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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,

2012a). Tensile strength (TS), percent elongation at break (EAB), and elastic modulus (EM) of the films were determined using Instron Universal Testing Machine (Model 5565, Instron Engineering Corporation, Canton, Mass, USA). The rectangular sample (2.54 cm × 15 cm) of each film was stretched with an initial grip separation and crosshead speed of 50 mm and 50 mm/min, respectively. Ten replicates were tested for each film and the average values were reported.

2.7. Water vapor permeability

Water vapor permeability (WVP) of the agar and agar/GSE films was determined gravimetrically using standard ASTM E96-95 method with slight modification (ASTM, 2012b). First, water vapor transmission rate (WVTR) was determined as follows: Film samples (7.5 cm × 7.5 cm) were mounted horizontally on the top of polymethacrylate cups (2.5 cm in depth and 6.8 cm of diameter) containing 18 mL of water. The cup with film sample was covered by the cup lid and crewed for sealing. Weight of the entire cup was measured and placed in an environmental chamber set at 25 °C with a constant RH of 50%. The weight loss of the cup was measured at every 1 h interval for 8 h. The WVTR was determined from the slope of the weight change of the cup *vs.* time curve. Then the WVP of the film was calculated using the following equation:

$$\mathrm{WVP} = \frac{\mathrm{WVTR} \times \mathrm{L}}{\Delta p} \quad (1)$$

where WVTR was the measured water vapor transmission rate ($g/(m^2\ s)$), L was the mean film thickness (m), and Δp was the partial water vapor pressure difference (Pa) across both sides of the film.

2.8. Affinity to water

2.8.1. Moisture content and water solubility

Moisture content (MC) of the agar and agar/GSE films was determined according to the method of Soradech, Nunthanid, Limmatvapirat, and Luangtana-anan (2012). Each film sample was cut into square of 3 cm × 3 cm. The initial weight (W_0) of sample was determined and dried at 105 °C for 24 h using a hot air oven. The final weight (W_l) of the film samples was measured and percent MC of the films were calculated as follows:

$$\mathrm{MC}(\%) = \frac{W_0 - W_l}{W_0} \times 100 \quad (2)$$

Water solubility (WS) of the agar and agar/GSE films was determined by measuring the weight of film samples before and after immersion into water. For this, a piece of film sample (3 cm × 3 cm) was cut and dried at 100 °C for 24 h. Initial weight of the film (W_0) was measured and directly immersed into the water (30 mL) in 50 mL of beaker with gentle stirring. After 8 h of immersion, the remaining piece of sample was taken from the beaker and remaining water on surface of the films were removed by filter paper. Then, the film was dried in hot air oven for 24 h to determine the final weight (W_f). The WS of the sample was calculated as follows:

$$\mathrm{WS}(\%) = \frac{W_0 - W_f}{W_0} \times 100 \quad (3)$$

2.8.2. Water contact angle

Contact angle of water on the film surface was measured to estimate surface hydrophobicity of the agar and agar/GSE films using a water contact angle (WCA) analyzer (Model Phoenix 150, Surface Electro Optics Co., Ltd., Kunpo, Korea). Film sample was cut into rectangular piece (3 cm × 10 cm) and placed on the horizontal movable stage (black Teflon coated steel, 7 cm × 11 cm) fitted with the WCA analyzer. Then, 10 μL of water was dropped on the surface of film sample using a micro-syringe (Rhim et al., 2006). The CA on both sides of the water drop was measured to assume symmetry and horizontal level.

2.9. Color and optical properties

The surface color of the agar and agar/GSE films was evaluated using a Chroma meter (Minolta, CR-200, Tokyo, Japan). A white color plate ($L = 97.75$, $a = -0.49$ and $b = 1.96$) was used as a standard background color. 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 film sample. The total color difference of the film (ΔE) was calculated as follows:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{0.5} \quad (4)$$

where ΔL, Δa, and Δb are the difference between the color plate and samples.

Optical properties of the agar and agar/GSE composite films were measured by UV–vis absorption and transmission spectra of the films. For this, a rectangular piece of film (4 cm × 4 cm) was cut from each film sample and absorbance was measured at wavelength ranged from 300 to 800 nm using a UV–vis spectrophotometer (Model 8451A, Hewlett-Packard Co., Santa Alara, CA, USA). Transparency of the films was determined by measuring the percent of transmittance at 280 and 660 nm using a UV–vis spectrophotometer.

2.10. Film microstructure analysis

Scanning electron microscopy (SEM) analysis was used to observe microstructure of the cross sectional area of the agar and agar/GSE films. Film sample was cut into small pieces and directly mounted on a specimen holder for the analysis. The cross sectional area of the films was analyzed using a Field Emission Scanning Electron Microscopy (FE-SEM, S-4800, Hitachi Co., Ltd., Matsuda, Japan) with an accelerating voltage of 5.0 kV.

2.11. Thermal stability

Thermal properties of the film samples were measured by thermogravimetric analysis (TGA) using a TGA analyzer (Hi-Res TGA 2950 Thermo Gravimetric Analyzer, TA Instrument, New Castle, DE, USA). About 5 mg of a sample was added into a standard aluminum pan and heated from 30 to 600 °C at heating rate of 10 °C/min under a nitrogen flow of 50 cm^3/min.

2.12. Statistical analysis

Antimicrobial activity and physical-mechanical properties of the films were measured in triplicate with individually prepared film samples. One-way analysis of variance (ANOVA) was conducted and significant difference ($p < 0.05$) between each mean property value was determined with the Duncan's multiple range tests using a statistical software package (SPSS 12.0, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Antimicrobial activity

The antimicrobial activity of agar and agar/GSE films was tested using disk diffusion method against food pathogens such as *E. coli*, *L. monocytogenes*, and *B. cereus* and the results were shown in

Table 1
Antimicrobial activity of the agar and agar/GSE composite films against common three foodborne pathogens.

Films	Zone of inhibition (mm)		
	L. monocytogenes	*E. coli*	*B. cereus*
Control	ND	ND	ND
0.6 μg/mL	13.03 ± 1.2^{b}	ND	ND
3.3 μg/mL	15.43 ± 1.2^{b}	ND	ND
6.6 μg/mL	17.66 ± 0.5^{c}	ND	5.33 ± 0.5^{b}
10.0 μg/mL	21.33 ± 1.5^{b}	5.23 ± 0.5^{a}	6.33 ± 0.5^{a}
13.3 μg/mL	23.66 ± 0.5^{b}	5.66 ± 0.5^{a}	8.66 ± 1.2^{a}

Bacterial growth inhibitory zone (mm) observed after 24 h of incubation at 37 °C. The values are represented as mean ± S.D. Values with the same superscript letter in the same column indicate that are statistically not different ($p > 0.05$). ND indicate the absence of growth inhibitory zone.

Table 1. As expected, the control film did not exhibit any antibacterial activity against *E. coli*, *L. monocytogenes*, and *B. cereus*. But, films incorporated with GSE showed antimicrobial activity against all the tested bacterial strains. Even the film contained the least concentration of GSE (0.6 μg/mL) showed very clear zone of growth inhibition (13.6 mm) against Gram-positive pathogen, *L. monocytogenes*. The zone of inhibition increased significantly as the concentration of GSE increased from 0.6 to 13.3 μg/mL (Table 1). The maximum zone of growth inhibition (23.6 mm) was observed with film containing 13.3 μg/mL of GSE. At the same time, Gram-negative pathogen, *E. coli* was found to be less susceptible to the agar/GSE films. Even though, the film containing the highest concentration of GSE (13.3 μg/mL) showed less inhibitory effect against *E. coli* compared with the Gram-positive bacteria. On the contrary, clear growth inhibition zones were observed in the agar/GSE composite films with higher concentration of GSE (10 and 13.3 μg/mL) against *E. coli*. This result indicates that Gram-positive *L. monocytogenes* was more susceptible to GSE than the Gram-negative bacterium, *E. coli*. This is probably due to the surrounding of additional external membrane on the cell wall of Gram-negative bacteria prevents the diffusion of hydrophobic or hydrophilic compounds through its lipopolysaccharide layer. Jang, Shin, and Song (2011) also reported that GSE incorporated rapeseed protein/gelatin films showed antimicrobial activity against Gram-positive and Gram-negative bacteria. They observed that Gram-positive *L. monocytogenes* was more highly inhibited by GSE compared with Gram-negative *E. coli* O157:H7. Song et al. (2012) also found that barley bran protein/gelatin/GSE films effectively inhibited the growth of *E. coli* and *L. monocytogenes*. The film containing GSE showed the maximum inhibition activity

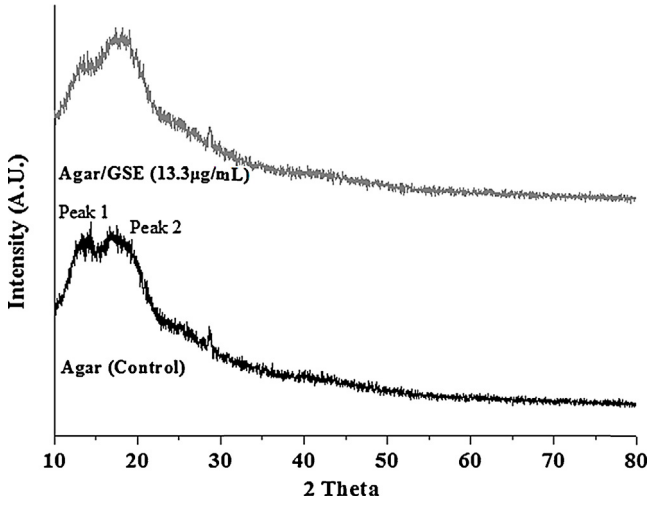

Fig. 1. XRD patterns of the agar and agar/GSE (13.3 μg/mL) composite films.

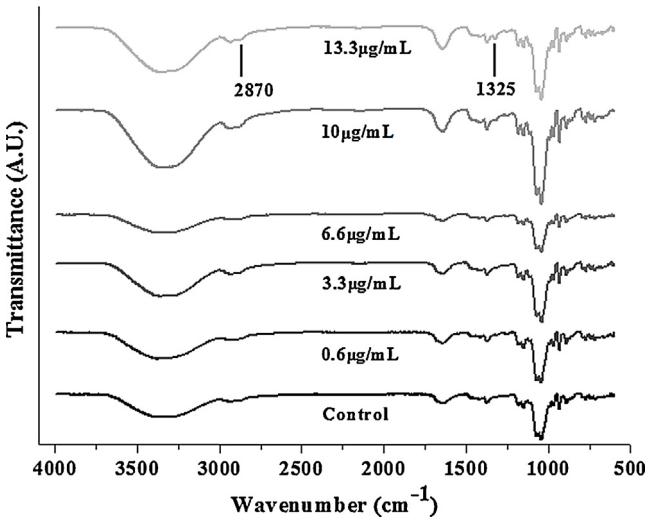

Fig. 2. FT-IR spectra of the agar and its different composite films.

against *L. monocytogenes*, while somewhat lower inhibition activity was observed against *E. coil*. Reagor, Gusman, McCoy, Carino, and Heggers (2002) reported that the GSE had strong antimicrobial effect against both *E. coli* and *L. monocytogenes*, but it had higher inhibitory activity against Gram-positive bacterium than Gram-negative one. Lim et al. (2010) prepared antimicrobial composite films by incorporation of GSE into *Gelidium corneum* (GC)/clay films and their antimicrobial activity against Gram-positive and Gram-negative bacteria. The GSE incorporated composite film showed better antimicrobial effect against Gram-positive *L. monocytogenes* than the Gram-negative *E. coli* O157:H7. Hong, Lim, and Song (2009) reported that GSE inhibited the activity of bacterial enzymes and weakened the cell wall and membrane of bacterium. Moreover, Corrales, Han, and Tauscher (2009) investigated antimicrobial effect of GSE incorporated pea starch films *in vitro* with pork loins infected by Gram-positive *Brochothrix thermosphacta*. The great reduction in bacterial cell viability (1.3 Log CFU/mL) was observed with GSE containing pea starch film.

3.2. X-ray diffraction and Fourier transform infrared spectroscopy analysis

X-ray diffraction (XRD) patterns of the agar and agar/GSE (13.3 μg/mL) composite films were shown in Fig. 1. The control film showed a peak at 19.0° and slight shoulder at 13.9° which indicated high degree of crystallinity of agar (Freile-Pelegrin et al., 2007; Wu et al., 2009). Though the major peaks of agar/GSE films shifted slightly to higher angle, the incorporation of GSE, as a whole, did not affect the degree of crystallinity of agar film.

Fourier transform infrared spectroscopy (FT-IR) spectra of the agar and agar/GSE composite films were shown in Fig. 2. The spectrum of agar exhibited various characteristic peaks in the range from 3354 cm^{-1} to 771 cm^{-1}. The characteristic broad absorption band at about 3354 cm^{-1} indicated stretching of hydroxyl (O-H) groups (Wu et al., 2009). The intense peak at 2925 cm^{-1} is attributed to stretching of C—H which is associated with the ring of methine hydrogen atoms. The peak appeared at 1642 cm^{-1} is due to the stretching vibration of the conjugated peptide bond formation by amine (NH) and acetone groups. The absorption band at 1371 cm^{-1} is assigned to ester sulfate. The characteristic absorption peaks at 1074, 1039 and 931 cm^{-1} indicates C—O stretching group of 3,6-anhydro-galactose. The peak at 886 cm^{-1} is due to the C—H stretching residual carbon of β-galactose (Wu et al., 2009). Peaks observed in the FT-IR spectrum of the control agar film are

Table 2
Thickness and mechanical properties of the agar and its various composite films.

Film	Thickness (μm)	TS (MPa)	EAB (%)	EM (MPa)
Control	36.7 ± 5.2^{a}	34.6 ± 2.7^{c}	25.6 ± 2.9^{a}	1004.9 ± 88.4^{d}
0.6 μg/mL	36.7 ± 5.2^{a}	34.1 ± 2.9^{c}	28.1 ± 1.7^{ab}	979.9 ± 43.8^{c}
3.3 μg/mL	38.3 ± 4.1^{a}	27.8 ± 4.5^{b}	29.0 ± 1.6^{abc}	684.9 ± 83.3^{c}
6.6 μg/mL	43.3 ± 5.2^{ab}	26.6 ± 3.5^{b}	32.1 ± 2.0^{cd}	591.2 ± 27.5^{b}
10.0 μg/mL	51.7 ± 7.5^{bc}	7.8 ± 0.6^{a}	33.0 ± 2.2^{d}	102.6 ± 10.7^{a}
13.3 μg/mL	53.3 ± 5.2^{c}	5.5 ± 0.4^{a}	29.5 ± 3.1^{bc}	73.7 ± 13.3^{a}

Results are represented as mean ± standard deviation and any two means in the same column followed by the same letter were not significantly different ($p > 0.05$) by Duncan's multiple range test.

relatively similar to those of agar/GSE film. However, some of the peaks were shifted to lower and higher frequency with increasing concentration of GSE. For example, peaks at 2925, 1371, 1074 and 931 cm^{-1} were shifted to 2931, 1370, 1071 and 928 cm^{-1}. These shifts are presumably attributed to the less interactions of polymer with GSE. Moreover, two more additional peaks were observed at 2870 and 1325 cm^{-1} in the FI-IR spectrum of agar/GSE composite films with higher concentration of GSE (10 and 13.3 μg/mL), which is probably due to the presence of phenolic compounds in GSE. As a whole, the FT-IR peaks of the agar/GSE composite films were similar to those of the control film which suggests that there were no significant chemical bond formed between the polymer matrix and GSE.

3.3. Thickness measurement

Table 2 shows thickness of the agar and agar/GSE composite films. Thickness of the films increased from 36.7 ± 8.2 to 43.3 ± 5.2 μm as the concentration of GSE increased from 0.6 to 13.3 μg/mL. Thickness of the agar films was strongly dependent on the concentration of GSE added. Rubilar et al. (2013) and Song et al. (2012) also reported similar trend of thickness for chitosan/GSE and barley bran protein/gelatin/GSE composite films.

3.4. Mechanical properties

The mechanical properties of the agar and agar/GSE composite films determined by means of tensile strength (TS), elastic modulus (EM) and elongation at break (EAB), and the results were shown in Table 2. The control and agar/GSE (0.6 μg/mL) films exhibited maximum tensile strength with values of 34.6 ± 2.7 and 34.1 ± 2.9 MPa, respectively. But, incorporation of GSE with more concentration decreased tensile strength of the agar films significantly ($p < 0.05$). Similar phenomenon has been observed by Gimenez et al. (2013) in the test of agar and agar/green tea extract (GTE) composite films. The incorporation of GTE into the agar film significantly decreased the tensile strength of the agar films from 18.4 ± 1.3 to 7.9 ± 1.0 MPa which was probably due to the reduction in the molecular interaction between the agar polymer strands and GTE. Similar results have been reported that the tensile strength of biopolymer films decreased with addition of GSE into the agar/nanoclay and chitosan films (Lim et al., 2010; Moradi et al., 2012). These results indicate that GSE is not so compatible with those biopolymers to make strong interaction between them. In general, the mechanical properties of the packaging films are closely associated with the distribution and density of inter- and intra-molecular interactions between the polymer chains in the film matrix (Chambi & Grosso, 2006).

Elastic modulus (EM) and elongation at break (EAB) indicates the intrinsic stiffness and flexibility of a film. As shown in Table 2, the control film showed higher values of EM (1004.9 ± 88.4 MPa) compared with that of agar/GSE composite films. However, the incorporation of GSE decreased EM of the agar films significantly ($p < 0.05$). As the concentration of GSE increased from 0.6 to 13.3 μg/mL, the EM of the films decreased substantially. This result is consistent with that of the TS measurement. On the contrary, the EAB of the agar films increased significantly ($p < 0.05$) by the addition of GSE. The maximum value (33.0 ± 2.2%) was observed for agar film containing 10 μg/mL concentration of GSE. Overall, the addition of GSE into the agar films greatly affected the mechanical properties of the films.

3.5. Water vapor permeability

The water vapor permeability (WVP) of the agar and agar/GSE composite films were shown Table 3. Though the control film showed lower WVP (1.33 ± 0.11 g m/(m^2 s Pa)) compared with those of agar/GSE composite films, the difference was not significantly different ($p < 0.05$). This result indicates that the incorporation GSE did not affect water vapor barrier property of the agar films. However, some controversial results on this have been reported. Hong et al. (2009) reported that the WVP of the agar rich red algae films decreased by addition of high concentration of green tea extract or GSE which was explained by the compact interaction between them forming less permeable polymer network. On the contrary, Song et al. (2012) and Lim et al. (2010) found that incorporation of GSE increased WVP of the barley bran protein/gelatin and *G. corneum* (GC)/clay composite films, probably due to the reduction in intermolecular interactions between the components and changing pore size of the films. Increase in WVP was also reported by Jang et al. (2011) for rapeseed protein/gelatin/GSE composite films.

Table 3
Moisture content, water solubility, water contact angle and water vapor permeability of agar and various composite films.

Film	WVP (×10^{-9} g m/(m^2 Pa s))	MC (%)	WS (%)	WCA (°)
Control	1.32 ± 0.11^{a}	18.73 ± 2.31^{a}	10.42 ± 2.10^{a}	66.54 ± 1.41^{e}
0.6 μg/mL	1.34 ± 0.11^{a}	22.45 ± 0.59^{b}	12.81 ± 1.03^{ab}	58.56 ± 0.72^{d}
3.3 μg/mL	1.41 ± 0.06^{a}	25.28 ± 0.98^{b}	23.73 ± 1.72^{c}	52.44 ± 1.56^{c}
6.6 μg/mL	1.49 ± 0.06^{a}	30.80 ± 1.18^{c}	41.12 ± 0.56^{d}	47.83 ± 0.60^{b}
10.0 μg/mL	1.74 ± 0.26^{a}	37.35 ± 0.76^{d}	52.40 ± 1.23^{e}	43.86 ± 2.68^{b}
13.3 μg/mL	1.48 ± 0.18^{a}	41.56 ± 0.07^{e}	61.56 ± 0.88^{f}	37.14 ± 1.19^{a}

Results are represented as mean ± standard deviation and any two means in the same column followed by the same letter were not significantly different ($p > 0.05$) by Duncan's multiple range test.

3.6. Moisture content, water solubility and water contact angle

Table 3 shows moisture content (MC) of the agar and agar/GSE composite films. MC is an important film property which is closely associated with the total water molecules occupied in the network microstructures of the composite films (Jiang, Li, Chai, & Leng, 2010). Neat agar film exhibited lower MC (18.7 ± 2.3%) compared with those of agar/GSE composite films. The incorporation of GSE into the agar film increased the MC of the composite films. Rubilar et al. (2013) also found that the addition of high concentration of GSE into the chitosan films increased the MC of the chitosan films, which was probably attributed to the hydrophilic nature of the GSE.

Water solubility (WS) of the agar and agar/GSE composite films was determined and their results were also shown in Table 3. WS of the films were greatly affected by the addition of GSE. The WS of the neat agar film was 10.4 ± 2.1%, but it increased significantly by blending with GSE. The WS of agar/GSE composite films increased linearly up to 61.6 ± 0.9% with increasing concentration of GSE. The linear regression equation between the WS *vs.* the GSE content was expressed as:

$$y = 25.56x + 11.1 \quad (R^2 = 0.99)$$

where x and y are GSE content (μg) and the WS (%), respectively.

As the concentration of GSE increased, the water solubility of agar films increased remarkably which is probably due to the incorporation of hydrophilic GSE. This result suggests that the addition of GSE could decrease water resistant of the agar films. The high WS of agar/GSE composite films may be due to the hydrophilic nature of GSE. Similarly, Gimenez et al. (2013) reported that the incorporation of green tea extract (GTE) into the agar films increased WS of the agar film from 24% up to 58%.

Surface hydrophobicity and wettability of the agar and agar/GSE films was also evaluated by measuring the water contact angle (WCA) of the films. In general, higher WCA indicates less hydrophilic nature of the film surface. Hydrophobicity of the agar and agar/GSE composite films was greatly affected by addition of GSE (Table 3). The incorporation of GSE had profound effect on the hydrophilic nature of the resulting composite films, which could be due to the hydrophilic nature of GSE. The control film exhibited higher water contact angle (66.5 ± 1.4°) compared with those of composite films. The significant decrease in the WCA was observed with addition of GSE with increasing concentrations into the agar films (Table 3). When water droplet is placed on the surface of a film, water contact angle is usually decreased over time due to the evaporation and absorption of water (Salarbashi et al., 2013). The evaporation is caused by the difference of water vapor pressure between the droplet and surrounding environment. The absorption is due to the sucking of water inside the polymer matrix. Consequently, hydrophobic character of the agar films decreased with incorporation of GSE. These results are in agreement with those of Moradi et al. (2012) who found that the surface hydrophilicity of chitosan film increased with addition of GSE.

3.7. Surface color and optical properties

The control and agar/GSE composite films were free-standing and flexible with smooth-surface. The control film was transparent without any color, while the film incorporated with GSE was less transparent with a slight yellow tint, which is clearly shown in the result of surface color properties and transparency of the films (Table 4). As shown in Table 4, Hunter *L*-values decreased, while Hunter *a*- and *b*-values increased after blending with GSE. Decrease in *L* values with increase in *b* values indicates that a yellowish tint appeared in the film. This result is in agreement with visible observation. The incorporation of GSE at increasing level resulted

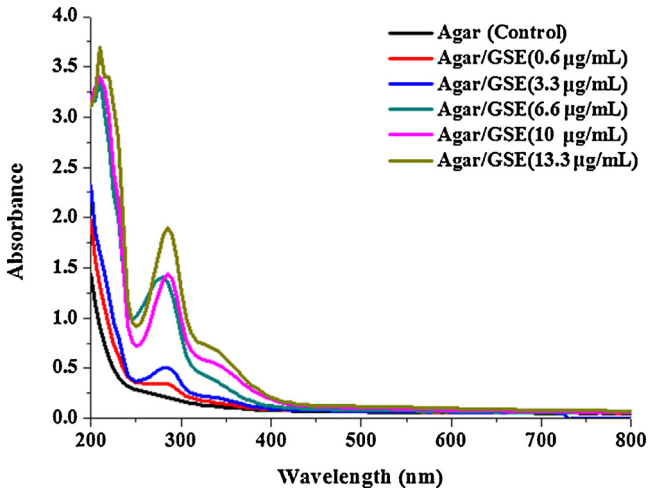

Fig. 3. UV–vis absorption spectra of the agar and its composite films.

remarkable increase in total color difference (ΔE) of the agar film from 2.15 ± 0.23 to 7.85 ± 0.75. Similar results were observed with addition of GSE into chitosan (Moradi et al., 2012) and pea starch film (Corrales et al., 2009). Changes in color were more pronounced with increasing concentration of GSE. Du et al. (2009) reported that type and concentration of the essential oil played an important role in the enhancement of color edible apple films.

The light absorption properties and transparency of the agar and agar/GSE composite films was determined by measuring absorption spectra (200–800 nm) and percent transmittance (280 and 660 nm) of the films. UV–vis absorption spectra of the composite films are shown in Fig. 3. The control film did not absorb radiation at scanned wavelength, but GSE incorporated agar films absorbed radiations at UV region (270–280 nm), probably due to the presence of phenolic compounds in GSE. Shojaee-Aliabadi et al. (2013) reported that the presence of the phenolic compounds in the essential oil might be absorbed light at lower wave length. The intensity of the absorption peak increased as the concentration of the GSE increased from 0.6 to 13.3 μg/mL.

Percent transmittance of the agar and agar/GSE composite films was measured at UV and visible regions at the wavelength of 280 and 660 nm, respectively, and the results are also presented in Table 4. The control film is clear and transparent as indicated in the high transmittance values of 60.3 and 87.8% observed at both UV ($T_{280\,nm}$) and visible ($T_{660\,nm}$) ranges, respectively. Transmittance of agar films decreased significantly after incorporation of GSE and the decrease in transmittance of both UV and visible light was more pronounced with increase in the concentration of GSE. Overall, transparency of the composite films was reduced by the addition of GSE. These results are in good agreement with those of Shojaee-Aliabadi et al. (2013) who reported that the addition of *Satureja hortensis* essential oil (SEO) greatly reduced the transparency of carrageenan films, which could be due to the opaqueness of SEO. The dispersion or distribution of the oil droplets in the whole polymer network influenced the light scattering, resulting in less transparent than the carrageenan control film. Pereda, Aranguren, and Marcovich (2010) also reported that the increased formation of opaqueness of the film can reduce transmission of light of the film. It is interesting to note that the differences in transmittance between both control and agar/GSE composite films were not so high at visible region, but remarkable differences were observed with dramatic decrease in transmittance at UV region. These results indicate that the GSE incorporated agar films could prevent penetration of UV efficiently without sacrificing see-through property of the packaging film. This great UV barrier property is a desirable

Table 4
Color and percent light transmittance of agar and various agar/GSE composite films.

Films	L	a	b	ΔE	$T_{280\,nm}$	$T_{660\,nm}$
Control	92.82 ± 0.26^{d}	-0.32 ± 0.01^{a}	4.35 ± 0.02^{a}	2.15 ± 0.23^{a}	60.26 ± 0.27^{f}	87.77 ± 0.13^{d}
0.6 μg/mL	92.49 ± 0.15^{cd}	-0.43 ± 0.38^{a}	4.83 ± 0.05^{b}	2.70 ± 0.07^{ab}	44.46 ± 0.30^{e}	87.27 ± 0.23^{d}
3.3 μg/mL	91.59 ± 0.32^{c}	-0.11 ± 0.29^{b}	5.32 ± 0.07^{c}	3.72 ± 0.25^{b}	31.38 ± 0.36^{d}	87.30 ± 0.31^{d}
6.6 μg/mL	89.81 ± 0.14^{b}	0.32 ± 0.23^{c}	5.97 ± 0.01^{d}	5.59 ± 0.19^{c}	4.76 ± 0.07^{b}	86.31 ± 0.17^{c}
10.0 μg/mL	87.81 ± 0.41^{a}	1.09 ± 0.26^{d}	5.72 ± 0.08^{d}	6.69 ± 0.59^{d}	3.88 ± 0.49^{c}	84.33 ± 0.37^{b}
13.3 μg/mL	87.27 ± 0.65^{a}	1.08 ± 0.26^{d}	5.70 ± 0.08^{d}	7.85 ± 0.75^{cd}	1.76 ± 0.22^{a}	81.35 ± 0.26^{a}

Results are represented as mean ± standard deviation and any two means in the same column followed by the same letter were not significantly different ($p > 0.05$) by Duncan's multiple range test.

functional property for food packaging films in order to prevent photochemical reactions caused by the UV light.

3.8. Microstructure

Fig. 4 shows SEM micrograph of the agar and agar/GSE composite films. Noticeable morphological difference was observed between the agar and agar/GSE composite films. The control film was found to be homogenous and smooth constituting continuous structures. However, agar/GSE films showed rough surface with cavity-like structures which could be due to the presence of hydrophilic compounds in the GSE. The roughness and size of the cavities in the agar film increased with increasing concentration of GSE. Similar microstructural behaviors have been observed with other composite films such as chitosan/GSE (Rubilar et al., 2013) and carrageenan/SEO films (Shojaee-Aliabadi et al., 2013).

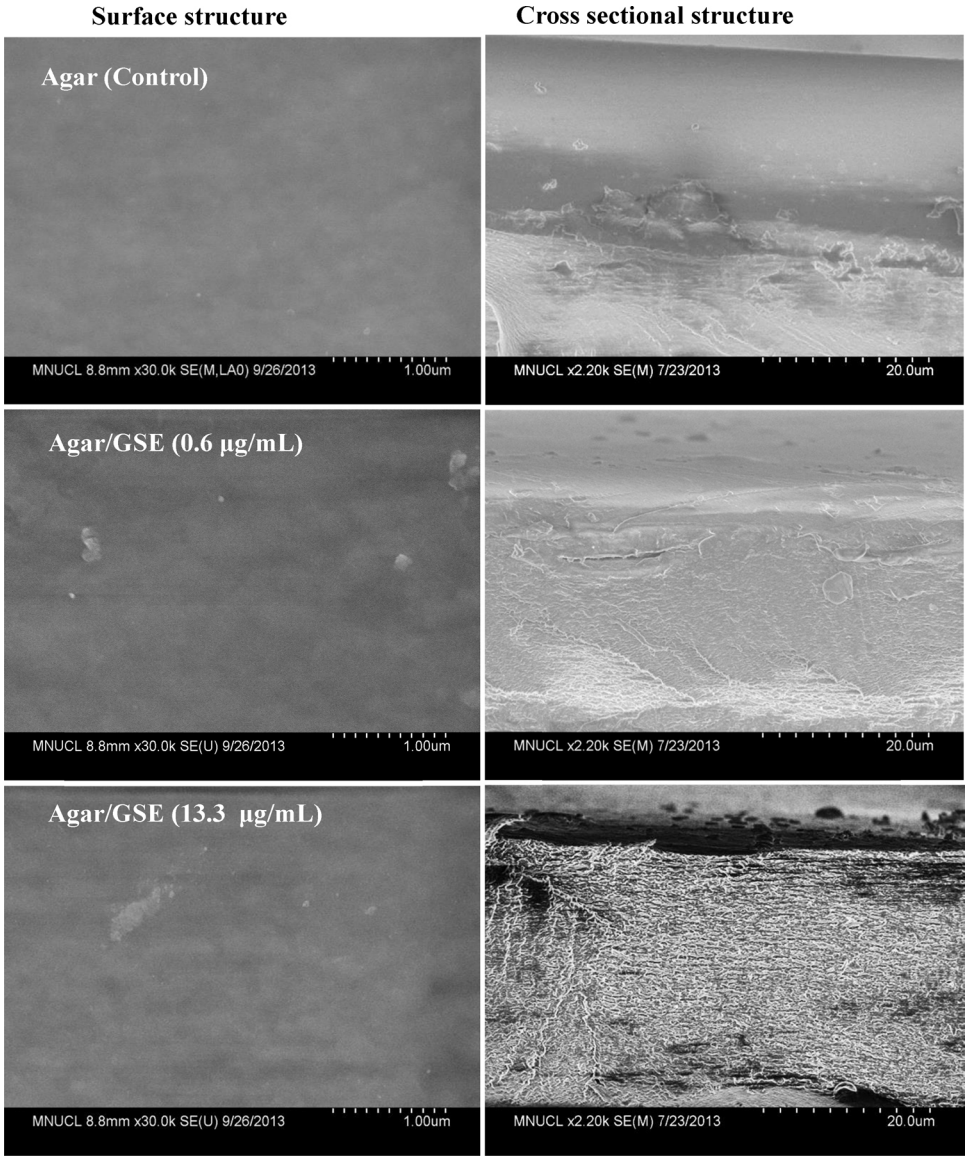

Fig. 4. SEM images of surface and cross section of the agar (control), agar/GSE (0.6 μg/mL) and agar/GSE (13.3 μg/mL) composite films.

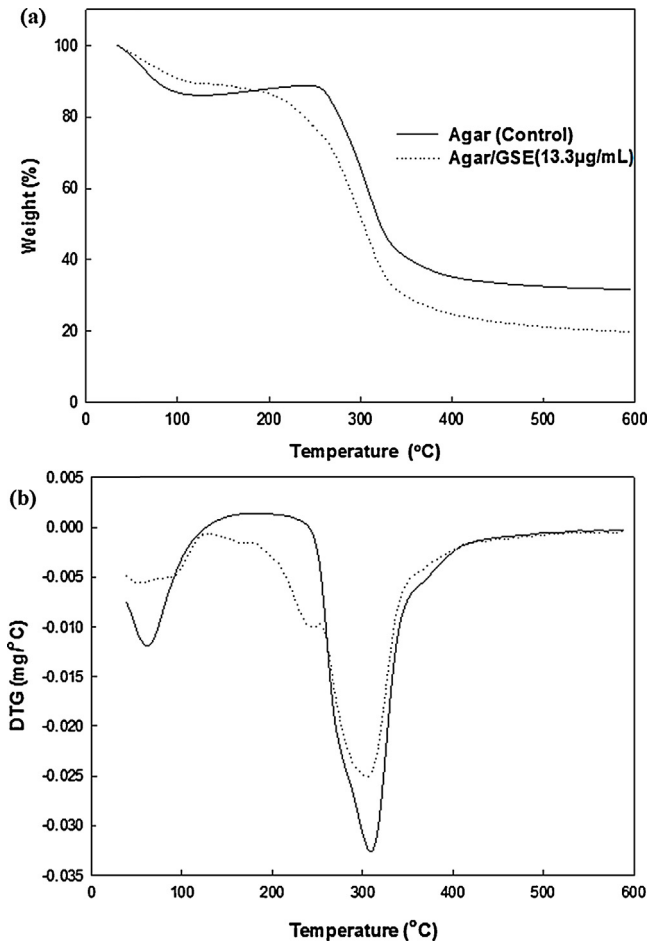

Fig. 5. TGA thermograms and DTGA curves of the agar and agar/GSE (13.3 μg/mL) composite films.

3.9. Thermgarvimetric analysis

Thermal property of the agar and agar/GSE (13.3 μg/mL) composite films was measured using a thermogravimetric analysis (TGA) and the results were shown in Fig. 5. As shown in the TGA thermograms, three distinctive steps of thermal degradation were observed in thermal destruction of agar and agar/GSE composite films. A series of thermal degradation of films were observed around 90 °C, 250 °C, and 325 °C for agar and 90 °C, 220 °C, and 320 °C for agar/GSE composite films, respectively. The first thermal degradation was mainly attributed to the evaporation of water remained in both the agar and agar/GSE films. The second and third step of degradation could be due to the decomposition of GSE, glycerol and agar polymer, respectively (Rhim et al., 2006). As shown in Fig. 5, the onset thermal degradation temperature of agar/GSE (13.3 μg/mL) film was little different from those of control film. This result indicates that the addition of GSE did not improve thermal stability of the agar films.

4. Conclusion

Antimicrobial films were prepared by incorporating GSE as natural antimicrobial agent with various concentrations into agar film for the use of active food packaging. Remarkable changes in color, transmittance, moisture content, water solubility, water barrier, hydrophobicity and mechanical properties were recorded. The agar/GSE composite film exhibited high UV barrier property which implies that the composite films have high potential for being used to protect food stuffs from UV light induced photochemical reactions. FE-SEM, FT-IR, XRD, and TGA were performed to characterize morphological properties of the resulting agar based composite films. Changes in film microstructures were observed in the agar/GSE films. XRD and TGA analysis indicated that the presence of GSE did not change crystallinity and thermal stability of agar film. The agar/GSE composite films exhibited strong antimicrobial activity against various Gram-positive and Gram-negative food-borne pathogens. The antimicrobial activity was dependent on the concentration of GSE and bacterial strains. The antimicrobial activity of the film is due to the migration of phenolic compounds in the GSE. The GSE entrapped by the polymer matrix might be released by replaced with water molecules to exert antimicrobial activity. The agar served as effective vehicle to carry antimicrobial agents. Conclusively, the agar/GSE films with remarkable functional properties can be used as an environmentally friendly active food packaging film as well as a promising alternative to synthetic polymer based packaging films. However, further studies are needed to investigate potential performance improvement for industrialized use of the film.

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