LWT - Food Science and Technology 80 (2017) 131–135

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Short communication

Betanin immobilized LDPE as antimicrobial food wrapper

Cynthya M. Manohar, Saurabh D. Kundgar, Mukesh Doble*

Department of Biotechnology, Indian Institute of Technology, Madras, Chennai 600036, India

ARTICLE INFO

Article history:
Received 2 April 2016
Received in revised form
3 June 2016
Accepted 6 July 2016
Available online 12 July 2016

Keywords:
Betanin
LDPE
UV crosslinking
Biological activity
Biofilm

ABSTRACT

Betanin was immobilized on LDPE by UV cross-linking and tested as food wrapper. The attachment of live *Staphylococcus aureu* and *Escherichia coli* bacterial colonies were 2.3 and 1.5 times less respectively on this film when compared to bare polymer. The corresponding reduction in the carbohydrate in the biofilm formed on the surface was 1.6 and 1.9 respectively and that of protein was 2.5 and 2.3 respectively. The modified polymer reduced the growth of *S. aureus* in cottage cheese and beef by three and four times respectively when wrapped with it for ten days when compared to the food wrapped with unmodified polymer. The phytochemical makes the surface antibacterial, antioxidant and anti-adhesive and can help in extending the shelf life of food.

1. Introduction

Food borne illnesses due to microbial contamination is a major global concern. Contaminated food also leads to wastage. Microbial contamination happens during packaging and post-processing; and occurs mainly at the surface (Muriel-Galet, López-Carballo, Gavara, & Hernández-Muñoz, 2012). Use of antimicrobial coatings (organic acids, peptides, spices, enzymes) on food wrappers helps to extend the shelf-life of the food by inhibiting the growth of microorganisms (Fuciños, Guerra, Teijón, Pastrana, & Rúa, 2012; Quintavalla & Vicini, 2002). *Escherichia coli* and *Staphylococcus aureus* are the common food borne pathogens reported (El-Hadedy & El-Nour, 2012). Antimicrobial substances when used as sprays or dips have limited life because they may diffuse during storage from the film surface into the food since they are not anchored to the polymer (Muriel-Galet et al, 2012). Hence immobilizing them can help in prolonging their activity and stability over a long period of time. Currently used cross linkers are toxic and cannot be used in food industry (Bräse, Gil, Knepper, & Zimmermann, 2005; Manohar, Prabhawathi, Sivakumar, and Doble, 2015). Use of natural products for such applications is desired than use of such chemicals since they may come in contact with the food. Betanin is found in red beetroot, is a natural food colorant and approved by European Union as safe to be used in food products (Hendry & Houghton, 1996). In addition it is known to possess antibacterial activity. Hence in this current study betanin is cross-linked to Low-density polyethylene (LDPE) using UV light to develop a stable antimicrobial packaging film and is tested with food. LDPE is the commonly used wrapper in food packaging industries (Rooney, 1995).

2. Materials and methods

2.1. Chemicals and bacterial strains

Sources of various chemicals: Betanin from Tokyo Industry co. (Japan), *Staphylococcus aureus* NCIM (National Collection of Industrial Microorganisms) 5021 and *Escherichia coli* NCIM 293 from National Chemical Laboratory, India. LDPE sheets from Marine industrial polymers, India; and other chemicals from Sigma (St. Louis, MO), and Super Religare Laboratories (India).

2.2. Biological activities of betanin

A standard graph was prepared between concentration of betanin (in phosphate buffer solution (PBS), pH 7) and the optical density of the solution (535 nm with Perkin-Elmer, Lambda 35, USA).

The minimum inhibitory concentration (MIC) of betanin against these strains was determined by microdilution broth assay (Sarker, Nahar, & Kumarasamy, 2007). The bacteria was grown for 8 h, centrifuged at 5000 rpm for 10 min, re-suspended in PBS (pH 7.2,

* Corresponding author.
E-mail address: mukeshd@iitm.ac.in (M. Doble).

http://dx.doi.org/10.1016/j.lwt.2016.07.020
0023-6438/

10 mM) and the absorbance was adjusted to 0.1. 100 μL of betanin in 10% (v/v) ethanol (stock of 1 mg/mL) was pipetted into the first row of a 96 well plate. To other wells 50 μL of nutrient broth was added. Serial dilutions were performed such that each well had 50 μL of betanin in serially descending concentrations. Appropriate controls were also taken. 10 μL of the above microbe solution was added in all the wells except the first row. The plate was incubated at 37 °C for 15 h. Resazurin dye (0.01% w/v) was added in all the wells and was incubated at 37 °C for 4 h in the dark. This dye stains dead cells blue and viable cells appear pink.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging assay is followed to evaluate the antioxidant activity of betanin with ascorbic acid as the standard (Duh, Tu, & Yen, 1999).

2.3. Immobilization of betanin

200 μL of betanin in ethanol (10% solution) was coated on LDPE film (1 × 1 cm) with a spin coater (APEX instruments Co Pvt Ltd., India), air dried for 2 h and exposed to UV light (365 nm and 500 W) in a cabinet at 30 °C for 24 h (Nguyen & West, 2002). It (BI-LDPE) was then washed with 25 mM of PBS (pH 7, 25 °C). The amount of betanin in this solution was determined and the difference from the initial amount taken for cross linking gave the immobilization efficiency. Betanin leached out from BI-LDPE stored in PBS for 30 d at 4 °C was estimated to determine its long term stability.

The samples were analysed with a Fourier Transform Infrared (ATR-FTIR) spectrometer (Perkin-Elmer PE 1600). The composition of the elements present on their surfaces was determined with a scanning electron microscope (SEM) equipped with an energy dispersive x-ray spectroscope (EDAX) (JEOL JSM 5600 LSV, Japan) and their surface wettability using a Goniometer (Kruss, Germany).

2.4. Characterization of biofilms

Bacterial strains were grown on a nutrient agar plate and incubated at 37 °C, and a single colony was inoculated into 25 mL of nutrient broth and incubated at 37 °C for 16 h in a shaker (Scigeneis Pvt., Ltd, India) at 120 rpm. 500 μL of the above preculture was inoculated into 20 mL of nutrient broth and cultured under the above conditions. After 16 h, the cultural broth was taken in a tube and centrifuged at 1000 g at 4 °C for 10 min. The pellet was diluted with 0.7% saline and its optical density (measured at 600 nm with a UV spectrophotometer (V-550, Jasco)) was adjusted to 0.1 (~10^7 cells/mL) which was used for further studies (Manohar & Doble, 2016).

BI-LDPE and LDPE (1 × 1 cm) pieces were immersed into separate conical flasks containing 25 mL of nutrient broth. 1 mL of the bacterial suspension was inoculated and stirred at 120 rpm for 24 h at 37 °C. Samples were then removed and washed twice with sterile water to detach the unbound bacterial cells. The strongly bound ones were dislodged by water-bath ultrasonication (Thomsan Pvt., Ltd, Ajmer, India) for about 10 min. The viable bacterial colonies were counted in a nutrient agar plate and reported as colony forming units (log CFU/mL on 1 cm^2 surface). Protein and exopolysaccharides content in the biofilm formed on the surfaces were determined by Lowry's (Lo & Stelson, 1972) and phenol sulphuric acid methods (Nielsen, 2010) respectively using crystalline bovine serum albumin and glucose as the standards respectively.

Another set of samples were washed with distilled water, fixed with 3% glutaraldehyde (in 0.1% PBS, pH 7.2) for an hour, rinsed twice with PBS, once with distilled water, dried overnight in a desiccator, coated with gold at 30 mA for a minute, and viewed under a SEM.

2.5. Food pack experiment

Fresh beef and cottage cheese (paneer) were purchased from a local market and cut into small pieces (1 g). Each was inoculated with ten million cells of *S. aureus*, left undisturbed for 5 min, wrapped with LDPE or BI-LDPE and incubated at 4 °C for 10 days. Later each sample was unwrapped, 0.1 g of it was homogenized in 1 mL of 0.7% saline and the number of live bacterial colonies was counted. The FTIR of the biofilm formed on the wrappers were also recorded.

2.6. Statistics

All the experiments were performed on three independent samples and were repeated thrice. *t*-test was carried out with MiniTab software (MiniTab Inc, USA) and $p < 0.05$ is considered as statistically significant.

3. Results

The immobilization of betanin on LDPE was 93%. It retained 80% of its initial activity after 30 days of storage at 4 °C in an incubator.

3.1. Physico-chemical characteristics of the polymer

FTIR spectra of betanin coated non-UV treated LDPE shows (Fig. 1a) characteristic bands at 2912, 2846 and 721 cm^{-1}; and bands at 3314, 1639, 1372 and 1024 cm^{-1} indicating phenolic and alcohol groups, aromatic amines, ester linkage and alkanes present in betanin respectively. All these bands are seen after UV treatment also (Fig. 1b), but their intensities are higher than the former suggesting the immobilization of betanin.

Water contact angles of LDPE and BI-LDPE are 89.9 ± 2° & 62.3 ± 3° respectively. EDAX of LDPE indicates 100% elemental carbon, while BI-LDPE in addition, also shows nitrogen (3.4%) and oxygen (42.4%) due to their presence in betanin.

3.2. Biofilm characteristics

The MIC of betanin against *S. aureus* and *E. coli* are 5.66 ± 0.45 and 11.32 ± 0.77 μM respectively. It exhibits DPPH scavenging activity of 43.70± 0.02% (ascorbic acid shows 84.32± 0.01%).

SEM indicates a reduction in the attachment of bacteria on BI-LDPE when compared to that on LDPE (Fig. 2). LDPE and BI-LDPE has 9.9 ± 9.2 log and 9.2 ± 8.3 log (CFU/mL) of live *S. aureus* attached respectively ($p = 0.013$) and 9.5 ± 8.0 log and 9.1 ± 8.3 log (CFU/mL) of *E.coli* respectively ($p = 0.045$) (Fig. 3a).

The amount of carbohydrates in the *S. aureus* biofilm on LDPE and BI-LDPE is 18.56 ± 0.49 and 11.28 ± 1.74 μg/mL respectively ($p = 0.02$), and in *E. coli* biofilm it is 26.26 ± 1.2 and 13.49 ± 0.48 μg/mL respectively ($p = 0.003$) (Fig 3b). The corresponding proteins in the *S. aureus* biofilm is 139.66 ± 8 and 54.50 ± 4 μg/mL ($p = 0.003$), and in *E. coli* biofilm they are 243.70 ± 7 and 106.13 ± 2.9 μg/mL respectively ($p = 0.001$) (Fig 3c).

3.3. Food wrap experiments

LDPE and BI-LDPE wrapped S. aureus contaminated paneer showed a colony count of 1.61 ± 0.45 log and 1.11 ± 0.77 log (CFU/mL) respectively after 10 d of storage at 4 °C ($p = 0.018$) (Fig 3 d). The corresponding colony count when wrapped on beef was 1.14 ± 0.18 log and 0.70 ± 0.24 log (CFU/mL) respectively ($p = 0.011$). FTIR spectra of the biofilm formed on the polymers wrapped with beef shows (Fig. 1c) bands characteristic of carbohydrate (3275, 3273 and 2917 cm^{-1}) and amide I and amide II (1636 and