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Supplementary files

A Facile In Situ Synthesis of Resorcinol-Mediated Silver Nanoparticles and Fabrication of Agar-Based Functional Nanocomposite Films

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Materials

Food grade agar was obtained from Gel-Tec Co., Ltd. (Seoul, Korea). Resorcinol (C6H6O2, 99%), ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)], DPPH (2,2-diphenyl-1-picrylhydrazyl), and methanol (99.8%) were procured from Sigma-Aldrich (St. Louis, MO, USA). Silver nitrate was purchased from Daejung Chemicals & Materials Co., Ltd. (Siheung, Gyeonggi-do, Korea). Tryptic soy broth (TSB) and brain heart infusion broth (BHI), and agar powder were obtained from Duksan Pure Chemicals Co., Ltd. (Ansan, Gyeonggi-do, Korea).

Characterization and properties of the films

SEM and FT-IR

The surface morphology of the composite films was tested using the field emission scanning electron microscope (FE-SEM, S-4800, Hitachi Co., Ltd., Matsuda, Japan) at an accelerating voltage of 2 kV. The piece of film was fixed on the SEM specimen holder and sputtered with platinum for 30 s.

The attenuated total reflectance-Fourier transform infrared (AT-FTIR) spectrophotometer (TENSOR 37 Spectrophotometer, Billerica, MA, USA) was used to measure the chemical structure of the film matrix in the 4000-500 cm^{-1} range.

Surface color and optical properties

The surface color of the composite films was analyzed using a Chroma meter (Konica Minolta, CR-400, Tokyo, Japan). The total color difference (ΔE) was calculated as the following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (1)$$

where ΔL, Δa, and Δb are the differences between the color of standard color plate and film samples.

The light absorption spectra of the nanocomposite films were determined over a range of 200-700 nm using a UV-visible spectrophotometer (Mecasys Optizen POP Series UV/Vis, Seoul, Korea). In addition, the UV-light barrier property and transparency of the film were determined by measuring the percent transmittance at 280 nm (T_{280}) and 660 nm (T_{660}), respectively.

Mechanical properties

The film thickness was measured by a hand-held digital micrometer (Digimatic Micrometer, QuantuMike IP 65, Mitutoyo, Japan). Instron Universal Testing Machine (Model 5565, Instron Engineering Corporation, Canton, MA, USA) was used to determine tensile strength (TS), elongation at break (EB), and elastic modulus (EM) of the films were tested using in tensile mode. An initial gauge separation and crosshead speed were set to 50 mm and 50 mm/min, respectively.

Water contact angle (WCA) and water vapor permeability (WVP)

The water contact angle (WCA) of the film was measured using a WCA analyzer (model Phoenix 150, Surface Electro Optics Co., Ltd. (Kunpo, Gyeonggi-do, Korea). About 10 μL of distilled water was placed on the surface of the film with a micro-syringe.

The water vapor permeability (WVP) of the film was determined following the standard method of ASTM E96-95 with modification. Each film (7.5 cm × 7.5 cm) was placed on the top of poly(methyl methacrylate) cup filled with 18 mL of distilled water. The WVP cups were stored in a humidity chamber controlled at 25

°C and 50% relative humidity (RH). The weight of the cups was obtained at 1 h intervals for 8 h. The water vapor transmission rate (WVTR) was determined from the slope of the weight loss plot of the WVP cup versus time and the WVP (g.m/m².s.Pa) of the film was calculated as follows:

$$WVP = (WVTR \times L)/\Delta p \quad (2)$$

where L was the mean film thickness and Δp was the water vapor pressure difference (Pa) across the film.

Thermogravimetric analysis

Thermogravimetric analysis (TGA) was examined using a thermogravimetric analyzer (Hi-Res TGA 2950, TA Instrument, New Castle, DE, USA). The samples were heated from 30 °C to 600 °C with a heating rate of 10 °C/min under a nitrogen flow of 50 mL/min. The maximum degradation temperatures were calculated from the first derivative form of the TGA (DTG).

Antioxidant activity

Antioxidant activities of the films were measured by assessing the free radical scavenging activity. 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical scavenging methods were used for the antioxidant activity assessment. For DPPH analysis, a prescribed amount of methanolic solution of DPPH was freshly made, and ~100 mg of tested film sample was added in a 10 mL DPPH solution and incubated at room temperature for 30 min and measured the absorbance at 517 nm. For the ABTS assay, a prescribed amount of potassium sulfate was added to the ABTS solution, followed by overnight incubation in the dark to make the ABTS assay solution. ~100 mg of tested film samples were added to 10 mL of ABTS assay solution, incubated at room temperature for 30 min, and measured the absorbance at 734 nm. The antioxidative activity of the agar-based nanocomposite films was calculated as follows:

$$\text{Free radical scavenging activity (\%)} = \frac{A_c - A_t}{A_c} \times 100 \quad (3)$$

where A_c and A_t were the absorbances of DPPH/ABTS of the control and test film, correspondingly. All the test was performed in triplicate, and the average value was reported.

Antimicrobial activity

The antimicrobial activity of the agar-based composite film was examined using a viable colony count method against two types of foodborne pathogenic bacteria, *L. monocytogenes* and *Escherichia coli*. The test strains were inoculated in 20 mL of BHI and TSB broth, respectively, for 16 h at 37 °C. Next, 200 μL of diluted inoculum (10^8-10^9 CFU/mL) was transferred to 20 mL of fresh BHI and TSB broth containing 100 mg film sample. The flask was stored in a shaker at 37 °C for 12 h. Samples were taken at intervals of 3 hours, and bacterial colonies were counted to measure each pathogen's cell viability. Antibacterial assay was performed in triplicate with individually prepared films.

Statistical analysis

Film properties were measured with individually prepared films in triplicate as replicated experimental units, and the results were provided with a mean ± standard deviation. In addition, a one-way analysis of variance (ANOVA) was performed, and the significant differences among the film properties were determined ($p < 0.05$) with Duncan's multiple range tests using the SPSS statistical analysis program (SPSS 21, SPSS Inc., Chicago, IL, USA).