Industrial Crops and Products 52 (2014) 664–670

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Biodegradable active packaging based on cassava bagasse, polyvinyl alcohol and essential oils

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ARTICLE INFO

Article history:
Received 23 July 2013
Received in revised form 16 October 2013
Accepted 20 November 2013

Keywords:
Oregano essential oil
Clove essential oil
Trays
Baking

ABSTRACT

The objectives of this work were to develop biodegradable trays from cassava bagasse and polyvinyl alcohol (PVA) incorporated with clove (CEO) or oregano (OEO) essential oils, to study their antimicrobial activity and to investigate the effects of incorporating these essential oils on the mechanical properties, water absorption capacity (WAC) and sorption isotherms of the tray with the best antimicrobial activity. The trays were produced by baking 97.5% (w/w) cassava bagasse with 2.5% (w/w) PVA. CEO or OEO was added to the trays using two methods: direct incorporation (6.5 to 10.0%) and surface coating (2.5 to 7.5%). Trays with OEO prepared by surface coating showed the highest antimicrobial activity, as they were effective against molds, yeasts, and Gram-positive and Gram-negative bacteria. The addition of OEO to the cassava bagasse-PVA matrix resulted in less resistant and more flexible trays, with a decrease in the water absorption and adsorption capacities.

1. Introduction

Petroleum-based conventional packaging is used in a wide variety of applications because of its high specific strength and durability, ease of processing and low cost. However, such packaging takes hundreds of years to decompose, causing serious environmental problems. Starch foams show promising characteristics for technological applications, but they suffer from several problems, including poor mechanical properties when stored under conditions of high relative humidity (Mali et al., 2010; Schmidt and Laurindo, 2010).

The development of biodegradable packaging based on starch and polyvinyl alcohol (PVA) has attracted an increasing amount of attention. The addition of PVA in the production of starch-based materials to improve moisture sensitivity and mechanical properties has been reported (Chiellini et al., 2009; Debiagi et al., 2011; Mali et al., 2010) to demonstrate good results.

Aiming to expand the applications of biodegradable packaging, antimicrobial agents can be incorporated directly into the packaging. Currently, there is high demand for natural compounds because consumers are more concerned about their health and the risks associated with the consumption of synthetic components, effectively leading to the emergence of active packaging (Campos et al., 2011; Kechichian et al., 2010).

Essential oils (EO) from plant extracts are natural antimicrobial agents. They are classified as generally recognized as safe (GRAS) compounds, and they are extensively used as flavoring agents in baked goods, sweets, ice cream, beverages and chewing gum (Rojas-Graü et al., 2006). The antimicrobial effect of EO is associated with the terpenoid and phenolic components present in their structure, such as carvacrol, eugenol and thymol (Burt, 2004), which have boiling points above 200 °C (Norwitz et al., 1986) and can be thermally processed without losing their antimicrobial activity as demonstrated by Pelissari et al. (2009).

Edible films combined with oregano (OEO) or clove (CEO) essential oils have exhibited a wide spectrum of antimicrobial activity against molds, yeasts and bacteria in numerous investigations (Benavides et al., 2012; Gómez-Estaca et al., 2010; Moreira et al., 2005; Pelissari et al., 2009; Sánchez-González et al., 2010). According to Pelissari et al. (2009), in addition to antimicrobial activity, the presence of OEO in films led to more flexible films and did not affect their thermal stability. Currently, there are no works concerning the use of biodegradable foam trays combined with essential oils as food packaging.

Biodegradable trays incorporated with EO are very attractive because of their ability to extend the shelf life of food products and prevent the occurrence of food-related diseases caused by pathogenic microorganisms. Thus, the objectives of this work were to develop biodegradable active trays based on cassava bagasse

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http://dx.doi.org/10.1016/j.indcrop.2013.11.032

and PVA incorporated with OEO or CEO, to study the antimicrobial activity of these trays, and also to investigate the effects of EO on the mechanical properties, water absorption capacity and sorption isotherms of the trays.

2. Material and methods

2.1. Materials

Cassava bagasse (CB) containing 14 wt% of fibers and 86 wt% of starch was obtained from cassava roots as the liquid residue after laboratorial starch extraction (Alves et al., 1999). The liquid residue was dried (50 °C/24 h) and milled to yield particles <0.35 mm.

Analytically pure glycerol, magnesium stearate and guar gum were purchased from Synth (Labsynth, Brazil). PVA (ACS grade, molecular weight of 72.000 and degree of hydrolysis of 86.5–89.5%) was purchased from Reagen (Quimibrás, Rio de Janeiro, Brazil). Oregano (*Origanum vulgare*) and clove (*Eugenia caryophyllata*) essential oils were purchased from Ferquima (Moldavia and Indonesia, respectively).

2.2. Microbial cultures

The following food-borne microbial strains were selected for use in the assays because of their relevance in the food industry: the Gram-positive bacteria *Staphylococcus aureus* ATCC 6538, Methicillin-resistant *S. aureus* (MRSA) N315, *Bacillus cereus, Streptococcus mutans* ATCC 25175 and *Enterococcus faecalis* ATCC 6569; the Gram-negative bacteria *Escherichia coli* ATCC 8739 and *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S.* Typhimurium) ATCC 14028; the yeast *Candida albicans* ATCC 90028; and the molds *Aspergillus niger* and *Penicillium citrinum* were obtained from the culture collection of the Laboratory of Basic and Applied Bacteriology and Laboratory of Medical Mycology, Department of Microbiology of the State University of Londrina.

2.3. Methods

2.3.1. Tray production by baking

To prepare the trays, cassava bagasse (97.5 g) and PVA (2.5 g) were mixed with water (210 mL) and additives (1 g of magnesium stearate and 1 g of guar gum for 10 min with a mechanical stirrer rotating at 18,000 rpm (Vithory-Brazil). The magnesium stearate was added to prevent the starch foam sticking to the mold and the guar gum was added to prevent solid separation (Salgado et al., 2008). Glycerol (10 g) was then added, and after stirring for an additional 10 min, 40 g of this formulation was homogeneously layered on a 100-mm long, 100-mm wide and 20-mm deep Teflon mold with a 1.0-mm thick metallic guide. A Teflon lid was placed over the mixture, and baking process was conducted using a hydraulic press (JOMAQ, São Paulo, Brazil) equipped with an electric heating system, a Pt100 temperature sensor and a proportional-integral-derivative (PID) controller. One pressing step was performed at 150 °C for 7 min and 100 bar. This tray formulation was chosen for the incorporation of EO because it had the best combination of mechanical and water barrier properties based on previous study aiming to develop biodegradable trays based on cassava bagasse and PVA (data not published).

2.3.2. Incorporation of essential oils on the trays

The antimicrobial essential oils (OEO and CEO) were added using two different methods: (1) direct incorporation (DI) method, in which the EO was previously mixed with cassava bagasse, PVA and additives during tray production at concentrations of 0, 6.5, 8.5 and 10.0% (w/w); (2) surface coating (SC) method, in which the EO was applied on all tray surfaces using a brush at concentrations of 0, 2.5, 5.0 and 7.5% (g oil/100 g tray), and then the trays were dried at room temperature for 24 h. Limited concentrations of OEC and CEO were chosen on the basis of previous results (data not published). All the formulations are described in Table 1. The produced trays were stored for 7 days at 25 ± 0.5 °C under 58% relative humidity (RH) before characterization, and the equilibrium moisture content of these samples after conditioning was 7.0 ± 0.35%.

2.3.3. Tray characterization

2.3.3.1. Antimicrobial activity. The disk inhibition zone assay was used to evaluate the antimicrobial activity of the trays according to Pelissari et al. (2009), with modification. The trays produced with and without (control) OEO and CEO were aseptically cut into 10 mm discs and placed on plates containing Mueller-Hinton (MHA) agar (Himedia, India), which had been previously spread with 0.1 mL of inoculums, each containing 1.5×10^8 CFU mL^{-1} of bacterial cultures, standardized using the McFarland scale. The plates were incubated at 37 ± 0.5 °C for 18–24 h. Sabouraud agar (Neogen, USA) was employed to investigate the antimicrobial activity of the trays against *C. albicans* culture, and the plates were incubated at 27 ± 0.5 °C for 18–24 h. For the molds (*A. niger* and *P. citrinum*), a spore suspension stock of each mold was diluted in a sterile solution to obtain 10^6 spores mL^{-1}, which were enumerated by direct counting using a Neubauer chamber. Thereafter, they were placed on plates with Sabouraud agar (Neogen, USA) and incubated at 27 ± 0.5 °C for 5 days. The diameter of the growth inhibition zones around the discs was measured using a metric ruler. The tests were carried out in triplicate for each formulation.

2.3.3.2. Stability of the antimicrobial activity of the trays. The stability of the antimicrobial activity of the trays prepared using the SC method with the addition of 5.0% (w/w) of oregano EO (OEO–SC5.0 formulation) was tested against the Gram-positive bacteria *S. aureus* ATCC 6538 and the Gram-negative bacteria *E. coli* ATCC 8739. The stability was determined every 3 days, over the course of 15 days, following the procedure described above (Section 2.3.3.1). The tests were carried out in triplicate.

2.3.3.3. Mechanical properties. A texture analyzer, Brookfield model CT3 (USA), with a 25-N load cell was used to determine the mechanical properties of the tray samples by means of tensile testing, according to a modified version of the Vercelheze et al. (2012) procedure. Tensile tests were performed using strips measuring 80 mm by 25 mm, with an initial grip separation of 80 mm and a crosshead speed of 2 mm s^{-1}. The stress–strain curves were recorded during extension, and the stress and strain at break were determined. Each formulation was assayed six times, and the reported values are the averages of six assays.

2.3.3.4. Water absorption capacity (WAC). The water absorption analysis was performed according to the Cobb method, ABNTNBR NM ISO 535 (1999). First, samples measuring 25 mm by 50 mm were weighed and soaked in 100 mL of distilled water for 1, 5, 10, 15, 20, 25 and 30 min. After removing the excess water using tissue paper, the samples were weighed again. The quantity of adsorbed water was calculated as the weight difference and expressed as the mass of absorbed water per mass of the original sample (ABNT, 1999). The reported values are the means of five measurements for each formulation.

2.3.3.5. Water sorption isotherms. Starch foam specimens measuring 25 mm × 25 mm were pre-dried for 14 days over anhydrous calcium chloride after which they were placed over saturated salt solutions at 25 °C in separate desiccators with the desired level of relative humidity (0, 11, 33, 45, 58, 75 and 90%) (Rockland, 1960) for 7 days. The equilibrium moisture was determined