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Development of Coffee Pulp Extract-Incorporated Chitosan Film and Its Antimicrobial and Antioxidant Activities

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Abstract

The aim of this work was to preliminary develop a coffee pulp extract- incorporated chitosan film. Coffee pulp (CP) was obtained from Doi Chaang Original Co. Ltd, Chiang Rai, Thailand. The CP was extracted with distilled water at the CP-to-distilled water ratio of 1:2, and then the mixture was filtered, centrifuged and dried by freeze drying to obtain the dried coffee pulp extract powder (CPE). Chitosan (CH) film incorporated with various concentrations of CPE (0-1.0 % w/v) was prepared by casting method and dried at different temperatures (ambient, 30 and 40 ^{o}C). The antimicrobial activity of the films against some potent foodborne microorganisms (*Bacillus cereus* KCCM 40133, *B. subtilis* KCCM 11316, *Escherichia coli* KCCM 21052 and *Pseudomonas fluorescence* KCCM 11362) was evaluated by disc diffusion method. The antioxidant activity (DPPH assay) and physical properties (water vapor permeability (WVP), solubility and color (*L**, *a** and *b**) of the film were determined. There was no inhibitory effect observed when the CH film without CPE was tested by disc diffusion method. The antimicrobial and antioxidant activities of the CPE-CH films significantly increased with the increasing CPE concentration ($p \leq 0.05$). The WVP and solubility of the CH film without the CPE was higher than those of the CPE-CH film. With the increasing CPE concentration in the CH matrix, the WVP and solubility of the CPE-CH film significantly decreased ($p \leq 0.05$). These results indicate that the CPE-CH film could be applied as an antimicrobial and antioxidant food packaging.

Keywords : *active packaging, by-product, edible film, natural extract*

Introduction

Microbial contamination is one of the most important concerns in the food industry. It can cause foodborne diseases, and consequently economic losses (1, 2). Packaging is reported to help reducing microbial contamination, particularly the antimicrobial (AM) packaging (3, 4). AM packaging is an active packaging which is usually produced in a form of edible film. The film is normally composed of film forming material, plasticizer and active agent. The film composition will be varied depending on the purpose of application (5, 6). The

antimicrobial agents which are incorporated into the packaging to develop the AM packaging include weak organic acids, enzymes, bacteriocins and natural extracts (7-9). As a result of the health concerns nowadays of the consumers, natural existing AM compounds are increasingly popular and substituted the synthetic ones (10, 11). Those natural AM compounds include the compounds from animal, plant and microorganisms, such as anthocyanin, caffeine, epicatechin, tannins, lactoperoxidase system, lysozyme, nisin, natamycin, etc. (7-9, 12, 13). Chitosan, a promising antimicrobial agent, is a cationic polysaccharide obtained from deacetylation of chitin, which is the major organic constituent of exoskeleton of crustaceans (11, 14). It was reported to be the most promising candidate as the film forming material (15, 16). Coffee pulp (CP) was reported as a potential source of phytochemicals having antimicrobial and antioxidant effects (17, 18). Those compounds are flavan-3-ols, caffeine, chlorogenic acid, hydroxycinnamic acid, anthocyanidins, tannins etc. (19-21). Wang et al. (22) reported that the phenolic compounds affected the microbial membrane causing structural and functional damage. This work, therefore, was aimed to exploit the CP antimicrobial activity, for the first time, to develop the antioxidant and antimicrobial CH film in order to pave the way of using these AM packaging to reduce the microbial contamination in food products.

1. Materials and Methods

1.1 Coffee Pulp

Arabica coffee (*Coffea arabica* L.) pulp was obtained from Doi Chaang Original Co. Ltd. in Chiang Rai, Thailand in January 2014 and kept at -40 ^{o}C until used.

1.2 Sample Preparation

The coffee pulp (CP) was blended with distilled water at the CP-to-distilled water ratio of 1:2 and then the mixture was filtered and centrifuged at 10,000 rpm for 5 min at 4 ^{o}C. The supernatant was collected and further dried by freeze drying. The dried coffee pulp extract (CPE) was used for proximal analysis and kept at -40 ^{o}C for further analysis.

1.3 Chemicals

All reagents were analytical grade. 2-2-diphenyl-1-picrryl-hydrazyl (DPPH) and chitosan were purchased from Sigma-Aldrich Co. Ltd., Germany.

1.4 Preparation of Films

Chitosan (CH, low MW, $\geq$ 75 % DD) was used as a film forming material in this work. The CH film suspension (150 mL) was prepared according to Sun et al. (23) with some modifications. Briefly, the CPE (0, 0.5, 0.75 and 1.0 % w/v) was dissolved in 150 mL of 1% acetic acid and stirred for 30 min. Then, CH powder (3 g) was added and mixed well. The mixture was stirred for 1 hr or until clear solution was obtained. After that, glycerol (0.90 g) was added to the film solution and mixed well. The film solution was casted onto a Teflon plate (22 $\times$ 29 cm^2) and allowed to dry at ambient temperature, 30 ^{o}C and 40 ^{o}C. The film was kept at 25 ^{o}C, 50 $\pm$ 5 % relative humidity for at least 48 hr before further testing.

1.5 Bacterial Suspension Preparation

Bacterial cell suspension for antimicrobial testing was prepared by re-suspending the active culture in normal saline to the 0.5 McFarland turbidity standards which is equivalent to the bacterial suspension of 1.5 $\times$ 10^8 CFU/mL (24).

1.6 Disc Diffusion Assay

The disc diffusion method was conducted according to the National Committee for Clinical Laboratory Standard (25) to evaluate the antimicrobial activity of the film. Within 15 min after adjusting the turbidity of a bacterial suspension, a sterile cotton swab was dipped into the suspension and swabbed on Mueller-Hinton Agar (MHA). The CH or CPE-CH film was cut using a sterile paper puncher (6.0 mm diameter), then the film was placed onto the microbial lawn together with tetracycline (30 µg/mL) as a positive control and sterile distilled water as a negative control. The plate was incubated at 37 °C for 24 hr. Then, the inhibition zone was determined and recorded (mm).

1.7 DPPH Radical Scavenging Activity (DPPH)

DPPH radical scavenging activity of the film was determined as described by Brand-Williams et al. (26) with some modifications. The dry film (0.2500 g) was soaked in 15 mL distilled water for 24 hr and extracted (Siripatrawan & Harte, 2010). The extract (3 mL) was mixed with 1 mL of 1 mM 2, 2-dipheny l-1-picryl hydrazyl (DPPH) in 95 % methanol. The mixture was mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of the resulting solution was determined at the wavelength of 517 nm using a UV-spectrophotometer. The blank was prepared in the same manner except that 95 % methanol was used instead of the DPPH solution. The antioxidant activity was calculated after the blank subtraction and expressed as scavenging activity (%) as shown in the equation (1) below.

$$\text{Scavenging activity (\%)} = \frac{A_{DPPH} - A_S}{A_{DPPH}} \times 100 \quad (1)$$

Where A_{DPPH} is the absorbance at 517 nm of DPPH methanol and A_S is the absorbance at 517 nm of the sample.

1.8 Water Vapor Permeability (WVP)

The WVP of the film was determined gravimetrically in triplicate according to the ASTM E96-01 method (27). The film was cut to a rectangular shape (60 × 60 mm^2) and used to close an aluminum cup (3.9 × 3.6 × 2.3 cm) containing 3 g of silica gel. The cup was placed in an incubator (50 ± 5 % RH, 25 °C) and weighed at an hour interval to 8 hr. The weight of the aluminum cup *vs* time was plotted. The slope was used to calculate water vapor transmission rate (WVTR) using equation (2) in order for WVP calculation by equation (3)

$$WVTR = \frac{Slope}{A} \quad (2)$$

Where, A is the sample area (m^2).

$$WVP = \frac{WVTR \times T}{\Delta P} \quad (3)$$

Where, T is the thickness of the film (mm) and ΔP is the partial pressure difference of the water vapour across the film.

1.9 Solubility in Water

Solubility is defined as the percentage of dry matter solubilized after 24 hr of film immersion in distilled water with respect to initial dry matter (28). The initial dry matter was determined by cutting and drying the film in a hot air oven at 100 °C for 24 hr. After that, the film was weighed and immersed in 50 mL distilled water,

shaken at 180 rpm for 24 hr at 25 °C, and then filtered. The residual film was taken out and dried (100 °C, 24 hr) to determine the final weight of dry matter. The solubility in water testing was conducted in triplicate and calculated using following the equation (4).

$$\text{Solubility}=\frac{\text{Initial weight-Final weight}}{\text{Initial weight}}\times 100\% \quad (4)$$

1.10 Color Measurement

The color of the film was determined with a Hunter Lab color meter and expressed as L^*, a^*, and b^*.

1.11 Statistical Analysis

All tests were conducted in triplicate for verification of the results and subjected to analysis of variance (ANOVA). A mean composition was carried out by Duncan's multiple range test. Statistical significance was set at P-value ≤ 0.05. All data analyses were performed using SPSS package (V16).

2. Results and Discussion

The color of the CH film containing 1.0 % CPE dried at ambient, 30 and 40°C were shown in Figure 1. It was found that the film color was darken with increasing temperature. The darker film might affect the consumer acceptability when the film is applied for further experiment as an edible film. The film was more brittle when the drying temperature increased. Therefore, the ambient temperature was used to prepare the film for further experiment. The film color increased with the increasing concentration of CPE in the CH matrix (Figure 2)

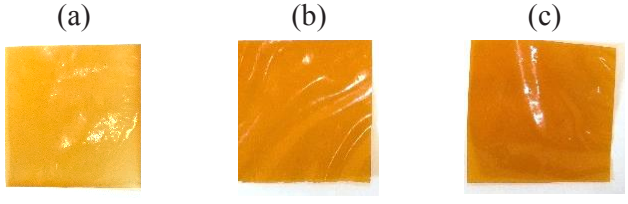

Figure 1. CPE (1% w/v)-CH Film dried at (a) Ambient Temperature (b) 30 °C and (c) 40 °C

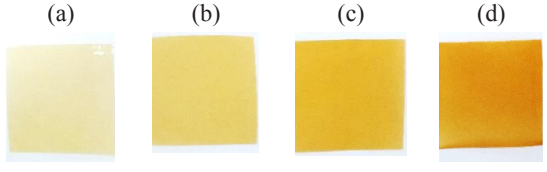

Figure 2. The CH Film Containing CPE at (a) 0 % w/v (b) 0.5 % w/v (c) 0.75 % w/v and 1.0 % w/v

Table 1. Inhibitory effect (inhibition zone, mm) of CPE-CH Film by Disc Diffusion Method

Microorganism	The CPE concentration (% w/v)			
	0	0.5	0.75	1.0
Gram-positive bacteria				
B. cereus KCCM 40133	NZ*	8.33 ± 0.58^{Ca}	10.33 ± 0.58^{Ba}	12.67 ± 0.58^{Aa}
B. subtilis KCCM 11316	NZ*	NZ*	9.33 ± 0.58^{Bb}	11.67 ± 0.58^{Aab}
Gram-negative bacteria				
E. coli KCCM 21052	NZ*	NZ*	8.33 ± 0.58^{Bc}	10.33 ± 0.58^{Ac}
P. fluorescence KCCM 11362	NZ*	NZ*	9.00 ± 0.00^{Bbc}	11.33 ± 0.58^{Abc}

Values are the mean ± SD , NZ* No inhibition zone
Means with different superscript block letters within a row are significantly different ($P \leq 0.05$)
Means with different superscript lowercase letters within a column are significantly different ($P \leq 0.05$)

2.1 Inhibitory Effect of the CPE-CH Film by Disc Diffusion Method

The inhibitory effect of the CH film containing various concentrations of CPE (0, 0.5, 0.75 and 1.0 % w/v) by disc diffusion method is summarized in Table 1. The initial diameter of all films was fixed at 6 mm. The first inhibitory effect of the CPE-CH film was observed when the film contained 0.5 % w/v CPE, but only *B. cerues* was inhibited. The film containing 1.0 % w/v of the CPE inhibited all the tested bacteria with *B. cereus* as the most susceptible bacterium. It was also found that the inhibitory effect of the CPE-CH film increased with the increasing concentration of the CPE in the film matrix. The increase of the film antimicrobial activity with the increasing CPE concentration in the film may due to its increasing positive charge of carboxylic group of chlorogenic acid (CGA) which was reported to be the main antimicrobial agent found in CPE. This positive charge interacts with the negative charge microbial cell membrane, consequently causing leakage of proteinaceous and other intracellular constituents from the microorganism cells (29-31). Gram positive bacteria were more susceptible to the CPE-CH film than gram negative bacteria. The effect of the film on the gram-negative bacteria is different from that of the CPE alone which might be due to the CPE and CH synergistic effect.

2.2 Antioxidant Activity (AOA)

DPPH assay was used to evaluate the antioxidant capacity of the CH films containing different CPE concentrations compared to the CH film without any CPE. The radical scavenging capability through the DPPH assay ranged from 5 - 23 % scavenging activity as shown in Table 2. The CPE was found to enhance the AOA of the film indicating by the higher AOA from DPPH assays when the CPE was incorporated. The AOA of the film significantly increased with the increasing CPE concentration ($P \leq 0.05$). This free radical can react with the phenolic compounds and residual amine group (NH_2) to form macromolecule radicals. The amine groups in CH, can form ammonium (NH^{3+}) groups by absorbing hydrogen ion from the solution (32, 33). The main polyphenols identified in the CPE were caffeine and CGA. These phenolic compounds exhibited a reducing activity which could enhance the antioxidant activity of the film (34).

Table 2. Antioxidant Activity of CH Film Containing Various CPE Concentrations by DPPH Assay

Active film	% scavenging activity
CH	5.07±0.34^{e}
0.50 CPE- CH	12.30±0.34^{c}
0.75 CPE- CH	16.85±0.45^{b}
1.00 CPE- CH	23.49±0.34^{a}

Values are the mean ± SD
Means with different superscript within a column are significantly different ($P \leq 0.05$)

Table 3. Thickness, Water Vapor Permeability, Solubility and Color of Chitosan Film with/ without CPE

Active film	Thickness (mm)	WVP (g.mm/m^2.h.kPa)	Solubility (%)	L*	a*	b*
CH	0.051±0.003^{a}	3.52±0.00^{a}	30.66±0.66^{a}	79.49±1.04^{a}	3.31± 0.15^{d}	23.59± 1.20^{d}
0.50 CPE-CH	0.051±0.003^{a}	2.32±0.31^{b}	29.36±0.58^{b}	74.33±2.67^{b}	7.76±0.54^{c}	35.11±1.07^{c}
0.75 CPE-CH	0.051±0.001^{a}	1.95±0.21^{c}	27.70±0.38^{c}	67.76±1.33^{c}	11.36±0.27^{b}	48.09±1.33^{b}
1.00 CPE-CH	0.052±0.001^{a}	1.48±0.00^{d}	25.03±1.47^{d}	60.33±3.14^{d}	16.14± 0.73^{a}	62.75± 1.35^{a}

Values are the mean ± SD
Means with different superscript within a column are significantly different ($P \leq 0.05$)

2.3 Thickness, Water Vapor Permeability, Solubility and Color

The thickness of the CH and CPE-CH film ranged from 0.051 - 0.052 mm. Generally, the thickness of the edible film is in the range of 0.010 – 0.100 mm based on its application (35-37). Addition of the CPE into the CH film resulted in no significant difference (P>0.05) of the film thickness while decreased the WVP and solubility as shown in Table 3. These decreases due to the increasing of hydrophobic group, when the CPE was added (38). These WVP and solubility reduction is due to the formation of hydrogen bonds between the -NH_2 group presented in CH and -OH group of the phenolic compounds in the CPE, thus reducing the availability of the hydrophilic group. These results were consistent with previous report when phenolic compounds were incorporated into the CH matrix (39). Similar results were also observed by Aljawish et al. (38) where the physicochemical interaction between phenolic compounds and CH was investigated. These also may be because the polyphenolic compounds may be able to fit into chitosan matrix and established interactions such as hydrogen or covalent bonding with reactive groups of chitosan (40). This fact may contribute to cross-linking effects of CPE compounds, leading to esters and/or amide groups of CH film. The incorporation of the CPE into CH film provided the darker color to the film as a result of the dark brown color of the oxidized anthocyanin which is the major pigment in the CPE (30). It was found that incorporation of the CPE into the CH film matrix caused a significant decrease of the L^* value ($P \leq 0.05$), whereas a^* and b^* values of the film significantly increased

($P \leq 0.05$). These results indicate the tendency towards redness and yellowness of the CH film when the CPE was incorporated.

3. Conclusion

From the results, the antimicrobial and antioxidant activities of CPE-CH films were found to significantly increase with the increasing CPE concentration ($P \leq 0.05$). Gram positive bacteria were more susceptible to the CPE-CH film than gram negative bacteria with *B. cereus* exerted the most sensitive bacterium. Incorporation of the CPE into the CH film resulted in no significant difference of the film thickness, whereas the WVP and solubility of the CPE-CH film significantly decreased ($P \leq 0.05$). And the film color increased with the increasing concentration of CPE in the CH matrix. It could be concluded that the CPE-CH film has a potential to be alternately applied as an antimicrobial and antioxidant food packaging to reduce the microbial contamination and to maintain food qualities. However, future research could be conducted to evaluate the toxicity of using these natural AM agents as edible films and coatings, and also characterize other physico-mechanical properties.

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