LWT - Food Science and Technology 47 (2012) 400–406

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

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

Controlled release of sorbic acid from bacterial cellulose based mono and multilayer antimicrobial films

Iuliana Mihaela Jipa*, Anicuta Stoica-Guzun, Marta Stroescu

University Politehnica of Bucharest, Department of Chemical Engineering, Polizu Str. 1-3, 011061 Bucharest, Romania

ARTICLE INFO

Article history:
Received 26 September 2011
Received in revised form
18 January 2012
Accepted 31 January 2012

Keywords:
Sorbic acid
Bacterial cellulose
Poly(vinyl) alcohol
Controlled release
Antimicrobial material

ABSTRACT

Biodegradable bacterial cellulose (BC) based films, incorporating sorbic acid (SA) as antimicrobial agent, have been obtained. Monolayer films, prepared using powdered BC (BCP) and poly(vinyl) alcohol (PVA), were coated with BC membrane to obtain multilayer films. Tests indicated that both SA and BCP concentration influenced sensitivity to water, release rate and antimicrobial ability of mono and multilayer films. Swelling degree, water vapour permeability and water solubility increased with SA content, but decreased with BCP addition. However in case of multilayer films, water solubility was negligible. Colour measurements indicated no degradation of SA during film preparation. The release of SA was faster when BCP concentration was higher but significantly slower, as a consequence of formed crystals dissolution, when antimicrobial concentration was increased. Furthermore, compared to the results for the monolayer films, an important decrease of SA release rate through the multilayer films was determined. The antimicrobial effect was tested against *Escherichia coli K12-MG1655*. The results obtained indicated that the new biocomposite films could be promising antimicrobial food packaging materials.

1. Introduction

Bacterial cellulose (BC) is a natural, renewable raw material synthesized as extra-cellular product by *Gluconacetobacter xylinus*, a Gram-negative acetic acid bacteria. During biosynthesis straight-chain glucose molecule polymer, linked at the β-1,4 position are aggregated into nanometre-sized fibrils (diameter between 20 and 50 nm and several 100 μm length) that are then crystallized into a microfibril (Grande et al., 2009; Huang, Chen, Lin, Hsu, & Chen, 2010). The obtained gel-like pellicles of high purity BC consisting of cellulose fibres interwoven in a mesh are formed at air–liquid interface of many substrates, including cheap raw materials (Shoda & Sugano, 2005). BC nanofibrils have high crystallinity, remarkable mechanical strength and high water holding capacity, biocompatibility and ultrafine reticulated structure (Iguchi, Yamanaka, & Budhino, 2000). Because of its distinctive properties, BC found numerous applications in cosmetics and medicine (Andrade, Costa, Domingues, Soares, & Gama, 2010; Jagannath, Raju, & Bawa, 2010; Wei, Yang, & Hong, 2011; Woehl et al., 2010). In recent years novel nanocomposites were developed using small amounts of BC and poly(vinyl) alcohol (PVA). Combining BC and PVA properties (odourless, nontoxic, chemical resistance, good mechanical properties), the obtained nanocomposites met the desired characteristics for biomedical applications (Millon, Oates, & Wan, 2009; Mohammadi, Boughner, Millon, & Wan, 2009). However, because high moisture sensitivity was considered a drawback in the use of both BC and PVA, only a few applications in the food packaging industry were reported: as active packaging to control contamination on the surface of products (Nguyen, Gidley, & Dykes, 2008; Sin, Rahman, Rahmat, & Samad, 2010; Su et al., 2010; Zhang et al., 2011) or oxygen-scavenger films using aerobic microorganisms (Altieri et al., 2004). Recently, our research group showed that PVA-BC composites could be used as biodegradable packaging materials (Stoica-Guzun et al., 2011). Due to their characteristics this materials could be proper packaging for food with high fat content and low water activity, such as desiccated or roasted seeds or spices.

Because the two most important causes of spoilage are represented by microbial growth and oxidation reaction occurring on the food surface (Guillard, Issoupov, Redl, & Gontard, 2009) the development of films carrying food additives represents a major research direction. Furthermore, functional packaging offers an effective protection not only for food but for additive as well. Thus, the degradation reactions of active substances incorporated in the packaging systems are minimized. In the same time the control of its release may be attained resulting smaller quantity of additives

* Corresponding author. Tel.: +40 021 402 3810.
E-mail address: iulia.jipa@yahoo.com (I.M. Jipa).

0023-6438/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.lwt.2012.01.039

brought into the product (Mascheroni, Guillard, Nalin, Mora, & Piergiovanni, 2010).

This paper investigates the development of antimicrobial mono and multilayered films as functional food packaging material, using BC, PVA and sorbic acid (SA) as the preserving agent. SA (2,4-hexadienoic acid) is considered a GRAS additive used in beverages, processed fruits and vegetables commonly used as model additive in release studies (Flores, Haedo, Campos, & Gerschenson, 2007; Guillard et al., 2009). Considering that antimicrobial activity depends mainly on the diffusion of the antimicrobial agent, an important part of this study is dedicated to kinetic evaluation of SA release. As presented by Berens and Hopfenberg (1978) cited by Uz and Altınkaya (2011) the overall process is described by two phenomenological independent contributions: the diffusion part, characterized by Fick's law and a structural part resulting from polymer relaxation/crystal dissolution.

The main objectives of the study were to: investigate the physical properties of films; determinate the diffusivity of SA incorporated in mono and multilayer systems, and evaluate antimicrobial efficacy of active films against *Escherichia coli K12-MG1655*.

2. Materials and methods

2.1. BC preparation

BC membranes (BCM) were obtained in a rotating biofilm contactor (2 L working volume), a horizontal bioreactor with a rotating plastic roller placed inside, partially immersed in the culture media, on which pellicles (cells and polymer) are forming during cultivation (Krystynowicz et al., 2002). A *Gluconacetobacter* xylinus culture isolated from the traditionally fermented vinegar in Microbiology Laboratory of Chemical Engineering Department of University Politehnica of Bucharest was used. Cultivation was performed on Hestrin–Schramm medium in ambient conditions (25 °C, atmospheric pressure) and 60 rpm for 10–12 days. The gel-like pellicles were purified by boiling in a 0.1 M NaOH aqueous solution for 60 min at 90 °C and then washed about four or five times with deionized water until neutral pH. Some of the purified wet BC pellicles, approximately 10 mm thick (determined with a Dial Thickness Gauge Gage Measuring Tool 0–20 mm), were used for the production of BCP in a colloidal mill as described in our earlier paper (Jipa et al., 2012). The other wet pellicles were subjected to a freeze-unfreeze cycle to diminish water content. After defrosting the wet BCM thickness was about 1 mm.

2.2. Composite films preparation

For the monolayer films PVA average molecular weight $M_w = 145{,}000\ g\ mol^{-1}$, 99% hydrolyzed (Mowiol 28–99) and SA, $M_w = 112\ g\ mol^{-1}$, purchased from Sigma–Aldrich Chemie GmbH Germany were used without further treatment or purification. A 7% (w/w) PVA aqueous solution was prepared by adding PVA flakes in water at 90 °C under vigorous stirring. BCP and SA, in different proportions, were then dispersed in cold PVA solution and vigorously stirred for 3 h at room temperature (25 °C). Films without antimicrobial agent are designated as: film 1 (F1) having a PVA/BCP 3/1 and film 2 (F2) for which PVA/BCP ratio is 3/1.5. To prepare the antimicrobial films, sorbic acid was added at different concentrations (0.1, respectively $0.5\ g\ 100\ g^{-1}$ dispersion) directly in PVA–BCP dispersion. For the antimicrobial films, after the name of the initial film is indicated A1 or A2 for low and respectively high concentration of SA. Air bubbles were eliminated using a vacuum deaeration system. Dispersion samples of 10 mL volume were then poured in plastic Petri dishes (10 cm diameter) and allowed to dry at room temperature 24 h. After drying, the films were removed from the cast plates and their thickness was measured to the nearest 0.001 mm by a micrometer (Mitutoyo Mfg Co. Ltd., Japan). The average values of measurements at five random positions were taken to calculate the properties of the film. Composition of the obtained films and blending ratio of the constituents are presented in Table 1.

Multilayer systems consist in three layer films: two external defrost BCM and an inner layer containing SA. The inner layer is one of the composites prepared before (monolayer films). To obtain the multilayer film, a previously dried film containing SA was coated on one side with a wet defrost BCM (approximately 90% humidity) and allowed to dry at 80 °C for 10 min. Then, the other side of active film was also coated with wet defrost BCM and dried for another 10 min at the same temperature. For a multilayer sample designation, the letter <m> was used after the initial film ID. For example, F1A1m corresponds to a BCM–F1A1–BCM three layer film.

2.3. Samples characterization

2.3.1. Swelling tests

In order to determine the swelling degree, membranes (triplicate) were dried to constant weight, cut into 2 cm × 2 cm square shapes, and immersed in distilled water at room temperature (25 °C) for 2 h. The mass of polymer dissolved into the distilled water was neglected considering the short time needed for the experiment. Also, the amount of SA, contained in the active films and released in aqueous media, was considered negligible compared to the amount of absorbed water. Swelling degree was obtained by measuring the initial weight, m_i and the weight of sample in swollen state, m_s using Eq. (1):

$$SD = (m_s - m_i)/m_i \quad (1)$$

The weight of swollen sample was measured after gently blotting film surface with tissue paper until the equilibrium was reached (Flores, Conte, Campos, Gerschenson, & Del Nobile, 2007).

2.3.2. Water solubility (WS)

WS was determined in triplicate according to a slightly modified method of Shen, Wu, Chen, and Zhao (2010). Samples of films (2 × 2 cm) were dried to constant weight and then immersed in 20 mL distilled water into glass beakers. The vessels were periodically shaken and kept at room temperature. After 72 h undissolved film pieces were removed from water and dried to constant weight again. The water solubility of films was calculated according to the following expression:

$$WS = \left(m_i - m_f\right)\Big/ m_i \quad (2)$$

where: m_i is the initial weight of film and m_f is the weight of dried undissolved film.

The initial weight of samples used for the determination of swelling degree and water solubility, was in the range of (0.04–0.06) g, for monolayer films and in the range of (0.05–0.08)

Table 1
Composition of monolayer PVA/BCP/SA composite films.

Sample ID	Composition	Blending ratio (w/w)
F1	PVA:BCP	3:1:0
F1A1	PVA:BCP:SA	3:1:0.04
F1A2	PVA:BCP:SA	3:1:0.2
F2	PVA:BCP	3:1.5:0
F2A1	PVA:BCP:SA	3:1.5:0.04
F2A2	PVA:BCP:SA	3:1.5:0.2