Journal of Food Engineering 136 (2014) 48–55

Contents lists available at ScienceDirect

Journal of Food Engineering

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

Preparation of a milk spoilage indicator adsorbed to a modified polypropylene film as an attempt to build a smart packaging

Jesica A. Cavallo, Miriam C. Strumia, Cesar G. Gomez *

Departamento de Química Orgánica, Facultad de Ciencias Químicas, (IMBIV-CONICET), Universidad Nacional de Córdoba, Edificio de Ciencias II, Haya de la Torre y Medina Allende, 5000 Córdoba, Argentina

ARTICLE INFO

Article history:
Received 23 December 2013
Received in revised form 25 February 2014
Accepted 22 March 2014
Available online 29 March 2014

Keywords:
Methylene blue
Colorimetric sensor
Polypropylene film
Chitosan
Polyacrylic acid

ABSTRACT

A colorimetric sensor of milk spoilage was built from adsorption of methylene blue (MB) onto a modified polypropylene film (PP). First, acrylic acid (AA) was grafted onto PP surface by photograft polymerization reaction, and then either MB or chitosan chains (Cs) was attached to polyacrylic acid (PAA). Three syntheses were researched in order to fix MB to PP-g-PAA–Cs into different levels of PAA–Cs layers. The growth of microorganisms and the generation of reducing substances take place during milk decomposition, where MB is reduced to its colourless form. Binding stability of dye on the film and its redox activity were confirmed by UV–vis spectrophotometry. Reduction kinetics of MB shows a response fast enough to work as a spoilage indicator against samples of liquid milk in different preservation states. This performance also describes these devices as suitable sensors to be used in the development of smart packagings.

1. Introduction

Over the last decades, researchers have shown a growing interest in developing different food safety measures to ensure the security and quality of food (Smigic et al., 2012). The food industry is constantly trying to enhance product safety by acquiring new technologies (Jung et al., 2012; Duncan, 2011). Microbial growth in food products results in a shelf-life reduction of food and an increase in the risk of food-borne diseases. Therefore, there is a special interest among the food industry, retailers, consumers, and their stakeholders in developing a device that is simple, low-cost, rapid, reliable, non-invasive and non-destructive to evaluate real-time freshness of food products (Kuswandi et al., 2012). An alternative concept to address this requirement is the development of smart packaging in the form of a food spoilage indicator to monitor freshness status of food products (Potyrailo et al., 2012). In the particular case of milk, historical data show that its pasteurization has contributed to public health and more recent data on occasional raw milk consumption indicate the hazard of bacterial infections, which could be avoided by heat treatment (Claeysa et al., 2013). Although milk and dairy foods represent a group characterized by a high nutritional value, most of them are highly perishable and cannot be controlled by using of preservatives due to current regulations (Valbuena et al., 2005). It is known that a natural bacterial growth takes place in pasteurized milk once its packaging is opened despite being kept in the refrigerator. Therefore, the development of a smart packaging to check its state of milk preservation is a topic that might prove a suitable tool for the quality control of safer foods (Maciel et al., 2012; Ensafi and Amini, 2010; Rastegarzadeh et al., 2009). Several colorimetric indicators implanted in the packaging have been used for food applications in recent years (Jung et al., 2012; Maciel et al., 2012; Ensafi and Amini, 2010; Zajko and Klimant, 2013). Usually these indicators are organic molecules whose structure is affected by external stimuli. In addition, MB is a thiazidic dye widely used in biomedical study and considered a leader compound in clinical areas, including therapy for malaria and schizophrenia, as well as photodynamic therapy cancer and, more recently, from microbial infection (photodynamic antimicrobial chemotherapy) (Buchholz et al., 2008; Wainwright et al., 2007). Moreover, this dye has low human toxicity and is able to exhibit efficient properties as a fotosensibilizator (Wainright, 2005). MB also shows good electrochemical properties, therefore, it has been widely used for electrochemical studies, i.e., electrocatalysis, solar cells, and biosensors (Xiao et al., 2011; Barou et al., 2012; Zhang et al., 2010). This dye also has redox activity; it acts as a hydrogen acceptor against a reducing substance in an anaerobic environment and takes the form of a leucobase. On the other hand, the kind of bacteria ordinarily found in milk consumes oxygen during its growth and multiplication. Here, many germs will quickly use up all the oxygen, while a small number will require much longer time.

* Corresponding author. Tel.: +54 351 5353867; fax: +54 351 4334170x150.
E-mail address: gom@fcq.unc.edu.ar (C.G. Gomez).

http://dx.doi.org/10.1016/j.jfoodeng.2014.03.021
0260-8774/

The addition of MB to a milk sample with a certain content of oxygen dissolved will reveal a blue colour until all the gas is consumed by bacteria; the sample will then almost immediately recover its white colour (Scheme 1) (Anderson et al., 2011). Therefore, the application of MB has become a helpful test to estimate the number of bacteria presents into liquid milk samples (Bapat et al., 2006; Lee et al., 2009). In this study a sensor device was built and its performance as a milk spoilage indicator was researched. First, MB was attached together with Cs onto the surface of polyacrylic acid-grafted PP films (PP-g-PAA) through electrostatic interaction with carboxyl groups of PAA (Cavallo et al., 2010). In order to avoid the diffusion of MB within food, PAA chains were crosslinked with Cs backbones, leading to formation of a mesh that held the molecule sensor (Scheme 2). Three synthesis techniques were compared, where the dye was deposited into different levels of PAA–Cs layers. Finally, the sensor performance was investigated from colour change of MB attached to film surface as a time function against samples of milk at different preservation states.

2. Experimental section

2.1. Reagents and equipment

Several reagents were used as purchased; chitosan (Cs), medium molecular weight and 85% of deacetilation degree, Aldrich (USA). Acetic acid p.a., Cicarelli (Argentina), methylene blue (MB) p.a., Anedra (Argentina). Instant full cream milk powder, Nestle Nido (Argentina). 20 μm thickness isotactic PP film was kindly supplied by Converflex S.A. (Argentina). Crystallinity degree (48%) was determined from the melting endotherm of the polymer measured with a Perkin–Elmer Pyris DSC calorimeter (USA) at a heating rate of 10 °C min^{-1}, assuming that the enthalpy of 100% crystalline isotactic PP was 138 J g^{-1}. Acrylic acid (AA) p.a., Merck (Germany), was purified by distillation under reduced pressure. Benzophenone (BP), p.a., Mallinckrodt (USA), was recrystallized by decreasing the temperature from a methanolic solution. Spectrophotometric measurements were recorded on a MultiSpec-1501 Shimadzu spectrophotometer (Japan). Fourier-transform infrared (FTIR) spectra of the samples were recorded on a Nicolet 5-SXC spectrometer (USA). pH determinations were carried out using a digital pH Altronix meter, TPXI 1584 (Argentina).

2.2. Photograft polymerization of AA onto PP films

The surface of the PP film was initially modified with AA using photograft polymerization at room temperature, BP as a radical initiator, and different reaction times (Scheme 3). A PP film with a surface area of 64 cm^2 was weighed and immersed into a Petri capsule with 1.00 mL of AAc/H_2O 50% by volume solution. This mixture was then irradiated under ultraviolet–visible light using a medium pressure UV lamp (Engenlhard, Hanovia – UK) and a nitrogen atmosphere at room temperature. Subsequently, the modified film was washed once with a NaOH aqueous solution at pH 8 in order to remove the PAA homopolymer and by-products. Finally, the grafted film was washed exhaustively with distilled water and dried under reduced pressure. The reaction yield is expressed as the grafting degree of PAA (G), which is calculated using Eq. (1) (Cavallo et al., 2010).

$$G/\text{wt.}\% = \left(1 - \frac{\text{PP}}{\text{PP-g-PAA}}\right) \times 100 \quad (1)$$

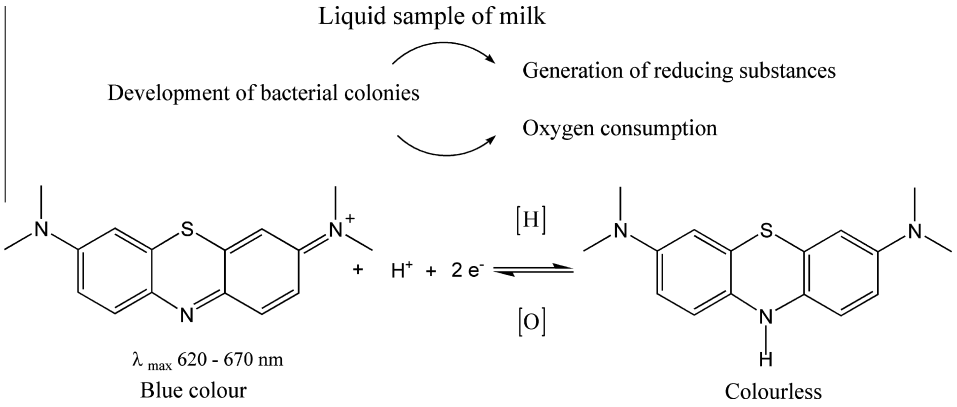
2.3. Chitosan immobilization onto PAA-grafted films

A sample of Cs was solubilized by addition of a 2 wt.% acetic acid aqueous solution under stirring at room temperature, using a 1:1 mol ratio of amino to carboxyl group (Cs/acetic acid). The final concentration of Cs aqueous solution with a pH value close to 5.0 was adjusted at 1 wt.% with distilled water. This polymer was immobilized mostly by electrostatic interaction between its ammonium groups and carboxylate groups of PP-g-PAA, when this film was left in contact with 45.0 mL of a Cs solution for 4 h at room temperature (Scheme 2). PP-g-PAA–Cs films were afterwards washed exhaustively in a beaker with distilled water under stirring at room temperature, and then dried under reduced pressure. Adsorption reaction of Cs took place on films with different G values and temperature reactions. Immobilization degree of Cs (I) was calculated according to Eq. (2) (Cavallo et al., 2010).

$$I/\text{wt.}\% = \left(1 - \frac{\text{PP-g-PAA}}{\text{PP-g-PAA} - \text{Cs}}\right) \times 100 \quad (2)$$

2.4. Adsorption of methylene blue to modified films

The dye was deposited on PP films with different G values using three synthesis techniques (Scheme 2). Technique A: the dye was mostly adsorbed to PP-g-PAA through electrostatic interaction between carboxylate groups of PAA and MB with a positive charge in its molecular structure. Here, the PP-g-PAA film (near 0.030 g, 16 cm^2) was left in contact with 20.0 mL of a 2.10^{-5} M of MB aqueous solution at pH 5.0 for 4 h at room temperature. The film was then exhaustively washed with distilled water in order to eliminate the dye unattached. Finally, Cs backbones were fixed to the PP-g-PAA–MB film using the procedure detailed in Section 2.3. Technique B: the MB molecule was mostly adsorbed to the more external carboxylate groups of PP-g-PAA–Cs, a 16-cm^2 film, where



Scheme 1. Oxidized and reduced MB species present in redox reactions.