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Chitosan Incorporated with Stingless Bee Propolis for Active Food Packaging Application

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Abstract: Food packaging is required in order to protect the food from contamination. The current globalizations on consumers demand towards food safety products have led to the innovation of food packaging technology, in which less synthetic chemical to be used. The use of natural substances such as chitosan has been greatly studied and incorporation of natural antimicrobial agent such as propolis helps to improve the functionality of the food packaging. In this study, chitosan films containing 0, 2.5, 5, and 10 % propolis extract concentration were developed. Changes in the Fourier transform infrared spectra of the films were observed when propolis extract was incorporated, showing some interactions occurred between chitosan and propolis polyphenols. The addition of the propolis has also resulted in reduction (~24 %) in contact angle measurement of the films. The antimicrobial results showed chitosan films containing propolis extract exhibited significant inhibition ring against *Escherichia coli* and *Bacillus cereus*.

Keywords: Food packaging, Antimicrobial, Chitosan, Propolis

1. Introduction

Chitosan is a product of different degrees of deacetylation from chitin, commercially obtained from crustacean shells [1] and used in food packaging manufacturing. Over the past few years, chitosan has gained its popularity due to its biological characteristics (biodegradability and low toxicity) and wide range of application in industries including food packaging [2].

Food packaging is designed for several purposes. Other than to contain and protect food, it is also functioned to keep the food safe and secure, to retain their quality and freshness and also to prolong the shelf life [3]. Due to increasing concerns of food quality and safety, new packaging innovation has been developed to perform some functions in food preservation, known as active packaging [4]. Other than that, the environmental concern on the non-biodegradability of existing food packaging materials has led to the use of alternative materials such as biopolymers to replace petrochemical-based plastics.

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The incorporation of antimicrobial agent into packaging film has been widely applied since microorganisms are prone to grow and deteriorate the food product. Propolis is one of the well-known natural antimicrobial agents. Propolis is a resinous substance collected from plants by bees and rich in numbers of chemicals such as polyphenols [5]. The polyphenol content of the propolis exhibit the antimicrobial effect and there are numerous studies that have been carried out for that function on various applications [6].

Active food packaging refers to the incorporation of some additives to packaging system either being added loosely, attached to or incorporated within the packaging materials with the aim to maintain or extend the quality and shelf-life of the product [7]. The packaging can be categorized as active when it performs the desired role in maintaining the quality of food in addition to supplying an inert barrier from external condition. Furthermore, active packaging is also defined as a change in the circumstances of packed food to extend shelf-life or improve the safety and sensory properties as it maintains the product quality. One of the innovations in active packaging is the potential release of antimicrobial agent from packaging materials. Antimicrobial agents that are included in the packaging material can help to prolong shelf life by preventing bacterial growth and food spoilage. In this system, antimicrobial agent will be released when needed and the system will only be activated in exceptional circumstances. Common examples of antimicrobial release in food packaging involve polysaccharides particulate that traps the antimicrobial compound. Bacteria will digest polysaccharides when they grow, and if contamination occurs, the packaging will release the antimicrobial compound and inhibit further growth of the microorganisms [8].

Chitosan, the deacetylated product of chitin, is soluble in dilute acids such as acetic acid and formic acid [9]. Chitosan is biodegradable and non-toxic and has some interesting biological activities, including excellent strength and elongation properties. Meanwhile, propolis is a complex resinous material collected by bees from plant exudates, beeswax, and bee secretions. Due to its waxy nature and mechanical properties, bees use propolis in the construction and repair of their hives for sealing openings and cracks and smoothing out the internal walls [10]. Propolis is a lipophilic in nature, hard and brittle material. It becomes soft, pliable, gummy, and very sticky when heated [11]. Propolis is not suitable to be used directly due to its complex chemical constituents [12]. Propolis is extracted commercially with suitable solvent. The most common solvents used for extraction are water, methanol, ethanol, chloroform, dichloromethane, ether, and acetone. The antibacterial properties of propolis was evidenced against Gram-positive bacteria and showed lower activity against the Gram-negative ones at small quantity or is inactive at all [13]. Thus, current study is conducted by applying propolis as antimicrobial agent in chitosan-based active food packaging. Secondly, the study is conducted to identify the antimicrobial effect of propolis at different concentrations (0%, 2.5%, 5% and 10%) towards *Escherichia coli* and *Bacillus cereus*.

2. Materials and Methods

2.1 Propolis Extraction

Propolis collected from beehive of stingless bee was extracted by adopting Siripatrawan et.al.'s techniques [14]. 100 grams of ground propolis were extracted using 100 mL of 30% ethanol aqueous solution. The solution was extracted at 50˚C in a water bath shaking incubator at a speed of 200 oscillation / min for 24 hours and then filtered through Whatman filter paper No. 1. The extract solution was concentrated using a rotary evaporator under reduced pressure at 45˚C [14].

2.2 Film Preparation

Low molecular weight chitosan powder was used to prepare chitosan-based films. The chitosan-based films were prepared according to the procedure with slight modifications [15]. A film-forming

solution was prepared by dissolving 2 grams of chitosan powder in 100 ml of 1 % acetic acid solution. Glycerol was added to the solution at 30 % w/w of chitosan. The solution was heated at 60˚C on a hot plate at 600 rpm for 30 minutes. The propolis extract was dissolved in the film-forming solution to obtain concentrations of 0, 5, 10 and 20 % w/w of chitosan. Each film-forming solution was casted on a glass plate. The obtained films were conditioned in a chamber prior to testing.

2.3 Fourier Transform Infrared (FTIR) Analysis

FTIR analysis was carried out to observe the structural interactions of chitosan and propolis polyphenols. The FTIR spectra of chitosan films were recorded from 4000 to 450 cm^{-1} using Perkin Elmer Spectron Two (OATR) (Perkin Elmer, USA).

2.4 Contact Angle Measurements

Water contact angle (Model Oca20, Data-Physics Instruments) was used to measure the contact angle of water in air on the surface of chitosan films. A film sample (10 mm × 10 mm) were placed on a movable sample stage and levelled horizontally; then a drop of about 3 µL of distilled water was placed on the surface of the film using a micro-syringe. The contact angle was measured in a conditioned room by recording contact angle values. Image analyses were carried out using SCA20 software.

2.5 Antimicrobial Activity Assay

Antimicrobial activity assay of chitosan films containing propolis was carried out using agar diffusion technique with slight modifications [14]. For the antimicrobial assay, a Gram-negative bacterium (*Escherichia coli*) and Gram-positive bacteria (*Bacillus cereus*) were used. Before use, the test strain was grown in nutrient broth. The bacteria cultures were then diluted to achieve the dilution of 10^6. The film was cut into a disc form of 10 mm in diameter. The film discs were placed on tryptic soy agar plates which had been previously seeded with inoculum containing indicator microorganisms. The plates were then incubated at 37 ˚C for 24 hours. The diameter of inhibitory zone surrounding film discs was measured.

3. Results and Discussion

The percentage yield of propolis extract which is solid and sticky is 10 %. This result was almost similar to the percentage of yield obtained by Shen and Kamdem [16].

3.1 Fourier Transform Infrared (FTIR)

FTIR was conducted to distinguish the intermolecular interaction between chitosan film matrices with phenolic compound that present in propolis extract. Figure. 1 showed that peaks of hydrogen-bond O-H stretch (3600-3100 cm^{-1}), C–H stretching (3000 -2850 cm^{-1}), C=O stretch (1750-1625 cm^{-1}), C-C=C asymmetric stretch (1500-1450 cm^{-1}), and C–O (1280-1000 cm^{-1}) can be observed in chitosan film. For films containing propolis extract, changes taking place at 1700 cm^{-1}, referring to C=O stretching within the carboxylic group, and peak at 1645 cm^{-1}, correlated to C=C stretching within the aromatic ring. The bands appeared at 2918 and 2849 cm^{-1} showed stronger peak intensity in films containing propolis extract when compared with those of pure chitosan film, probably due to stretching vibrations of the C-H bond in $-CH_2$ and $-CH_3$ groups, respectively [17-18]. The stretching of C=O, C-C=C- [(in-ring) aromatic], and C-O (esters, ethers) found in the phenolic components were observed at 1800-1000 cm^{-1} of the FTIR spectra.

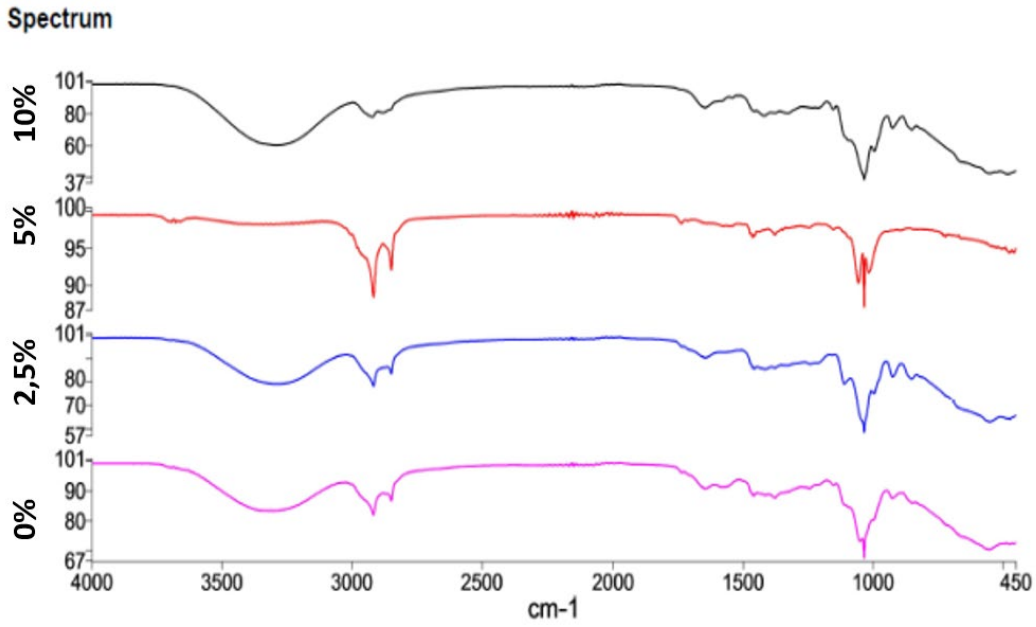

Figure. 1. Infrared spectra of chitosan-propolis film containing different concentrations of propolis extract; (A) 0%, (B) 2.5%, (C) 5%, and (D) 10%.

3.2 Contact Angle (CA)

CA measurement is used to investigate the effect of different concentration of propolis extract towards membrane hydrophilicity. In general, the CA refers to the surface hydrophobicity. Higher CA value demonstrates higher hydrophobicity of the film surface. In this study, the value of CA slightly decreases with decreasing of chitosan content in the film due to the addition of glycerol and propolis extract in the mixture (Table 1). Propolis extract is hydrophobic in nature due to hydrophobic constituents of propolis [19]. This may affect the result of the contact angle value as the concentration of the propolis increases. However, previous studies have shown that the addition of 30 % of glycerol has resulted in significant decrease in contact angle value of chitosan film due to hydrophilic nature of the plasticizer [20].

Table 1. Contact angle (CA) value of chitosan-propolis sample film at different concentration of propolis extract

Sample	CA ± standard deviation (°)
Propolis 0%	59.21 ± 7.54
Propolis 2.5%	86.76 ± 8.39
Propolis 5%	40.94 ± 2.23
Propolis 10%	45.20 ± 0.06

3.3 Antimicrobial test

The antimicrobial test of chitosan-propolis extract films was carried out via agar diffusion technique against two types of bacteria (*Escherichia coli* and *Bacillus cereus*). The inhibition zone obtained has shown that the addition of propolis extract has promoted the antimicrobial activity of the film. According to Uzel *et. al*., the components in propolis extract consist of polyphenols such as

pinocembrin, pinostropin, pinobanksin, galangine and chrysin [21]. The bioactive phenolic compounds will employ the physiological changes of the cell membrane, followed by the death of the cell [22].

Table 2. Results of inhibition zone on target bacteria

Bacteria	Inhibition zone (mm)		
	0 %	5 %	10 %
E. coli	n.a.	1.0	0.5
B. cereus	n.a.	2.0	2.0

*n.a. – not available

By comparing the results obtained in this study (Table 2), the inhibition zone for *B. cereus* colonies are slightly larger than the inhibition zone for *E. coli* colonies. This is because the difference of the cell structure between Gram-positive bacterium (*B. cereus*) and Gram-negative bacterium (*E. coli*). Both tested bacteria have cell membrane that protected the intracellular structure. However, gram-negative bacterium has an additional layer that is made up of phospholipids, proteins and lipopolysaccharides that is impermeable to most substances [15]. However, chitosan film without propolis extract showed no inhibition towards *E. coli* and *B. cereus* in this study. Siripatrawan *et. al*. reported that there was possibility of the antimicrobial activity of the chitosan itself to be considered as negligible when it was in film form [15].

4. Conclusion

The incorporation of ethanolic propolis extract to the chitosan film has produced a soft and brittle film due to the waxy nature of the propolis. The antimicrobial property of film however, was evidenced against *E. coli* and *B. cereus* at concentration of 10 %, but much stronger in *B. cereus* bacteria. The incorporation of propolis has also reduced the hydrophobicity of the chitosan film which means the film has increased the water-liking property. In short, from this study, the character of the resultant thin film still needs further improvement and tests to be able to rule out as an active food packaging material.

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