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Development and characterization of carrageenan/grapefruit seed extract composite films for active packaging

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ABSTRACT

Carrageenan-based antimicrobial films were developed by incorporation of grape fruit seed extract (GSE) at different concentration into the polymer using a solvent casing method and their physical, mechanical, and antimicrobial properties were examined. The carrageenan/GSE composite films appeared yellowish tint due to the polyphenolic compounds in the GSE. SEM analysis showed rough surface with sponge like structures on the cross section of the films. FT-IR results indicated at GSE had good compatibility with carrageenan. The amorphous structure of polymer films was not changed by the incorporation of GSE. But, the addition of GSE increased moisture content, water vapor permeability, and surface hydrophilicity of the films. The tensile strength and elastic modulus decreased with increasing content of GSE, however, the elongation at break increased significantly up to 6.6 μg/mL of GSE then decreased thereafter. Thermal stability of the films was not influenced by GSE incorporation. The carrageenan/GSE composite films exhibited great antibacterial activity against food borne pathogens. These results suggest that the carrageenan-based composite films have a high potential for being used as an antimicrobial or active food packaging applications.

1. Introduction

Food spoilage or contamination is one of the most life threatening problems worldwide. One of the commonly used methods to control the growth of bacterial pathogens is to spray antimicrobial agents on the food surface [1,2]. However, diffusion or interaction of antimicrobial agents with large food stuffs has limited the wide usage of this method. Moreover, consumers are increasingly demanding for the minimally processed ready to eat and chemical preservative free fresh foods [3–5]. In this scenario, packaging technology was introduced to protect the food stuffs from the microbial contamination and environmental conditions like light, moisture, dust and oxygen [6]. Variety of packaging systems has been used to provide secure food using petroleum-based synthetic plastic packaging materials [7]. But, increasing disposal and glut of synthetic polymers and plastics based packaging waste materials have been created serious environmental problems due to their poor degradability and sustainability [8].

Therefore, there is a growing interest in the development of natural biopolymer based packaging system to address these problems. The biopolymer based packaging films offers several advantages due to their good biodegradability, biocompatibility, environmentally-friendliness, and even edibility [9]. The biopolymer films have been showed excellent physical and mechanical properties and barriers to oxygen, aroma, carbon dioxide, lipids and flavors. In addition, they can be used as suitable carriers system for various active ingredients such as antibacterial, antifungal, antioxidant and coloring agents [10–12]. The biopolymers such as lipids (beeswax), proteins (gelatin, casein, gluten, soya and whey protein), and polysaccharides (starch, alginate, agar, carrageenan, pectin, cellulose and its derivatives, and chitosan) are widely being employed for the production of biodegradable and environment friendly packaging films in food industry [13,14]. Owing to the unique colloidal nature, good film forming property, moderate oxygen and moisture permeability, polysaccharide-based films are particularly interests in packaging industry.

Carrageenan is a water-soluble natural sulfated polysaccharide extracted from the species of red sea weed (Rhodophycae) and it has been extensively studied in food and pharmaceutical industry as gelling, stabilizing and emulsifying agents [15]. It is composed of alternating copolymer of α-(1-3)-D-galactose and β-(1-4)-3,6-anhydro-D or L-galactose and classified into three sub groups (κ, λ, and ι) based on the number and distribution of sulfated ester pattern on 3,6-anhydro-D or L-galactose residues. The κ-carrageenan

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had been shown better film forming property with high water barrier and mechanical properties [16,17]. During the cooling of film solution, random coil structure of carrageenan forms double helical structure, which results in formation of compact films [18,19]. The carrageenan has been blended with various other polymers to improve functional properties of the packaging films that include starch [20], sodium alginate [21], and locust bean gum [22]. It has also been used as an effective carrier or delivery system for essential oils [18], drugs [19], and nanoclay [22].

The incorporation of antimicrobial or antioxidant agents into the polymeric films can help to maximize the shelf-life of perishable food by providing the protection against microbial growth, enzymatic browning, vitamin losses, and oxidation [23]. Despite the availability of many antimicrobial agents, natural antimicrobial agents obtained from plant extracts are particularly attractive in food packaging industry due to their promising antibacterial, antifungal and antioxidant properties. Grapefruit seed extract (GSE) is a natural extract derived from the seeds, pulps and peel of grapefruit (*Citrus paradise Macf. Rutaceae*). It is known to possess broad spectrum of antibacterial, antifungal, antiviral, antiparasitic, anticancer, antioxidant and antifeedant properties [24,25]. GSE contains various polyphenolic compounds such as flavonoids, citric acid, ascorbic acid, tocopherol, limonoid and some other trace compounds [26]. The addition of GSE into whey protein isolate extended the shelf life of fish products [27].

The main objective of this study was to prepare effective antimicrobial composite films for active packaging by incorporation of GSE into biopolymer carrageenan. The color, optical, water vapor barrier, hydrophobicity, and mechanical properties of the resultant composite were investigated. In addition, SEM, FT-IR, XRD, and TGA analysis were performed for characterization. The resultant composite films were tested against food-borne pathogens to explore their antimicrobial activity.

2. Materials and methods

2.1. Materials and bacterial strains

The polymer carrageenan was purchased from Hankook Carragen (Whasoon, Jeonnam, Korea). Grapefruit seed extract (GSE, DF-100) was obtained from Komipharm International Co., Ltd. (Seoul, Korea). Bacteriological media such as brain heart infusion broth (BHI), tryptic soy broth (TSB), and agar powder were procured from Duksan Pure Chemicals Co., Ltd (Gyeonggi-do, South Korea). Glycerol was procured from Daejung Chemicals & Metals co., Ltd (Siheung, Gyonggido, South Korea). Food-borne pathogenic strains such as *Escherichia coli* O157:H7 ATCC 43895, *Listeria monocytogenes* ATCC 15313, *Staphylococcus aureus* ATCC 29213 and *Bacillus cereus* ATCC 21366 were purchased from Korean Collection for Type Cultures (KCTC) in South Korea. These four bacterial strains were cultured in TSA and BHI agar media and subsequently stored at 4 °C for further analysis. Ultra-filtered high pure deionized water was used for the preparation of all chemicals and composite films.

2.2. Film preparation

The solution casting method was used for the preparation of carrageenan based composite films. To prepare stock solution of GSE (v/v), 10 mL of extract was dissolved in 10 mL of distilled water under stirring at room temperature for 10 min. Different concentration of seed extracts (0.1, 0.5, 1, 1.5, and 2 mL) were mixed with 150 mL of water and stirred at 90 °C for 30 min using a magnetic stirrer to obtain the final concentration of 0.6, 3.3, 6.6, 10, and 13.3 μg/mL of GSE, respectively. Air bubbles were removed by standing at room temperature for 30 min. To these solutions, 3 g of carrageenan and 0.9 g of glycerol as plasticizer were added separately and continued to stir at 90 °C for 30 min. Completely solubilized and well dispersed film forming solutions were cast evenly onto a leveled Teflon film (Cole-Parmer Instrument Co., Chicago, IL, USA) coated glass plate (24 cm × 30 cm), and allowed to dry at room temperature (22–25 °C) for 2 days. All dried composite film samples were peeled off from the glass plate and preconditioned at 25 °C and 50% RH for 48 h in a constant temperature humidity chamber (Temp & Humidity Chamber, HB-105 MP, Hanbaek Scientific Co., Seoul, Korea) to normalize the moisture content prior to further characterization.

2.3. Color properties

The color of the film surface was measured using a Chroma meter (Minolta, CR-200, Tokyo, Japan). Prior to measuring the film surface color, the Chroma meter was calibrated under a standard white color plate (L^* = 97.75, a^* = −0.49, and b^* = 1.96). The color parameters such as L^*, a^*, and b^* (L^* = lightness, a^* = red/green, and b^* = yellow/blue) values were determined by average of five readings from each samples. The total color difference (ΔE) of the films was calculated as follows:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5} \quad (1)$$

where ΔL^*, Δa^*, and Δb^* are the variations between the color parameters of the film samples and white color standard plate used as a film background

2.4. Optical properties

Optical properties of the carrageenan and carrageenan/GSE composite films was determined by UV–vis absorption and percent transmittance spectra of the films. A rectangular piece of each film sample (4 cm × 4 cm) was directly mounted between the two spectrophotometer magnetic cell holders. The absorbance spectra of the films were measured at selected wavelength ranges from 300 to 800 nm using a UV-vis spectrophotometer (Model 8451A, Hewlett-Packard Co., Santa Alara, CA, USA). Transparency of the pure carrageenan and its composite films was determined by measuring light transmittance at UV (280 nm) and visible (660 nm) regions using a UV–vis spectrophotometer. Three measurements were made for each sample and the average values were reported.

2.5. Scanning electron microscopy (SEM)

Microstructure on the cross section of the carrageenan and it composite films (0.6 and 13.3 μg/mL) were characterized by SEM analysis. The film samples were cut into small pieces and then mounted on a cylindrical aluminum specimen holder and examined using a Field Emission Scanning Electron Microscopy (FE-SEM, S-4800, Hitachi Co., Ltd., Matsuda, Japan) with an accelerating voltage of 5.0 kV.

2.6. Measurement of FT-IR and XRD

Fourier transform infrared (FT-IR) spectra of the carrageenan and carrageenan/GSE composite film samples were analyzed using FT-IR spectroscopy (TENSOR 37 spectrophotometer with OPUS 6.0 software, Billerica, MA, USA) operated at a resolution of 4 cm^{-1}. To this analysis, the film samples were placed on the ray exposing stage and the spectra were recorded at wave number of 500–4000 cm^{-1}.

X-ray diffraction (XRD) pattern of the carrageenan and carrageenan/GSE composite films were measured using X-ray diffractometer (PANanalytical X-pert pro MRD diffractometer,