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Antimicrobial and physical-mechanical properties of agar-based films incorporated with grapefruit seed extract

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ABSTRACT

The use of synthetic petroleum based packaging films caused serious environmental problems due to their difficulty in recycling and poor biodegradability. Therefore, present study was aimed to develop natural biopolymer-based antimicrobial packaging films as an alternative for the synthetic packaging films. As a natural antimicrobial agent, grapefruit seed extract (GSE) has been incorporated into agar to prepare antimicrobial packaging film. The films with different concentrations of GSE were prepared by a solvent casting method and the resulting composite films were examined physically and mechanically. In addition, the films were characterized by FE-SEM, XRD, FT-IR and TGA. The incorporation of GSE caused increase in color, UV barrier, moisture content, water solubility and water vapor permeability, while decrease in surface hydrophobicity, tensile strength and elastic modulus of the films. As the concentration of GSE increased from 0.6 to 13.3 μg/mL, the physical and mechanical properties of the films were affected significantly. The addition of GSE changed film microstructure of the film, but did not influence the crystallinity of agar and thermal stability of the agar-based films. The agar/GSE films exhibited distinctive antimicrobial activity against three test food pathogens, such as Listeria monocytogenes, *Bacillus cereus* and *Escherichia coli*. These results suggest that agar/GSE films have potential to be used in an active food packaging systems for maintaining food safety and extending the shelf-life of the packaged food.

1. Introduction

Concerns on environmental waste problems and depletion of natural resources caused by non-biodegradable petroleum-based plastic packaging materials as well as consumer's demand for safe and high quality foods triggered an increased interest in the development of innovative food packaging materials using biopolymers (Duncan, 2011; Rhim & Ng, 2007; Sorrentino, Gorrasi, & Vittoria, 2007; Tang, Kumar, Alavi, & Sandeep, 2012). Biopolymers from various natural resources have been considered as attractive alternatives for non-biodegradable petroleum-based plastic packaging materials, since they are abundant, renewable, inexpensive, environmentally friendly, as well as biodegradable and biocompatible (Sorrentino et al., 2007; Tang et al., 2012). Biopolymer-based packaging materials have some beneficial properties, when incorporated with active compounds, such as improving food quality, securing food safety, and extending shelf-life of food (Rhim, Wang, & Hong, 2013; Yu et al., 2013). Since food quality and safety are major concerns in the food industry, biopolymer-based antimicrobial packaging has been considered as an emerging technology that have a significant impact on maintaining food quality and extending shelf-life of packaged foods.

Packaging materials with antimicrobial function have long been recognized as one of the most promising active packaging systems for extending shelf-life of food, maintaining food safety, quality and improving storage stability by destroying or inhibiting spoilage and pathogenic microorganisms that contaminate foods (Han, 2000; Falguera, Quintero, Jiménez, Muñoz, & Ibarz, 2011). A number of naturally derived polymers such as polysaccharides, proteins and lipids have been widely used to develop biodegradable packaging films. Among them, polysaccharide based packaging films are particularly attractive due to their better film forming property, moderate oxygen and moisture permeability, and unique colloidal nature. Polysaccharides such as cellulose, cellulose derivatives, carrageenan, agar, chitosan, pectin, starch and alginate have been frequently used for making biodegradable antimicrobial packaging films (García, Pinotti, Martino, & Zaritzky, 2004; Rhim, Hong, Park, & Ng, 2006; Rhim, 2011; Rhim & Ng, 2007). Agar is one of the most promising polysaccharide for developing biodegradable antimicrobial packaging films (Gimenez, Lopez de Lacey, Perez-Santín, Lopez-Caballero, & Montero, 2013; Wu, Geng, Chang, Yu, & Ma, 2009). Agar is a gelatinous polysaccharide which is extracted from marine red algae, such as *Gelidium* and *Gracilaria* spp. It has been widely used for the preparation of packaging films due to its

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high mechanical strength and moderate water resistant properties (Gimenez et al., 2013) and also been used for blend with some other biopolymeric materials such as starch (Phan, Debeaufort, Luu, & Voilley, 2005), protein (Letendre, D'Aprano, Lacroix, Salmieri, & Sr-Gelais, 2002), carrageenan (Rhim, 2013), and gelatin (Gimenez et al., 2013), to improve physical and mechanical properties of the packaging films. In addition, agar has been used to carry antimicrobials such as silver nanoparticles, nanoclays and green tea extract (Rhim, 2011; Rhim et al., 2013; Gimenez et al., 2013).

Usually, antimicrobial packaging films are produced by incorporating antimicrobial compounds into the polymeric materials or mixing them during polymer processing step. The substances such as organic acids, bacteriocins, spice extracts or essential oils, fatty acids, plant seed extract, enzymes, nano-sized metal and metallic oxides have been used as effective antimicrobials to produce active packaging films (Balasubramnian, Rogenberg, Yam, & Chikindas, 2009; Han, 2000). Among them, natural antimicrobials such as essential oils, spice extracts, and fruit seed extracts are widely used particularly in the food packaging sector due to their potent antimicrobial activity and compatibility with biopolymer matrices (Han, 2000). As one of such natural substances grapefruit seed extract (GSE) is interesting since GSE is known to have powerful antimicrobial activity (Cvetnić & Vladmir-Knežević, 2004). The GSE is usually extracted from the seed and pulp of grapefruit (*Citrus paradisi* Macf., Rutaceace), and it contains large quantities of polyphenolic compounds, flavonoids (mainly naringin), citric acid, ascorbic acid, tocopherol, limonoid and some other trace compounds (Cho, Seo, Choi, & Joo, 1990). The beneficial actions of GSE have partly been attributed to the antioxidant activity of citrus flavonoids. However, GSE has become a subject of controversy since some commercially available products are not completely natural. Artificial preservatives, such as benzethonium chloride, triclosan, and methylparabens, were identified in some commercially available products (Ganzera, Aberham, & Stuppner, 2006; Takeoka, Dao, Wong, Lundin, & Mahoney, 2001). It has been claimed that antimicrobial activity of GSE has been attributed to the synthetic preservative agents added in the GSE (von Woedtke, Schlüter, Pflegel, Lindequist, & Jülich, 1999). On the other hand, Cvetnić and Vladmir-Knežević (2004) demonstrated that pure ethanolic extract of grapefruit seed and pulp exhibited strong antimicrobial activity against *Salmonella enteritidis* and other pathogenic or non-pathogenic microorganisms. GSE has been applied for the preservation and extension of shelf-life of food such as fish products (Cho et al., 1990), fruits (Cho et al., 1991), and minimally processed vegetables (Xu et al., 2007). However, only a few works on the application of GSE for the preparation of antimicrobial food packaging films are available in the literature (Lim, Jang, & Song, 2010; Song, Shin, & Song, 2012).

Therefore, this research aimed to develop a biodegradable antimicrobial packaging film by incorporating GSE into agar films. The effect of GSE incorporation was assessed on the antimicrobial activity and physical-mechanical properties of agar films including X-ray diffraction, Fourier transform infrared spectroscopy, mechanical resistance, water vapor permeability, affinity to water (solubility, moisture content, contact angle), color and optical properties, microstructural analysis, and thermal stability.

2. Materials and methods

2.1. Materials

Agar was purchased from Fine Agar-Agar Co., Ltd. (Damyang, Jeonnam, Korea). Grapefruit seed extract (GSE, DF-100) was purchased from Komipharm International Co., Ltd. (Seoul, Korea). Microbiological media such as brain heart infusion broth (BHI), tryptic soy broth (TSB), and agar powder were obtained from Duksan Pure Chemicals Co., Ltd (Gyeonggi-do, South Korea). Glycerol was procured from Daejung Chemicals & Metals Co., Ltd (Siheung, Gyonggido, South Korea). All solutions were prepared using ultra-filtered high purity deionized water.

2.2. Preparation of agar and agar/GSE films

Antimicrobial agar films were prepared using a solution casting method. First, GSE solution was prepared by dissolving 10 g of GSE in 10 mL of distilled water under stirring at room temperature for 10 min. Different concentration of GSE solutions (0.6, 3.3, 6.6, 10 and 13.3 μg/mL) were mixed with 150 mL of water, then 3 g of agar and 0.9 g of glycerol were added into the solution and dissolved the agar by vigorous mixing using a magnetic stirrer at 90 °C for 30 min. The film 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. Dried films were peeled off from the glass plate and preconditioned at 25 °C and 50% RH for 48 h to normalize the moisture content prior to further analysis. The control agar film was prepared with the same method without GSE.

2.3. Microorganisms and antimicrobial activity assay

The antimicrobial activity of the films was tested using a disk diffusion method using *Listeria monocytogenes* (ATCC 15313), *Bacillus cereus* (ATCC 21366) and *Escherichia coli* O157:H7 (ATCC 43895) as target microorganisms. All the strains were purchased from Korean Collection for Type Culture (KCTC, Seoul, Korea) and aseptically inoculated into brain heart infusion (BHI) and tryptic soy broth (TSB) broths and incubated at 37 °C. After 16 h of incubation, 100 μL of cultured broth was serially diluted into two folds using sterile water (900 μL). 100 μL of the diluted culture broth were spread on TSA and BHI agar media. Then, various film discs (4.0 mm diameter) were placed on the surface of the agar media and subsequently incubated at 37 °C for 24 h. Bacterial growth inhibition zones around the discs were measured.

2.4. XRD and FT-IR analysis

X-ray diffraction (XRD) pattern of the agar and agar/GSE films was analyzed using a X-ray diffractometer (PANanalytical X-pert pro MRD diffractometer, Amsterdam, Netherlands). Film samples were cut into rectangular shape and mounted on a glass slide and the XRD spectra were recorded using Cu-Kα radiation and a nickel monochromator filtering wave at a voltage and current of 40 kV and 30 mA, respectively. The diffraction patterns were obtained at diffraction angles between 10° and 90°.

Fourier transform infrared (FT-IR) spectra of the agar and agar/GSE films 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}. Film sample was placed on the ray exposing stage and the spectrum was recorded between the wave number ranges of 500–4000 cm^{-1}.

2.5. Measurement of thickness

Thickness of the agar and agar/GSE films was measured using a hand-held micrometer (Dial Thickness gauge 7301, Mitutoyo Corporation, Kanagawa, Japan) with an accuracy of 0.01 mm. The measurements were made at least six random locations on each film sample and the average values were calculated.

2.6. Mechanical properties

Tensile properties of the agar and agar/GSE films were measured according to the standard test method ASTMD-882-88 (ASTM,