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Characterization of konjac glucomannan-ethyl cellulose film formation via microscopy

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ABSTRACT

Konjac glucomannan-ethyl cellulose (KGM-EC, 7:3, w/w) blended film shows good mechanical and moisture resistance properties. To better understand the basis for the KGM-EC film formation, optical microscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) were used to observe the formation of the film from emulsion. Optical microscopy images showed that EC oil droplets were homogeneously dispersed in KGM water phase without obviously coalescence throughout the entire drying process. SEM images showed the surface and cross-sectional structures of samples maintained continuous and homogeneous appearance from the emulsion to dried film. AFM images indicated that KGM molecules entangled EC molecules in the emulsion. Interactions between KGM and EC improved the stability of KGM-EC emulsion, and contributed to uniformed structures of film formation. Based on these output information, a schematic model was built to elucidate KGM-EC film-forming process.

1. Introduction

Edible films are a thin layer made of food grade proteins, lipids, polysaccharides or their composite. They are frequently applied in food packaging, and can help solve the environmental impact caused by use of plastic packaging bags [1]. Many recent studies have focused on preparation, characterization, and application of edible films, e.g., starch- [2], chitosan- [3], pectin- [4], and protein-based [5] films. The mechanical properties, structural characterization, and gas permeability of these films were also tested. Edible film formation is a process related to formulation, composition compatibility, film-forming solution stability, solvent evaporation, polymer-polymer/polymer-solvent interactions, and structural changes. Film-forming processes of biopolymers are few reported and have only been investigated by two-dimensional correlation ATR-FTIR spectroscopy as for pullulan film formation [6].

Microscopic techniques have been widely used to evaluate morphological changes in the surface of polymer latex film and dried edible films. For examples, atomic force microscopy (AFM) and scanning electron microscopy (SEM) were used to analyze latex film formation [7] and bitter vetch protein film morphology [8]. SEM was applied to microstructure observation of the dried basil seed gum film [9]. Polarized light microscopy and SEM were used to observe the micro-structural characterization of yam starch films [10].

Konjac glucomannan (KGM) and ethyl cellulose (EC) are polysaccharides with good film-forming ability, and therefore are widely used in film preparation for application in food and drug fields [11,12]. Previous reports have focused on preparation and characterization of these films [12–14], and a KGM-EC blending ratio 7:3 for blend film preparation is suggested due to good mechanical properties, high moisture resistance and homogeneous film surface [14]. However, film-forming processes of biopolymers analyzed by microscopy has been little studied despite its importance for their understanding the resulting network structure of films, which essentially determine their properties [15].

Therefore as a continuation of our previous work on KGM-EC blended film [13], we observed the film-forming process of KGM-EC emulsion via microscopy. This study aimed at a deeper understanding of the basis for KGM-EC film formation. The structure changes related to the whole film formation were investigated combined with optical microscopy, TEM, AFM, and SEM.

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2. Materials and methods

2.1. Materials

Konjac glucomannan (KGM) (5.44×10^5 g/mol) was provided by Licheng Biological Technology Co., Ltd. (Wuhan, China). Ethyl cellulose (EC) (CP grade, 48.0–49.5% (w/w) ethoxy groups, and 5.18×10^4 g/mol), ethyl acetate, and dibutyl sebacate were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. KGM-EC emulsion preparation

KGM-EC emulsion (7:3, w/w) preparation for microscopic observation followed a published method [14]. Briefly, KGM water phase (0.7%, w/w) was prepared by dissolving 0.7 g KGM in 100 mL deionized water with stirring using an electric mixer (JJ-IA, Zhongda Instrument, Jintan, China) at 600 rpm at 60 °C for 2 h. EC oil phase (1.0%, w/w) was prepared by adding 0.3 g EC and 0.06 g dibutyl sebacate to 30 ml ethyl acetate with stirring at 60 °C for 15 min. The EC oil phase was slowly added to KGM water phase which was being stirred, and then the mixture solution was stirred at 60 °C for 30 min. This was termed KGM-EC emulsion (1.06%, w/w), and ethyl acetate oil droplets homogenously dispersed in KGM water phase were termed EC oil droplets. KGM water phase and EC oil phase were all diluted to 10 μg/mL for measuring the value of oil/water interfacial tension with a drop tensiometer (Tracker, Teclis Instruments, France). And the value of interfacial tension of EC oil/KGM water was measured to be 7.0 mN/m.

2.3. Urea addition into KGM-EC stabilized emulsion

In order to confirm existence of hydrogen bond interactions between KGM and EC molecules, effect of urea addition on KGM-EC emulsion was investigated. KGM-EC-urea mixtures containing different urea concentrations (0, 0.1, 1.0, and 4.0 mol/L) were made by directly adding urea powder into KGM-EC emulsions with continuous stirring at 60 °C for 10 min. Then the mixture was dropped onto a glass slide and observed by optical microscopy (BT1600, Dandong Bettersize Instruments, Dandong, China) at room temperature. Samples were tested at least in triplicate and eight images were obtained with each replication.

2.4. Sample preparation for the film-forming process analysis

Previously we suggested a KGM-EC (7:3, w/w) blended film preparation, and the blended film exhibited high tensile strength and elongation at break [14]. Thus in this study, the film-forming process analysis was based on this ratio. 45 g of KGM-EC emulsion (7:3, w/w) was poured into a plastic Petri dish (diameter 9.0 cm), and placed in a drying oven at 60 °C. Samples were taken out at 0, 1, 3, 5, 7, and 9 h (completely dried film), and were immediately frozen using liquid nitrogen and freeze-dried, coded as KE_0, KE_1, KE_3, KE_5, KE_7, and KE_9 for later SEM imaging. In this way, we can 'arrest' the structure state of composites in solution at a predetermined time in the film-forming process. KGM, EC, and KGM-EC blend films were prepared by drying KGM water phase (1%, w/w), EC oil phase (1%, w/w), and KGM-EC emulsion (1.06%, w/w) for 9 h at 60 °C for SEM and AFM imaging test, respectively. All above samples were cut into 1 cm × 1 cm pieces using a medical blade, and the cross-sections of samples were obtained by breaking samples after freezing in liquid nitrogen.

2.5. Microscopy

2.5.1. Optical microscopy

KGM-EC emulsion was dropped onto a glass slide, and then its entire film-forming process (0–120 min) was *in situ* photographed by optical microscopy (BT1600, Dandong Bettersize Instruments, Dandong, China) at room temperature. At least three replications were made on KGM-EC emulsion preparation, and eight images were obtained for imaging test of each replication.

2.5.2. Scanning electron microscopy (SEM)

Samples prepared in 2.4 were mounted on a metal stub and coated with gold (Bio-Rad type SC 502, JEOL Ltd., Japan), and then observed by SEM (JSM-6390LV, JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 30 kV. Samples were tested at least in triplicate and eight images were obtained with each replication.

2.5.3. Transmission electron microscopy (TEM)

KGM water phase and EC oil phase prepared in Section 2.2 were immediately diluted to 2–5 μg/mL at 60 °C, then they were pipetted onto TEM copper grids covered with a porous carbon film which were preheated to 60 °C in a dry box. The samples were then observed using a TEM (JEM-2100, JEOL Ltd., Japan) with a Gatan 830CCD camera at an accelerating voltage of 200 kV. Gatan DigitalMicrograph (version 3.7) software was used to analyze the images. Samples were tested at least in triplicate and eight images were obtained with each replication.

2.5.4. Atomic force microscopy (AFM)

KGM water phase, EC oil phase, and KGM-EC emulsion prepared in Section 2.2 were immediately diluted to 2–5 μg/mL at 60 °C, then they were pipetted onto mica which was preheated to 60 °C in a dry box. Samples were observed by tapping mode AFM (Nanoscope IIIa Multimode, Veeco Co., Santa Barbara, CA) equipped with an E-scanner. Tapping mode cantilevers with nominal spring constant of 5–100 N/m and nominal resonance frequencies of 10–320 kHz were employed. The KGM, EC, and KGM-EC blend films were attached to mica surfaces. They were examined by tapping mode AFM (Nanoscope IIIa Multimode, Veeco Co., Santa Barbara, CA) in air at 25 °C with 65% relative humidity. The AFM was equipped with an E-type scanner and a rectangular silicon cantilever with a resonance frequency of 290–320 kHz. NanoScope software (NanoScope Analysis v140r1sr2) was used for all AFM images processing, and the roughness parameters such as *R*a and *R*q of films were determined. Samples were tested at least in triplicate and eight images were obtained with each replication.

3. Results and discussion

3.1. Microscopic observation of KGM-EC film forming process

KGM-EC emulsion was found to be stable as no phase separation was visually observed at 25 °C for 21 days aging (Fig. 1a and b). Optical microscopy images showed that EC oil droplets were homogeneously dispersed in KGM water solution and no phase separation was observed during the entire drying process (Fig. 1c, 0–120 min). Dried film was formed on the glass slide at the time of 120 min. The appearance of little EC coalescence in all images (Fig. 1c) may be caused by EC deposition in the dry process of KGM-EC emulsion, and may also be related to linking of the two polysaccharides components between neighboring droplets, but it was quite low. The high KGM-EC emulsion stability may be caused by hydrogen bond interactions between KGM and EC. In order to confirm this hypothesis, urea, which can effectively break inter-molecular hydrogen bonds [16], was added to the emulsion. Compared to KGM-EC emulsion, no visual difference