coatings

MDPI

Supplementary Materials

Pullulan/Agar-Based Functional Film Containing Eucalyptus Essential Oil and Rutin

Swarup Roy [1,2,*] and Jong-Whan Rhim [1,*]

[1] BioNanocomposite Research Center, Department of Food and Nutrition, Kyung Hee University, Seoul 02447, Republic of Korea
[2] School of Bioengineering and Food Technology, Shoolini University, Solan 173229, India
* Correspondence: swaruproy2013@gmail.com (S.R.); jwrhim@khu.ac.kr (J.-W.R.)

Experimental

Characterization and Properties of the Film

Surface Color and Transmittance

The surface color of the films was measured using a Chroma meter (Konica Minolta, CR-400, Tokyo, Japan) with a white color plate (L = 97.75, a = -0.49, and b = 1.96) as a standard background for color measurement. The total color difference (ΔE) was calculated using the following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad \text{(S1)}$$

where ΔL, Δa, and Δb are the difference between each color value of the standard color plate and film sample.

The UV-vis transmission spectra of the composite films were recorded using a spectrophotometer (Mecasys Optizen POP Series UV-vis spectrophotometer, Seoul, Korea). The UV-barrier and transparency properties of the film were assessed by determining the percent light transmittance of the film at 280 nm (T_{280}) and 660 nm (T_{660}), respectively [1].

Surface Morphology and FTIR

The film's surface morphology was observed using field-emission scanning electron microscopy (FE-SEM, SU 8010, Hitachi Co., Ltd., Matsuda, Japan). FTIR spectra of the film samples were noted at a wavenumber of 4000–500 cm^{-1} with the resolution of 32 scans at 4 cm^{-1} using a TENSOR 37 Spectrophotometer with OPUS 6.0 software (Billerica, MA, USA).

The Mechanical Properties

First, five rectangular strips of film samples (2.54 cm × 15 cm) from each film were cut using a precision double-blade cutter (model LB.02/A, metrotec, S.A., San Sebastian, Spain). The film sample's thickness was measured using a hand-held digital micrometer (Digimatic Micrometer, QuantuMike IP 65, Mitutoyo, Japan) with an accuracy of 1 μm. The film thickness was measured at five random locations of each film sample, and their average was used. The film's mechanical properties, such as tensile strength (TS), elongation at break (EB), and elastic modulus (EM), were determined following the standard method of ASTM D 882-88 using an Instron Universal Testing Machine (Model 5565, Instron Engineering Corporation, Canton, MA, USA). The Instron machine was operated with an initial grip separation of 50 mm and a crosshead speed of 50 mm/min [2].

Water Vapor Permeability (WVP)

The WVP of the composite films was determined gravimetrically using a WVP cup following the ASTM E96-95 standard method. At first, the WVP cup was filled with a

prescribed amount of water, then covered by the films, sealed, and kept in the controlled environmental chamber at 25 °C and 50% RH. After equilibration, the WVP cup's weight was measured at every one-hour interval, and the weight loss was calculated. The WVTR ($g/m^2 \cdot s$) was determined from the slope (linear) of the steady-state portion of weight loss of the cup versus the time curve. Then, the WVP of the films was calculated in $g.m/m^2.Pa.s$ as follows [3]:

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

L was the film's thickness (m), and Δp was the partial water vapor pressure difference (Pa) across the film.

Thermal Stability

The films' thermal stability was determined using a thermogravimetric analyzer (Hi-Res TGA 2950, TA Instrument, New Castle, DE, USA). For the measurement, ~10 mg of film sample was taken in a standard aluminum pan and scanned at a heating rate of 10 °C/min in a temperature range of 30–600 °C under a nitrogen flow of 50 cm^3/min with an empty pan as a reference.

Antioxidant Activity

Antioxidant activities of the films were measured by assessing the free radical scavenging activity. The 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical scavenging methods were used for the antioxidant activity assessment [4]. For DPPH analysis, a prescribed amount of a methanolic solution of DPPH was freshly made, and ~50 mg of the tested film sample was added in a 10 mL DPPH solution and incubated at room temperature for 30 min, and the absorbance was measured 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. Then, ~50 mg of the tested film samples were added to 10 mL of the ABTS assay solution, incubated at room temperature for 30 min, and the absorbance was measured at 734 nm. The antioxidative activity of the pectin/agar composite 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 absorbance of DPPH/ABTS of the control and test film. All the tests were performed in triplicate, and the average value was reported.

Statistical Analysis

The film properties were measured in triplicate with individually prepared films. One-way analysis of variance (ANOVA) was performed to compare the differences among the samples, and the significance of each mean property value was determined ($p < 0.05$) by Duncan's multiple range test using the SPSS statistical analysis computer program for Windows (SPSS Inc., Chicago, IL, USA).

References

1. Roy, S.; Rhim, J.-W. Preparation of Carrageenan-Based Functional Nanocomposite Films Incorporated with Melanin Nanoparticles. *Colloids Surf. B Biointerfaces* **2019**, *176*, 317–324, doi:10.1016/j.colsurfb.2019.01.023.
2. Rhim, J.-W. Physical-Mechanical Properties of Agar/κ-Carrageenan Blend Film and Derived Clay Nanocomposite Film. *J. Food Sci.* **2012**, *77*, N66--73, doi:10.1111/j.1750-3841.2012.02988.x.
3. Rhim, J.-W. Effect of Clay Contents on Mechanical and Water Vapor Barrier Properties of Agar-Based Nanocomposite Films. *Carbohydr. Polym.* **2011**, *86*, 691–699, doi:10.1016/j.carbpol.2011.05.010.
4. Roy, S.; Rhim, J.-W. Preparation of Antimicrobial and Antioxidant Gelatin/Curcumin Composite Films for Active Food Packaging Application. *Colloids Surf. B Biointerfaces* **2020**, *188*, doi:10.1016/j.colsurfb.2019.110761.