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Physical and antimicrobial properties of banana flour/chitosan biodegradable and self sealing films used for preserving Fresh-cut vegetables

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ABSTRACT

Kluai Namwa is a local species of banana grown in Thailand. Flour made from this banana was mixed with chitosan, a by product of the shrimp industry, for casting films as it has valuable characteristics including antimicrobial effects. Utilization of excess banana available in high season and chitosan can from the waste crustacean shells could help reduce waste while making available a value added product. Banana/chitosan films were produced using 0.5–2 g banana flour and 0.5 g chitosan in 100 ml aqueous solution. Film water vapor permeability, tensile properties, solubility and morphology were investigated. The composite yellowish film exhibited great water permeability of 38.81–41.66 g mm/m^2 day kPa. Tensile strength and elongation were in the range of 5.19–14.22 MPa and 1.64–2.59%, respectively while the solubility obtained was 40.90–64.21%. The presence of starch in the composite film makes possible water soluble and sealable bags or wraps, while the presence of chitosan gives them the antimicrobial property. The composite bags were found to protect asparagus, baby corn and Chinese cabbage against *Staphylococcus aureus* activity by serving as a good barrier and as a antimicrobial agent. With these properties the edible bag with its contents can be processed together during food preparation making its use very convenient.

1. Introduction

The world's annual consumption of plastic materials has increased continuously and is presently nearly 100 million tons today. An estimated 56% of all plastics waste is used for packaging (Wasteonline, 2007). The disposal of plastic bags is difficult and hence has a very significant environmental impact. They create visual pollution problems and can have harmful effects on animals because they are non-degradable and take a long time to break down. One option that is being considered to solve this problem is the use of biodegradable plastics.

Banana is an important food crop that is grown widely in many tropical countries including Thailand. They are grown in around 106,947 ha in Thailand (DAE, 2003) and about 160.4 Mtons are exported (DAE, 2005). The Kluai Namwa variety is grown in the largest quantities and account for 77% of total banana produced in Thailand (DAE, 2003). However this variety is not exported so much, because it has considerable domestic consumption. Banana flour can be produced from unripe banana at a low cost thus reducing loss due to spoilage in the glut season. Banana flour has 73.4% starch content (Dangaran, Renner-Nantz, & Krochta, 2006) and might be an alternative form of starch flours for novel applications.

Chitosan is a natural biopolymer derived from chitin that is found in crustacean shells, fungi cell walls and other biological materials. Chitosan can be extracted from the wastes of the frozen sea food industry as a by-product. There is considerable potential to use this by-product in many applications. Chitosan is a polymer composed of β–(1,4)-2-deoxy-2-amino-D-glucopyranose units (Kim, No, Kim, & Prinyawiwatkul, 2006). Chitosan has good flow and film forming characteristics and is nontoxic, biodegradable, has bio-functional property, is biocompatible and has important antimicrobial properties (Pranoto, Rakshit, & Salokhe, 2005). The well-known applications of chitosan include water treatment, dietary foods, seed coatings, encapsulation of food ingredients, immobilization of enzymes and film forming (Lazaridou & Biliaderis, 2002). Due to its good film forming properties no plasticizers are required (Nadarajah, Prinyawiwatkul, No, Sathivel, & Xu, 2006). It can be applied in many products to prolong the shelf life of food and agriculture products due to its antimicrobial activity. (Aydinli & Tutas, 1999; Dutta, Tripathi, Mehrotra, & Dutta, 2009; Pranoto et al., 2005; Zivanovic, Chi, & Drughon., 2005). The aim of this study was

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to make use of the sealing properties of banana starch and the antimicrobial characteristics of chitosan to produce an edible film with innovative characteristics.

2. Materials and methods

2.1. Materials

Commercial chitosan powder with degree of deacetylation of 85% and 65 kDa average molecular weight was purchased from NNC Product Ltd. (Bangkok, Thailand). Glycerol (analytical grade) was provided by U&V Holding Co., Ltd. (Nontaburi, Thailand). Banana flour was produced from green peel banana c.v. Kluai Namwa. Fresh mature green bananas were removed from their bunches, washed, peeled and sliced at the average thickness of 1 cm. The banana pieces were then soaked in a solution of browning inhibitor consisting of citric acid (1 g/100 ml) (FFTC, 2005) for 30 min. The liquid was then drained and the banana oven drying at 55 °C (Leite, Mancini, & Borges, 2007) for 8 h. The dried chips were then milled using a hammer mill and ground to 80 mesh size. The banana flour produced in this way had a moisture content of 6.6–7.7% on a wet basis, 549.94 RVU peak viscosity and 37.63% amylose content. This sample of flour was used throughout the study.

2.2. Film preparation

Banana flour solution was prepared by dissolving banana flour (1, 2, 3 and 4% w/w) in water. The banana flour solution was heated and stirred on a hot plate to 85 °C for 20 min. A chitosan solution was prepared by dispersing 1 g chitosan powder in 100 ml aqueous solution of glacial acetic acid while stirring on hot plate at 50 °C until it dissolved completely. The solution was then filtrated through filter paper (Whatman No. 4) to remove remaining small particles from solution. The banana flour and chitosan solutions were then mixed to achieve concentrations of banana to chitosan ratios of 0.5:0.5, 1:0.5, 1.5:0.5 and 2:0.5. Glycerol (0.5 ml) was then added and the banana/chitosan solution stirred. 100 ml of the final solution was poured into 15 cm diameter superlene plates made of polyamine Nylon-6 (Paisan Superlene Co, Ltd., Bangkok, Thailand). The solutions in the plates were dried at 40 °C for 24 h and the film samples produced were peeled at ambient temperature and kept in plastic bags held in desiccators before being examined further.

2.3. Film testing

2.3.1. Thickness

The thickness of the films was measured with an accuracy of 0.01 mm using a Dial Thickness Gauge (7301 Mitutoyo, Mitutoyo Ltd., Japan) at three random positions of the film sample. The average values for each sample were then used in calculation of water vapor permeability and other mechanical properties.

2.3.2. Water vapor permeability

Water vapor permeability (WVP) tests were performed using the gravimetric modified cup method based on ASTM E96 (2000) and using a specially designed permeation cell which where maintained at 24 °C. As recommended by the method, the test cups were filled with 30 ml distilled water to achieve 100% relative humidity on one side of the film. Film ($\varnothing = 6$ cm) was placed on the cup rim and was sealed with sealant ring. The distance between film and water surface was 1.8 cm. The samples cup were weighed and kept in a chamber with controlled air velocity of 0.01 m/s. Silica gels were used to control the relative humidity on the other side of film. The temperature and relative humidity were maintained at 24 °C and 65%, respectively. The sample cups were weighted every 1 h up to 8 h. The outside atmosphere had a stable RH 75% RH. As the test dish had to be taken out of the desiccator every hour for weighing the test dish, the relative humidity inside the desiccator increased and was found to be in the range of 65–75% throughout the test period. An accurate weight loss (g) versus time (h) was plotted to obtain a straight line and its slope measured. The WVP was calculated by the following equation:

$$WVP = \frac{slope \times x}{A \times \Delta p} \quad (1)$$

where *slope* is the gradient of the plot of weight loss versus time (g/day), A is the film surface area (m^2), x is the film thickness (mm), and Δp is the vapor pressure difference between inside and outside the cell (kPa).

2.3.3. Tensile strength and elongation at break

Tensile strength (TS) and elongation (E) of the films were determined using a Texture analyzer (Stable Micro Systems Ltd., UK) using the standard testing method ASTM D882 (1997). Films were cut into 1 × 15 cm^2 pieces and initial gauge separation and crosshead speed was fixed at 50 mm and 50 mm/min, respectively. Tensile strength was calculated from the maximum force by dividing it by the area of cross section and the elongation at break was calculated from the ratio of increase in length, expressed in percentage.

2.3.4. Solubility

Solubility tests were performed in boiling water. Films were cut into 1.5 × 1.5 cm pieces and were weighted (A). 50 ml distilled water was heated to 100 °C on a hot plate then accurately weight films were soaked for 4 min in it (Perez-Gago & Krochta, 2001). The samples were filtrated to keep the residual films that were dried in the hot air oven at 70 °C for 24 h to constant weight. The final films were then weighted again (B). The percentage solubility ($\%S$) tests were calculated from 3 replicated tests. The percentage solubility in each case was calculated as follows:

$$\%S = \frac{(A - B) \times 100}{A} \quad (2)$$

2.3.5. Surface morphology

Morphology of film surface was analyzed using scanning electron microscope (SEM, model 6400, JEOL, Germany) at the Suranaree University of Technology, Thailand. 5 × 1 mm^2 of film pieces were mounted on bronze stubs using tape and were coated with gold allowing surface visualization. All samples were observed using an accelerating voltage of 10 kV.

2.3.6. Sealability test

Sealability was tested using a hand-press standard plastic heat sealer. Bags that were 10 cm long and 5 cm wide were produced for application.

2.3.7. Microbial counts of Fresh-cut vegetables in sealable bags

Vegetables including asparagus (*Asparagus officinalis*), baby corn (*Zea mays*), oyster mushroom (*Pleurotus ostreatus*) and Chinese cabbage (*Brassica pekinensis*) were purchased from the local supermarket. The vegetable samples were cut with a sharp knife into pieces 5 cm in length and then sealed in the 5 × 10 cm^2 banana flour/chitosan composite film bags. Each sample was placed on an aluminum tray and stored in a refrigerator at 10 °C for 1–3 days. The *Staphylococcus aureus* content in the vegetables was determined by the dilution method (BAM, 2001).