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Physical and antimicrobial properties of starch-carboxy methyl cellulose film containing rosemary essential oils encapsulated in chitosan nanogel

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ABSTRACT

This study was set to prepare a new active film by using a biodegradable bio-based source, i.e., corn starch. To achieve that, benzoic acid (BA) and chitosan (CS) were covalently bound and CS-BA nanogel was then obtained using self-assembly method. Subsequently, rosemary essential oil (REO) was encapsulated in CS-BA nanogel. Finally, REO in both free and encapsulated forms were incorporated in starch-carboxy methyl cellulose (CMC) films and their physical, mechanical and antimicrobial properties were studied. The films incorporating CS-BA nanogel had a higher water vapor permeability compared with the films containing REO. Moreover, film containing 0.2% CS-BA nanogel had the highest transparency and tensile strength. The REO and nanogel alone had inhibitory effects against *Staphylococcus aureus* (*S. aureus*) and by encapsulation, the inhibitory effect of REO was increased. By encapsulating REO in nanogel, both immediately (REO) and gradual (Nanogel) antimicrobial effect against *S. aureus* in the starch-CMC suspensions were obtained.

1. Introduction

The widespread pollution caused by the vast utilization of plastics in food packaging has triggered a growing interest in using edible and biodegradable films made from natural polymers [1]. Among the various natural polysaccharides used as a source of polymers, starch is considered promising for preparing biodegradable films, because it is extensively available, relatively easy to handle, biodegradable, and economic [2]. The application of native starch such as corn starch to produce edible films is limited owing to its hydrophilic nature leading to the formation of films with both poor mechanical properties and poor water resistance. To address this challenge, different materials such as glycerol, cellulose, carboxymethyl cellulose (CMC), and chitosan have been employed to improve the properties of starch films [3–5].

Staphylococcus aureus (*S. aureus*) is one of the most important human pathogens that can cause different kinds of illnesses from minor skin infections to life threatening diseases. *S. aureus* is one of the five most common causes of infections after injury or surgery [6]. Traditionally, antimicrobial agents were directly added to foods to avoid such contaminations. However, direct application of antibacterial substances in foods offers limited benefits because active substances may be neutralized, partially inactivated, sensorially deteriorated or easily diffused when coming in contact with the food mass. Therefore, the use of packaging films, in which antimicrobial or antioxidant agents are incorporated, can be more efficient, allowing effective agents to be released gradually from the package during an prolonged storage period [7].

Among the antimicrobial and antioxidant agents commonly used in active packaging, essential oils (EOs) are gaining increasing interest due to their potent antimicrobial properties and the fact that these compounds are classified as Generally Recognized as Safe (GRAS) [8]. Recently, nanoencapsulation of EOs has been revealed to improve the EOs bioactivity through the activation of passive cell absorption mechanisms [9]. Among various EOs, *Rosmarinus officinalis* (Rosemary) essential oil is a known EO with powerful antibacterial properties [10].

Various encapsulation nanomaterials have been investigated but nanogels due to their high loading capacity, high stability, and release properties are considered very promising [11]. Nanogels are three dimensional, cross-linked networks of polymer chains formed via covalent linkages or self-assembly processes [9]. Chitosan (CS) is a cationic polysaccharide that could be used for the production of nanogels [11].

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Benzoic acid (BA) is one of the oldest chemical preservatives used in cosmetics, pharmaceutical, and food industries. Sodium benzoate was the first chemical preservative approved for use in foods by the U.S. Food and Drug Administration (FDA) [12]. BA occurs naturally in several foods and commodities and has been shown to possess an antimicrobial activity against various bacteria such as *S. aureus* [13].

To the best of our knowledge, there is no information reported on the combined effect of REO and CS-BA nanogel on the properties of starch-CMC films. Therefore, the objectives of this study were (i) to prepare CS-BA nanogel through the formation of linkages between the existing amino groups of CS, and the carboxyl group of BA, (ii) to investigate the combined effects of encapsulated REO in CS-BA nanogel on physico-mechanical properties of starch-CMC films, and finally, (iii) to examine the antimicrobial activity of the fabricated films against *S. aureus* as a foodborne pathogen.

2. Materials and methods

2.1. Materials

CS (75–85% deacetylated and low molecular weight) and BA were purchased from Sigma-Aldrich (Germany). Ethylene dichlo1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was obtained from Fluka (USA). Acetic acid, Tween 20, glycerol, CMC (molecular weight of ~28,000 and degrees of substitution = 0.51) and ethanol were purchased from Merck (Germany). The EOs of *R. officinalis* (Rosmary) was purchased from Barij Essence Co. (Iran). The main components of the REO were described by the manufacturer as α-pinene (23.67%), β-pinene (1.14%), borneol (6.50%), *P*-cymene (1.47%), limonene (4.28%), cineole (11.04%) camphor (7.68%). The corn starch (12% moisture and 24% amylose) was purchased from Glucosan Co. (Iran). Mueller–Hinton Agar (MHA) and Nutrient Broth (NB) were purchased from Himedia (India). *S. aureus* (ATCC: 6538) was provided by the Pasteur Institute of Iran (Iran).

2.2. Nanogel formulation and analysis

CS-BA nanogel was prepared through the formation of amide bonds between BA and CS through an EDC-mediated reaction [14]. To verify the formation of CS-BA nanogels, Fourier transformation infrared (FTIR) spectrum at 20 °C and at the range of 500–4000 cm^{-1} was performed using an FTIR-430 (Jascow, Japan). The size and morphology of the obtained CS-BA nanogel were analyzed using a scanning electron microscopy (SEM) on a Philips: XL30 (model:KYKY–EM3200, China). The encapsulation of REO in CS-BA nanogel was carried out according to the method elaborated in our previous study [11]. In brief, the REOs was dissolved in ethanol (1:1, v/v) and the mixtures of the nanogels (10,000 mg/l) and the REOs (5000 mg/l) were prepared by sonication (70 kHz) for 5 min.

2.3. Preparation of films

For preparing different films, first starch (2.5% w/v) was mixed with distilled water and glycerol as a plasticizer (60% w/w of starch) at 25 °C for 5 min. Then mixture was then transferred to a water bath at 90 °C for over 30 min, and was agitated by a magnetic stirrer at 300 rpm. Subsequently, CMC (0.25% w/v) was added to the suspension and was mixed at 75 °C for 20 min [15]. Dispersions were then cooled to 40 °C and then, free REO previously mixed with Tween 20 (25% w/w, based on essential oil) at levels of 0.25%, 0.5% and 0.75% (w/v) as well as encapsulated REO in CS-BA nanogel at levels of 0.00125%, 0.0025% and 0.00375% (w/v) were added to the starch-CMC suspensions. The resultant formulations were mixed by a magnetic stirrer (600 rpm) for over 45 min. In the next step, various suspensions prepared were cast on framed glass plates (20 × 15 cm), and were then dried at 45 °C for about 30 h. Finally, dried films were manually peeled off the glass plates and stored inside desiccators at 25 °C and 53% relative humidity (RH) until evaluation. This RH was achieved by using a saturated magnesium nitrate solution. A starch-CMC film without REO, was also prepared in the same way and was used as control.

2.4. Microstructure of film surface

Small strips (5 mm × 5 mm) of the starch-CMC films were mounted on aluminum stubs, coated with a thin layer of gold and observed using a Scanning Electron Microscope (Philips: XL30, model:KYKY–EM3200, China), at an accelerate voltage of 25 kV.

2.5. Determination of physical properties of films

2.5.1. Film thickness

Film thickness was measured using a hand-held micrometer (No. 7326, Mitutoyo Manufacturing Co., Ltd., Tokyo, Japan) to the nearest 0.00254 mm (0.0001 in). Five thickness measurements were taken on each testing specimen and the average value was used in both tensile strength (TS) and water vapor permeability (WVP) calculations as well as when determining transparency property.

2.5.2. Solubility in water

The solubility in water (SW) of the films was measured according to the method of Almasi et al. [15]. Briefly, the films were first cut into 3 × 3 cm^2 pieces. Then, the initial dry weight of the films was determined by thermal processing at 105 °C to constant weight. Then, the solubility of the films in water was measured by immersion in 50 mL of distilled water in an incubator shaker for 24 h at 25 °C. Finally, the remaining pieces of the films after immersion were dried at 105 °C to constant weight (final dry weight). WS% was calculated using the following Eq. (1):

$$\mathrm{WS}\ (\%) = [(\mathrm{Wo}-\mathrm{Wf})/\mathrm{Wo}] \times 100 \quad (1)$$

where Wo is the initial dry weight of the film sample after reaching a constant weight, and Wf is the dry weight of the unsolved film. Three replications were used to calculate the solubility in water.

2.5.3. Water vapor permeability

The water vapor permeability (WVP) of films was determined according to the method of Shojaee-Aliabadi et al. [16]. Briefly, circular glass cups with a diameter of 5.1 cm and a depth of 5.4 cm were used. After placing 25 ml of water in a cup for providing a relative humidity (RH) of 100%, the cup was covered with the film in three replicates. Film was cut circularly with a diameter of 6.1 cm and was sealed with melted paraffin. The cups were weighed with their contents and placed in a desiccator kept at 25 °C. Cups were weighed every 12 h and the weight loss was determined. The WVP (g Pa^{-1} s^{-1} m^{-1}) was calculated by using the following Eq. (2).

$$WVP = \frac{\Delta m}{A\Delta t}\frac{x}{\Delta p} \quad (2)$$

where $\frac{\Delta m}{\Delta t}$ is the weight of the moisture gain per unit of time (g/s), X is the average film thickness (mm), A stands for the area of the exposed film surface (m^2), and Δp denotes the water vapor pressure difference between the two sides of the film (Pa). WVP was measured for three replicated samples for each type of film.

2.6. Mechanical properties of films

To determine the mechanical properties of the films, film specimens were cut into rectangular shapes that were 2 cm wide and 5 cm long. The tensile strength (TS) and elongation at break (E) of the films were determined using an Instron Universal Testing Machine (Model 5566,