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Properties of lignin extracted from sugarcane bagasse and its efficacy in maintaining postharvest quality of limes during storage

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ABSTRACT

Lignin extracts from sugarcane bagasse by alkaline extraction in the presence and absence of ethanol were investigated for their efficacy on maintaining fresh fruit shelf life. For lignin extraction, the highest lignin yield was obtained from 40% (w/w) NaOH extraction in water. Addition of ethanol gave relatively lower yield of lignin but less hemicellulose contamination. FT-IR intensity of lignin-to-carbohydrate transmittance ratios of extracted lignin from 40% (w/w) NaOH in distilled water were similar to the ratios of lignin standard from Kraft process. From gel permeation chromatography (GPC), lignin isolated from 40% (w/w) NaOH in distilled water yielded high amount of both large and small molecules of lignin. The weight loss ratio between lignin and hemicellulose was analyzed by thermogravimetric analysis (TGA). It was confirmed that isolated lignin from 40% (w/w) NaOH in distilled water contained highest amount of hemicellulose in lignin extract. The test of coating formulas on limes demonstrated that coating solutions of 0.8% (w/w) xanthan gum and 1.5% (w/w) extracted lignin from 40% (w/w) NaOH had potential to maintain weight loss and color change. This coating formula also exhibited higher antifungal activities. Limes coated with extracted lignin showed higher antifungal activity than limes coated with commercial lignin.

1. Introduction

Lignocellulose materials (i.e. sugarcane bagasse) are composed of cellulose, hemicellulose and lignin. Sugarcane bagasse is second abundant agro-industrial residue in Thailand which contains 25–28% lignin, 61–63% carbohydrate, 5–6% extractives and 6–7% ash (Sakdaronnarong, Onsrithong, Suwankrua, & Jonglertjunya, 2012). Among all constituents in sugarcane bagasse, lignin is more resistant to most forms of biological attack than cellulose and hemicellulose. In vitro study on antimicrobial and antifungal activity of lignin extracts was recently reported (Doherty, Mousavioun, & Fellows, 2011). Thus, lignin extract is natural and potent substances to use as coating agent for fresh fruit preservation after post-harvesting.

Lime [*Citrus aurantifolia* (Christm.) Swingle] is typical fruit originated in South East Asia (Win, Srilaong, Heyes, Kyu, & Kanlayanarat, 2006). According to its special taste and odor particular for Asian cuisine, lime is widely consumed and exported to exotic markets worldwide. It is categorized into the genus citrus which additionally includes oranges, mandarins, lemons and grape fruits. Lime is more aromatic in flavor, greenish-yellow smooth skin, and juice is highly acidic. It is used for culinary and non-culinary purposes, primarily for its juice. Its flavor is a key ingredient in drinks and foods. Color is an important criterion of quality and consumer acceptability. The green color of lime is due to the presence of chlorophylls in mature green fruit. Its flavor is excellent especially when used in the green stage (Win et al., 2006). In order to get premium prices of limes, the maintenance of green color in peel of lime throughout the postharvest supply chain is required.

Fruit ripening is characterized by a number of biochemical and physiological processes involving chemicals/enzymes such as glycosidases, glycanases and beta-D-galactosidase (Baloch, Bibi, & Jilani, 2011; Toivonen & Brummell, 2008). The chlorophyll degradative process is a consequence of alterations in the physiological and biochemical processes occurring in the flavedo tissue of the lime and has resulted in yellow color in peel of lime (Win et al., 2006). Fruit ripening continues after harvest and is relatively rapid at ambient temperature during marketing. Moreover, significant fruit losses after harvest resulted from decay caused by

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microorganisms (Plooy, Regnier, & Combrinck, 2009). Due to the low pH values of most fruits, the typical microorganisms growing on them were molds and yeasts (Oms-Oliu et al., 2010). The decay of most citrus fruit was from green and blue molds caused by *Penicillium digitatum*, *Penicillium italicum*, *Botrytis cinerea* and *Alternaria* sp. (Youssef, Ligorio, Nigro, & Ippolito, 2012). Rapid ripening processes and infection caused by microorganisms limit the shelf-life of fresh fruit. In order to extend the marketing distances and holding periods for fresh horticulture goods after harvest, fresh fruits have been coated with different edible/non-edible materials such as wax (Youssef et al., 2012), calcium alginate (Costa, Conte, Buonocore, Lavorgna, & Del Nobile, 2012), glycerol and essential oils (Rojas-Graü et al., 2007) and gum arabic (Ali, Maqbool, Ramachandran, & Alderson, 2010). Youssef et al., 2012 studied wax coating on three citrus cultivars, '*Comune*' clementines, '*Tarocco*', and '*Valencia late*' oranges. Treatment with wax alone increased decay incidence compared to the water treated control. However, decay incidence was significantly lower when fruit treated with wax combined with different salts. Moreover, the fresh-cut carrots were dipped into 4% sodium alginate solution containing silver-montmorillonite nanoparticles and then dipped for another minute into a 5% $CaCl_2$ solution to promote the alginate gel-forming process (Costa et al., 2012). Rojas-Graü et al., 2007 studied the effect of glycerol, lemongrass, oregano oil and vanillin incorporated in apple puree-alginate coating. Coatings with calcium chloride and N-acetylcysteine could enhance firmness and color. Lemongrass (1% w/w) and oregano oil (0.5% w/w) had antimicrobial activity against *Listeria innocua*. Ali et al., 2010 studied gum arabic coating on tomato and found that 10% (w/v) gum arabic could delay in changes of some parameters such as weight, firmness, decay percentage and color development. In summary, coatings can prevent quality changes in foods by acting as barriers to control moisture transfer, oxygen uptake, lipid oxidation and loss of volatile flavors and aromas, and therefore, the benefits of applying coatings to fresh fruits and vegetables include controlled ripening and browning, and delayed color, flavor, moisture and firmness loss (Toğrul & Arslan, 2004).

In the present study, the lignin was extracted from sugarcane bagasse, abundant waste from sugar industry in Thailand, by different alkaline and alkaline/organosolv methods. Apart from antimicrobial properties of lignin extracts, dissimilar ratios of lignin and hemicellulose in lignin extracts from different extraction conditions was believed to influence the shelf life of coated fruits. A proper mixture of lignin which is hydrophobic substance and hemicellulose which is hydrophilic substance was proposed to influence moisture change and weight loss of coated fruits. Moreover, lignin molecular weights were first tested for their efficacy on coating limes. Changes in fruit color, weight loss and antimicrobial activity that enhanced the shelf life of fresh fruits were monitored.

2. Materials and methods

2.1. Materials

Limes used in the experiment were purchased from Salaya market, Phuttamonthon, Thailand on the same day after harvest. They were selected for their uniformity, size, color and shape, and for being free of damage and fungal infection. Before coating was applied, fruits were washed with diluted "St Andrews" washing solution, which consisted of 0.0048% (w/w) sodium lauryl ether sulfate and 0.0012% (w/w) alkylpolygylcoside for 1 min and air-dried at ambient temperature.

In this study, the antifungal activity of commercial lignin and lignin extracts on the growth of *Alternaria alternate* was investigated. Commercial lignin was purchased from Sigma–Aldrich (St. Louis, MI, USA). Sugarcane bagasse supplied from a sugar factory in Nakorn Ratchasima province, Thailand. Lignin was extracted from sugarcane bagasse by alkaline/organosolv extraction at moderate temperature.

A. alternate (TISTR 3435) was grown in Sabouraud Dextrose Agar (SDA) with composition of 40 g/L dextrose, 10 g/L Neopeptone, and 15 g/L agar.

2.2. Lignin extraction and separation

Sugarcane bagasse (*Saccharum* sp.) was dried at 60 °C for 3 h, cut to 5-cm pieces and finally milled by centrifugal mill. Milled sugarcane bagasse was sieved by sieve shaker to mesh −12/+16 (1.70 mm average diameter) and dried at 60 °C for 2 h to a constant weight. 20 g sugarcane bagasse was subjected to hydrothermal pretreatment in 200 mL solution (10% consistency) containing 40% (w/w) NaOH and the solutions used in this study were distilled water and 50% (v/v) ethanol. The extraction was performed in a 500-mL Erlenmeyer flask. The reaction was carried out in a water bath at 90 °C for 4 h and stirred every 15 min. After pretreatment, suspension was cooled down to room temperature. Liquid and solid parts were separated by using vacuum pump and separation funnel equipped with filter paper (Whatman no. 1). Liquid part so called black liquor containing mainly lignin was kept in refrigerator for analysis of lignin and sugar released.

For lignin separation, the volume of black liquor from each pretreatment was recorded and black liquor was concentrated using rotary evaporator equipped with vacuum pump. For ethanol removal, the pressure at 125 mbar and temperature at 50 °C was applied at 150 rpm of rotation speed. The pH of concentrated solution was adjusted to 1.0 using concentrated sulfuric acid and the precipitated solid lignin was separated by centrifugation at 3000 rpm for 15 min. Liquid phase was kept in refrigerator for analysis of sugars released. Solid lignin was washed with 0.1 M HCl until pH was equal to 2.0. Solid lignin was finally separated by centrifugation and dried at 50 °C for 2 h in a vacuum oven. The dry weight of solid lignin and lignin yield were determined.

2.3. Lignin characterization

Characterization of extracted lignin was performed by Fourier-transform infrared (FT-IR) spectroscopy, enzymatic assay for hemicellulose contamination, Thermogravimetric analysis (TGA) and molecular weight distribution using gel permeation chromatography (GPC). Functional groups of lignin (lignin:KBr ratio of 1:99 by weight) were analyzed by FT-IR spectroscopy (Spectrum 2000, PERKIN ELMER, USA) at 4000–400 cm^{-1} wave number at 4 cm^{-1} resolution and 128 numbers of scan. The FT-IR spectra were averaged from triplicate measurement. Hemicellulose contamination was determined by hemicellulase hydrolysis (2 mL of black liquor: 10 mL of sodium acetate buffer pH 4.8: 3 mL of Accellerase 1500 enzyme) at 50 °C for 48 h. The reducing sugar released was measured by DNS assay (Miller, 1959) reported as glucose equivalence. Gel permeation chromatography for the lignin molecular weight distribution determination was performed in LiCl/DMF system (Sjöholm, Gustafsson, & Colmsjö, 1999). The black liquor (2 μL) was subjected to GPC on a 1.5 × 100 cm Sephadex LH 20 column in DMF containing 0.8% (w/v) LiCl in order to exclude the hydrophobic ability of lignin molecules. Fractions (1.5 mL) were collected and the lignin quantity was assayed by UV–VIs spectroscopy at 280 nm in terms of absorbance. Thermogravimetric analyzer (Pyris Diamond TG/DTA, PerkinElmer, USA) was used to determine thermal stability of lignin samples with a heating rate of 10 °C/min in nitrogen atmosphere and the temperature ranging between 30 and 500 °C.