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Characterization of nanobiocomposite kappa-carrageenan film with *Zataria multiflora* essential oil and nanoclay

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ABSTRACT

In this study, we aimed to improve the physical, mechanical and water-vapor permeability (WVP) properties of kappa-carrageenan (KC) films by including montmorillonite (MMT) nanoclay in the film-forming solution. To further improve these properties, the combined effect of *Zataria multiflora* Boiss essential oil (ZEO) and MMT was also investigated. The incorporation of MMT improved the physical and mechanical properties of KC film. Film made from KC alone had a tensile strength (TS) of 26.29 MPa, while the KC film with 10% nanoclay had a TS of 34.67. Further analysis was provided by X-ray diffraction and scanning electron microscopy that confirmed the dispersion of MMT in the KC matrix. It was also shown that the combined effect of nanoclay and ZEO significantly improved the TS and EB of KC films. ZEO decreased the WVP of the nanocomposite films; for example, 3% ZEO reduced WVP by around 78%. The antimicrobial activity of nanocomposite films was also studied using the overlay and vapor-phase methods; the films effectively inhibited the growth of five pathogens tested. Thus, the incorporation of both nanoclay and ZEO into KC films is a promising way to manufacture films with better mechanical, antimicrobial and WVP properties.

1. Introduction

Concerns about environmental impact and exhausting natural resources caused by non-biodegradable, petrochemical-based plastic packaging has raised interest in the use of biodegradable alternatives originating from renewable sources [1]. The interest in the study of edible and biodegradable natural-polymer films has witnessed a steady increase due to their excellent biodegradability, biocompatibility and edibility, and the range of their potential applications [2]. Recent decades have seen extensive investigation into biodegradable films prepared from various protein, polysaccharide and lipid-based biopolymer materials [3,4]. Kappa-carrageenan (KC) is one of the most interesting biopolymers; it is composed of a linear chain of sulfated galactans and extracted from certain species of red seaweed. It shows good potential for development as a source of biodegradable or edible films [5]. However, KC films do not have good mechanical and water-vapor barrier properties, as they are intrinsically hydrophilic. As a result, they might not be used for widespread applications in the food industry. Several strategies have been proposed to overcome these problems, such as blending with synthetic or natural polymers [6], blending with hydrophobic compounds [7] and cross-linking [8]. Furthermore, the water permeability and mechanical characteristics of edible films may be improved via inclusion of natural antimicrobial compounds such as plant essential oil. In particular, the addition of these antimicrobial compounds allows improvement of food safety and extension of shelf-life by reducing the growth of pathogenic and spoilage microorganisms [9]. In a previous study [7], we prepared KC films incorporated with *Satureja hortensis* essential oil. In that study, the film properties were improved significantly through emulsification, and the water-vapor barrier and water-resistance properties were further modified. However, even after such modifications, the physical and mechanical properties of these films were still inadequate for many applications. To compete with synthetic plastics, they should have comparable mechanical and/or barrier properties. Thus, further improvement is needed to increase their physical and mechanical properties.

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Currently, one of the most effective alternatives for improving the barrier and mechanical properties of packaging materials, either synthetic or natural, is the formation of nanocomposites [10,11]. Among the various inorganic nanoparticles, those based on layered-silicate clay minerals have been more widely investigated due to their commercial availability, low cost, significant enhancements and relatively simple process ability [12–14]. Montmorillonite (MMT), a hydrated alumina-silicate layered clay consisting of an edge-shared octahedral sheet of aluminum hydroxide between two silica tetrahedral layers, is the most commonly used layered silicate [15]. Due to their high aspect ratios and high surface area, if clay particles are properly dispersed in the polymer matrix, they exhibit extraordinary enhancement of mechanical, thermal, physicochemical and barrier properties at a low concentration.

Polymer nanocomposites have begun to receive much attention. However, only a few studies on the preparation of carrageenan-based nanocomposite films have been performed. Recently, Savadekar et al. [16] investigated the effect of using nanofibrillated cellulose on the properties of KC-based films. They obtained improved thermal stability and mechanical and barrier properties for KC films. Rhim et al. [17] prepared agar/kappa-carrageenan-blend nanocomposite films using different concentrations of a natural MMT (Cloisite Na^+), and reported that the hydrophilic Cloisite Na^+ was the most compatible with an agar and kappa-carrageenan polymer matrix. Although significant improvements in the properties of KC nanocomposite films have been reported in these studies, the films are still not comparable to synthetic plastic films. This indicates that more-efficient methods or a combination of methods are needed to improve the films' properties to where they can substitute for non-biodegradable plastic films for packaging.

Our recent publication has shown that *Zataria multiflora Boiss* essential oil (ZEO) is compatible with a KC polymer matrix [18]. It seems that the combination of nanoclay and ZEO in KC films can be an appropriate way to produce antimicrobial films with improved barrier and mechanical properties, due to the antimicrobial activities of ZEO and the structural integrity imparted by the nanocomposite matrix. Therefore, the objectives of this study were to improve the physical, mechanical and barrier properties of carageenan-based films by incorporating nanoclays into the film-forming solution. Furthermore, the antimicrobial activity of nanoclay composite carageenan film containing ZEO against five pathogens was also studied.

2. Materials and methods

2.1. Materials

Kappa-carrageenan was provided from Rico Philippines Industrial Corporation. ZEO was obtained from Barij Essence Pharmaceutical Co., Kashan, Iran, and stored in a dark container at 4 °C until used. Commercial MMT nanoclay (Cloisite Na^+) without organic modification was supplied by Southern Clay Products (Gonzales, TX, USA). Mueller-Hinton agar (MHA), Mueller-Hinton broth (MHB), glycerol and Tween 80 were bought from Merck Co. (Darmstadt, Germany). Magnesium nitrate and sodium chloride (used to equilibrate films at 50% RH and 75% RH respectively) were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents used were of analytical grade.

2.2. Bacterial strains

Staphylococcus aureus ATCC 25923, *Bacillus cereus* PTCC 1154, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 14028 were provided by the Iranian Research Organization for Science and Technology (Tehran, Iran). Stock cultures of the studied bacteria were grown in MHB at 30 °C for 24 h before the tests.

2.3. Preparation of films

KC-based films were prepared as described in our previous study [7]. A solution of KC was prepared by dissolving 1 g of powder in 100 ml of distilled water at 82 °C under magnetic stirring for 30 min. Once complete dissolution was achieved, glycerol (45%, w/w based on KC) as a plasticizer was added, and mixing was continued for another 30 min. The film solutions were allowed to cool to 65 °C to remove air bubbles and dissolved air [8]. They were then cast onto the center of a rimmed circular area (177 cm^2) of clean and leveled glass plates, and dried at 30 °C for 30 h. The dried films were peeled off the casting surfaces and stored in plastic bags in desiccators at 25 °C and 53% relative humidity (RH) for further testing. Nanocomposite films with various clay contents (3, 5, 10 and 15 g/100 g KC) were prepared using a casting-solution method following the procedure of Rhim et al. [19]. First, precisely weighed nanoclay (Cloisite Na^+) was dispersed into distilled water (100 mL) and stirred using a magnetic stirrer overnight to reach complete swelling of the clay. The solution was then homogenized using a rotor-stator homogenizer (IKA T25-Digital Ultra Turrax, Staufen, Germany) at 13,500 rpm for 10 min at 80 °C. This was followed by sonication using a high-intensity ultrasonic processor, which was used to aid intercalation of the clay. The KC solution (prepared as described above) was then added slowly into the pretreated clay solution, and the mixture was stirred for 1 h. Thereafter, the procedure for the film preparation was identical to that described previously. To prepare nanocomposite films containing ZEO, a selected amount of clay content (5 g/100 g KC) was prepared following the same procedure as that used for nanocomposite formulation. KC solution (prepared as described above) was added to the homogenized and ultrasonicated clay solution, and the mixture was stirred for 1 h. The nanoemulsions were obtained by adding ZEO to the solution to reach final concentrations of 1, 2 and 3% (v/v). Tween 80 was added as an emulsifier in quantities proportional to the ZEO (0.1, 0.2 and 0.3%, v/v) and homogenized with Ultra-Turrax (IKA T25-Digital Ultra Turrax, Staufen, Germany) at 13,500 rpm for 3 min. Then, the emulsions were cooled to 65 °C to remove all bubbles incorporated during homogenization. Nanocomposite films incorporated ZEO were prepared and stored as previously described (25 °C and 53% RH). For each formulation, 3 different samples were prepared and replicates of each type of film were evaluated.

2.4. X-ray diffraction (XRD)

XRD patterns of the nanocomposite KC films were taken using a Philips X'Pert MPD Diffractometer (Eindhoven, Netherlands) operated at 40 kV and 30 mA, equipped with Co Kα radiation at a wavelength of 0.1541 nm. Samples were scanned over a diffraction angle (2θ) range of 2–10°, with a scanning rate of 0.02°/s at room temperature. The interlayer d-spacing (d_{001}) was calculated using Bragg's diffraction equation, $\lambda = 2d \sin\theta$, where λ is the wavelength of the X-ray radiation used (0.1542 nm), d is the spacing between diffractional lattice planes and θ is the measured diffraction angle.

2.5. Film thickness

Film thickness was determined using a hand-held digital micrometer (Mitutoyo No. 293-766, Tokyo, Japan) having a precision of 0.001 mm. Five thickness measurements were taken on