL films were the only ones that exhibited antibacterial efficiency. Antifungal activities against *Alternaria alternata* and *Monilinia fructigena* were detected for CHL and CHL50/GEL50 films.

1. Introduction

Biopolymers play an increasingly significant role in packaging engineering by substituting non-biodegradable and non-renewable petrochemical polymers. Depending on the formulation and production methodology, bio-based packaging can be biodegradable, compostable or even edible, which opens new application opportunities. Edible packaging can be used wherever the application of synthetic polymers is limited, e.g. layers separating various components in complex food products, casing and coatings that are not removed for cooking and eating, carriers for food additives, microcapsules, etc. Every biopolymer has its own material-specific properties. Consequently, optimization of material process selection is becoming increasingly important in the engineering design and product manufacturing process. Biopolymers for packaging purposes are expected to satisfy the functional requirements of desirable barrier (to water, gases, oil), mechanical (being tough, stress-resistant, elastic) and visual (translucency, colorless) properties. The choice of raw materials should also include other aspects such as cost, availability, health issues, processing (i.e. packaging fabrication route), susceptibility to microbial decomposition, bioactivity.

Chitin is known to be the second most abundant biopolymer in nature that occurs as the major organic skeletal substance of invertebrates and as a cell wall constituent of fungi and green algae. Chemical structure of chitin consists of linear repeating units of 2-acetamido-2-deoxy-D-glucopiranose attached through β-1,4 linkages. Due to rigid crystalline structure chitin is very difficult to dissolve in common solvents; however, fusion of chitin with alkalis gives acid-soluble chitosan [1–3]. Water-soluble chitosan salts, such as acetate, ascorbate, lactate and malate can be also obtained, and these are one of the forms of the polymer used most frequently in application and implementation studies [4,5]. Chitosan and its derivatives have found application as a safe excipient and functional material in many fields including pharmaceutical, food, cosmetics, biomedical, agricultural, paper, textile, and water treatment industries. The polymer is currently of interest because of its wound healing activity and drug-delivery potential [5,6]. In

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food applications semi-permeable chitosan coatings can modify the internal atmosphere, thereby delaying ripening in fruits and vegetables [7]. The polymer inhibits the growth of bacteria and fungi which qualify it for food protection [8,9]. Theoretically, chitosan should be an ideal preservative coating material based on the numerous positive effects. However, considering the cost of chitosan preparation, it seems important to combine it with other film forming biopolymers such as plant and animal proteins as well as polysaccharides [10].

Starch is a very attractive base for new packaging materials due to its abundance, low-cost, and ability to be processed on conventional plastic processing equipment. In order to increase the industrial applications, the starches are modified by the physical, chemical or enzymatic treatments. The oxidation results in partially converted or degraded starches that have unique functional properties such as low viscosity, high stability, clarity, and excellent film forming properties. Blending of chitosan with starch gives the composite films with good mechanical properties. According to Xu et al. [11], the highest mechanical strength of the films occur at the starch to chitosan ratio of 1:1, suggesting the maximum integrity of these two components.

Gelatin (GEL), commonly used as film former for shells of pharmaceutical capsules, is obtained by partial hydrolysis of collagen – one of the most prevalent and widely distributed protein in the animal kingdom. Chitosan–GEL blend films have been shown to be homogeneous due to the good miscibility between both biopolymers leading to improved material properties of the blend films as compared to those obtained from pure GEL [12,13].

Although some studies dealing with chitosan and chitosan composite films have been performed, to the best of our knowledge, there is no previous report on the films made with commercially available water-soluble chitosan lactate (CHL). The objective of this paper was to characterize the bactericidal and fungicidal activity of CHL, as well as the physicochemical and antimicrobial properties of films based on CHL and its blends with oxidized potato starch (OPS) and GEL. Furthermore, morphology and structure of the films were examined by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR).

2. Materials and methods

2.1. Materials

Commercial food-grade biopolymers were used in this study: chitosan lactate with degree of deacetylation 80–95% (Heppe Medical Chitosan GmbH, Germany), oxidized potato starch LU-1404-3 (WPPZ S.A. Luboń, Poland), pork gelatin (McCormick-Kamis Polska S.A., Poland). Sorbitol (Sor) was purchased from Sigma Chemical Co., St. Louis, MO, USA.

2.2. Minimal inhibitory concentration (MIC) and minimal bactericidal/fungicidal concentration (MBC/MCF)

Bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Pectobacterium carotovorum*) and phytopathogenic fungi (*Botrytis cinerea* Pers. ex Nocca & Balb, *Monilinia fructigena* (Aderhold et Ruhland) Honey, *Alternaria alternata* (Fr.) Keissler, and *Rhizopus nigricans*) were maintained at 4 °C on nutrient agar (BTL, Poland) or potato dextrose agar (BTL, Poland) slants, respectively.

The MIC of CHL was determined by the tube dilution method. Initial bacteria inocula corresponding to 0.5 McFarland standard were prepared in sterile saline. Aqueous solutions of CHL in concentrations 1–50 mg/ml were mixed with nutrient broth (for bacteria) or malt broth (for fungi), then 0.05 ml of inocula was added into each tube. The final concentrations of CHL ranged from 0.1 to 10 mg/ml.

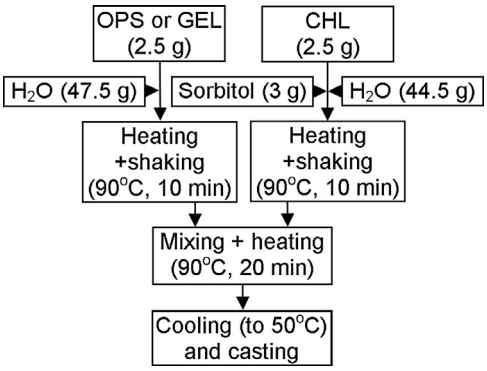

Fig. 1. Schematic diagram showing the blending procedure of chitosan lactate (CHL) with oxidized potato starch (OPS) and gelatin (GEL).

Test tubes were incubated at 37 °C (for *E. coli*, *S. aureus*, *B. cereus*) and 30 °C (for *P. carotovorum*) for 48 h. The MIC, defined as the lowest concentration of antimicrobial that inhibited the visible growth of a microorganism after incubation, was determined by turbidmetric method. Each test included a control – the medium without CHL in which a good growth of bacteria was observed. MIC for fungi was determined similarly, but spore suspensions were used as initial inocula. Tubes were incubated at 28 °C for 2–7 days and every day were visually analyzed for the growth of mycelia. Additionally, all microorganisms were transferred on agar plates in order to check the MBC/MFC, defined as the lowest concentration of antimicrobial that prevent the growth an organism after subculture on to antimicrobial-free media. Measurements were performed in triplicate.

2.3. Film preparation

Films were obtained from 5% (w/w) aqueous biopolymer solutions containing 3% (w/w) sorbitol. CHL was mixed with sorbitol and distilled water, then heated in a water bath at 90 °C for 30 min with constant stirring. The blend film-forming solutions (FFSs) contained CHL/OPS or CHL/GEL mixtures, 50:50 proportionally (Fig. 1). OPS and GEL FFSs without CHL were also prepared. After cooling to 50 °C, the pH of FFS was determined by using a pH meter. The FFSs were cast onto leveled polycarbonate trays (12 × 12 cm) and dried at ~50% relative humidity (RH) and ~25 °C for ~24 h. A constant amount of the 0.011 g of total solids was cast onto 1 cm^2 of tray area, in order to maintain film thickness. The films were peeled from the trays and cut prior to testing.

2.4. SEM

The microstructures of the film components, FFSs and film surfaces were tested using a scanning electron microscope (Zeiss Ultra Plus, Oberkochen, Germany). Cryo-SEM was used to examine the FFSs. Before viewing the samples were dusted with gold (powders and films) or platinum (FFSs). Imaging of samples was performed in high vacuum (5×10^{-4} Pa), using a secondary electron detector at 3 kV.

2.5. FTIR

The film samples were dried in a desiccator containing $CaCl_2$ for 2 weeks before analysis. IR spectra were measured using an FTIR-ATR Nicolet 6700 spectrophotometer coupled to a PC with OMNIC analysis software. The films were placed in contact with attenuated total reflectance (ATR) on the small sampling area of diamond