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Original Article

Rambutan *(Nephelium lappaceum)* peel extract: Antimicrobial and antioxidant activities and its application as a bioactive compound in whey protein isolate film

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Abstract

This study focused on assessment of the bioactivities and characterization of rambutan peel extract (RPE) and the feasibility of RPE as a bioactive compound for antimicrobial and antioxidant applications in whey protein isolate (WPI)/cellulose nanocrystal film. The RPE inhibited against nine strains of pathogenic and food spoilage bacteria (*Micrococcus luteus*, *Bacillus* sp., *Escherichia coli*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus* sp. and *Staphylococcus aureus*). The extract also exhibited higher antioxidant activities than those of butylated hydroxytoluene (BHT) (via DPPH, ABTS and FRAP assays), α-tocopherol (via DPPH and ABTS assays) and butylated hydroxyanisole (BHA) (via hydroxyl radical and superoxide anion radical scavenging assays). The WPI/cellulose nanocrystal film containing 3% RPE exhibited moderate antimicrobial activity and strong antioxidant activity, which can be applied as a bioactive film for food packaging application.

Keywords: rambutan peel extracts, antimicrobial activity, antioxidant activity, whey protein isolate film, cellulose nanocrystal

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1. Introduction

Rambutan (*Nephelium lappaceum*) is a commercial, tropical fruit in Thailand and Southeast Asia, which can be consumed fresh or in processed products. However, its peels or husks were the wastes generated after industrialization. There are many studies reporting the extraction of bioactive compounds from rambutan peels (Thitilertdecha, Teerawut gulrag, & Rakariyatham, 2008; Thitilertdecha, Teerawutgul rag, Kilburn, & Rakariyatham *et al.*, 2010). Those researchers found that rambutan peel extracts (RPEs) consisted of bioactive compound such as phenolic compounds, flavonoids and tannin (Sekar *et al.*, 2014; Thitilertdecha *et al.*, 2010). However, the main bioactive compounds are geraniin, corilagin and ellagic acid (Lim, 2013; Rohman, 2017; Thitilertdecha *et al.*, 2010). These bioactive compounds of RPEs showed high potential biological activities (antimicrobial, antioxidant, antiviral, anti-inflammatory and cytotoxic), which may be advantageous for further applications (Chingsuwanrote, Muangnoi, Parengam, & Tuntipopipat, 2016; Ma, Guo, Sun, & Zhuang, 2017; Perera, Appleton, Ying, Elendran, & Palanisamy, 2012; Thitilertdecha *et al.*, 2008, 2010).

Nowadays, due to environmental concerns, there is greater interest in biodegradable packaging. Whey protein isolate (WPI) is an interested biopolymer for food packaging application (Pereira *et al.*, 2016; Qazanfarzadeh & Kadivar, 2016; Sukyai *et al.*, 2018). Our previous study found that WPI-based film had poor mechanical properties and water barrier properties due to its hydrophilic nature. Reinforcement of WPI film with sugarcane bagasse cellulose nanocrystals (CNCs) could overcome those drawbacks and improve the physical and mechanical properties of the film due to the strong hydrogen bonding and high surface area of the CNCs (Sukyai *et al.*, 2018). The addition of natural extracts with potential as antimicrobial and antioxidants are of special interest from the safety aspect of the customer to improve the functional attributes and application of biodegradable WPI film.

Therefore, the aims of this study were to extract and to characterize bioactive compounds from rambutan peel and to evaluate the antimicrobial and antioxidant activities of the extracts. Moreover, the feasibility was also studied of RPEs to improve the antimicrobial and antioxidant properties of WPI film.

2. Materials and Methods

2.1 Materials

2.1.1 Raw materials

Rambutan (*Naphelium lappaceum*) peel samples were received from Malee Group Public Company Limited, Nakhon Pathom, Thailand. Sugarcane bagasse (SCB) was obtained from Kaset Thai International Sugar Corporation Public Co., Ltd., Nakhon Sawan, Thailand. Whey protein isolate (WPI) was purchased from Mighty International Co., Ltd., Thailand.

2.1.2 Microorganisms and cultures

Nine bacterial strains were obtained from the Thailand Institute of Scientific and Technological Research (TISTR), Bangkok, Thailand (*Escherichia coli* TISTR 117, *Pseudomonas fluorescens* TISTR 358, *Salmonella typhimurium* TISTR 1469, *Enterococcus faecalis* TISTR 379, *Leuconostoc* sp. TISTR 944, *Streptococcus* sp. TISTR 1030, *Micrococcus leteus* TISTR 2374, *Bacillus* sp. TISTR 1323, and *Staphylococcus aureus* TISTR 746).

2.1.3 Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), α-tocopherol (vitamin E), butylated hydroxytoluene (BHT), ascorbic acid (vitamin C), butylated hydroxyanisole (BHA) and ethylenediamine tetraacetic acid (EDTA) were purchased from Sigma-Aldrich, U.S.A. Glycerol, barium chloride, sodium acetate, ferric chloride and ferrous sulfate were purchased from Ajax Finechem, New Zealand. Nitroblue tetrazolium (NBT) was purchased from Chemimpex, U.S.A.

2.2 Rambutan peel crude extraction

After drying and grinding the rambutan peels, the rambutan powder was extracted with 95% ethanol using a Soxhlet extractor for 16 h. The extract was then concentrated using a vacuum rotary evaporator (Buchi, Switzerland) according to the method of Nanthakumar, Udhayasankar, Asha devi, Arumugasamy and Shalimol (2014).

2.3 Identification of bioactive components in RPE

Bioactive components in the RPE were quantified using a high performance liquid chromatographer (HPLC) (Shimadzu, Japan) equipped with an Inertsil ODS-3 C18 (4.6x150 mm, 5 μm) column. The experiment was conducted using a gradient elution of 0.4% formic acid in methanol with a flow rate of 1.0 mL/min and a column temperature of 30 °C. The peaks of sample were measured at 280 nm using a variable wavelength detector. The amount of the extract was calculated from the known standard calibration curves. The accuracy of the analysis of the main active compounds in RPE was confirmed by a spike method. In particular, the rambutan extract (1 mg/mL) was spiked with all standard solutions (100 μg/mL) of the extraction solvent. The spiked samples were then quantified in accordance with the methods mentioned above.

2.4 Antimicrobial activity assay

2.4.1 Agar disc diffusion

The antimicrobial activity was examined using the agar disc diffusion method (CLSI, 2006). Briefly, bacteria were incubated on the nutrient agar plates at 35 °C for 24 h and then inoculated into NaCl (0.85%w/v) to obtain the inoculum suspension which the turbidity match with McFarland No. 0.5 (10^8 CFU/mL). *Streptococcus* sp. and *Leuconostoc* sp. were inoculated on Lactobacillus MRS broth, while the other bacteria were inoculated on Mueller-Hinton agar. After that, 10 μL of RPEs dissolved in dimethylsulfoxide were loaded onto sterile paper discs (6 mm

diameter) prior to being placed on inoculated agar plates. These plates were then incubated at 37 °C for 24 h. The results were expressed as the average diameter of the inhibition zone (mm ± SD).

2.4.2 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of RPE against microorganisms

The MIC and MBC of rambutan extracts were determined in 96-well microplates using the broth microdilution method (CLSI, 2012). Lactobacillus MRS broth was used as diluent for *Streptococcus* sp. and *Leuconostoc* sp., while Mueller-Hinton broth was used as diluent for the other bacteria. The positive control was composed of diluent and bacterial growth, while the negative control contained only bacteria. Microplates were inoculated at 37 °C for 24 h. The MIC was defined as the lowest concentration of RPEs that completely inhibited the growth of the bacteria in the well as detected by resazurin (0.02%). To determine the MBC, 10 µL of bacterial suspension were removed from each well after overnight growth, (before adding the resazurin working solution) and spread onto Mueller-Hinton agar prior to incubation at 37 °C for 24 h. The MBC was defined as the lowest concentration of RPEs at which 99.9% of the inoculated microorganisms were killed (Sukatta, Rughtaworn, Tuntawiroon, & Meaktrong, 2011).

2.5 Antioxidant activity assays

2.5.1 2,2-Diphenyl-2-picrlhydrazyl radical scavenging activity (DPPH)

The DPPH assay was evaluated with some modification from the method of Zhu, Zhou, and Qian (2006). The RPEs were diluted to different concentrations. A sample of 1 mL of extract and 1 mL of 0.1 mM DPPH in 95% ethanol was mixed and incubated in the dark at room temperature for 30 min. The mixture absorbance was measured at 517 nm. BHT, α-tocopherol and vitamin C were used as standards. The antioxidant activity was calculated as the radical scavenging effect (%) using Equation (1).

$$\text{Radical scavenging effect (\%)} = [(A_0\text{-}A_1)/A_0] \times 100 \quad (1)$$

where, A_0 is absorbance of the standard and A_1 is the absorbance of the sample. IC_{50} values were calculated from the plot of radical scavenging effect percentages against sample concentration, where IC_{50} is the concentration of sample at which 50% of free radical scavenging activity occurs.

2.5.2 3-ethylbenzothiazoline-6-sulfonic acid radical scavenging activity (ABTS)

The ABTS assay was carried out according to the method of Re *et al.* (1999). The ABTS radical cation ($ABTS^{\bullet+}$) solution was prepared by mixing ABTS agent (7 mM) with 2.45 mM potassium persulfate ($K_2S_2O_8$) and stored in the dark for 12–16 h. The $ABTS^{\bullet+}$ solution was diluted to obtain sample absorbance around 0.70 ± 0.02 at 743 nm. After that, 2 mL of diluted $ABTS^{\bullet+}$ solution was mixed with 20 µL of diluted sample and incubated for 6 min prior to measuring its absorbance at 734 nm. Butylated hydroxytoluene (BHT), α-tocopherol and vitamin C were used as positive controls. The % $ABTS^{\bullet+}$ activity was calculated using Equation (1).

2.5.3 Ferric reducing antioxidant power (FRAP)

The FRAP reagent was prepared by mixing 10 µL of acetate buffer (300 mM), 1 mL of $FeCl_3.6H_2O$ (20 µM), 1 mL of TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution in HCl (40 µM) and 1.2 mL of deionized water. The antioxidant activity was measured using a reducing agent. Briefly, 60 µL of diluted samples, 180 µL of deionized water and 1.8 mL of FRAP reagent were mixed and incubated at 37 °C for 4 min to perform the reaction. The absorbance of each solution was recorded at 595 nm. The antioxidant capacity was calculated using the standard curve of standard solutions (ferrous sulfate at various concentrations from 0.1 to 1.0 mM). The antioxidant capacities of the samples were expressed as µM of Fe (II)/g of sample. BHT, α-tocopherol and vitamin C were used as positive controls.

2.5.4 Hydroxyl radical scavenging activity

The hydroxyl radical-scavenging activity was determined using the 2-deoxyribose modified method of Özyürek, Bektaşoğlu, Güçlü, and Apak (2008). The reaction mixture consisted of 0.8 mL of potassium phosphate buffer (pH 7.4), 0.2 mL of EDTA (1 mM), 0.2 mL of $FeCl_3$ (0.5 mM), 0.2 mL of deoxyribose (10 mM) and 0.2 mL of sample and was mixed at 37 °C. The mixture was then incubated at 37 °C for 1 h prior to adding 0.2 mL of ascorbic acid (1 mM) and H_2O_2 (10 mM). Finally, 2 mL of TBA (1%) and TCA (2.8%) were incorporated. The mixture was further incubated at 100 °C for 15 min. The absorbance of each sample was measured at 532 nm. BHA and gallic acid were used as positive controls. The % hydroxyl radical ($OH^{\cdot}$) radical scavenging activity value was calculated using Equation (1).

2.5.5 Superoxide anion radical scavenging activity

The reaction mixture consisted of 1 mL of nitroblue tetrazolium (NBT) solution (78 µmol of NBT in 100 mmol phosphate buffer, pH 7.4), 1 mL of nicotinamide adenine dinucleotide hydride (NADH) solution (234 µm in 100 mmol phosphate buffer; pH 7.4) and 1 mL of sample. The reaction was started by adding into the mixture 0.1 mL of phenazine methosulphate solution (30 µmol in 100 mmol phosphate buffer; pH 7.4). The reaction mixture was then incubated at room temperature for 5 min and the absorbance was measured at 560 nm (Kumar *et al.*, 2008). BHA, gallic acid, trolox and vitamin C were used as positive controls. The % superoxide anion radical scavenging activity value was calculated using Equation (1):

2.6 Preparation of whey protein isolated film containing RPEs

WPI film incorporated with cellulose nanocrystals

(CNCs) and RPEs was prepared using a solution casting method following our previous study (Sukyai *et al.*, 2018). Briefly, WPI (5% by weight of solution) was mixed with glycerol (50% solid) while 5% (w/w) of CNC and 3% (w/v) of RPE (a little bit higher from the MIC result) were firstly mixed before being combined in whey solution. The CNC in the present work was prepared using acid hydrolysis following previous methods (Lam, Chollakup, Smitthipong, Nimchua, & Sukyai, 2017; Saelee, Yingkamhaeng, Nimchua, & Sukyai, 2016). The mixture was then cast on an acrylic plate with had a Teflon surface prior to drying at 50 °C for 15 h. After that, films were stored in a desiccator containing saturated potassium carbonate salts at 30 °C (43.2% relative humidity) for 48 h prior to further analysis. Besides, the WPI film with the same components as above without CNC and the other WPI film with the same components without RPE were prepared for comparison the effect of antioxidant and antimicrobial activities of the sample WPI films.

2.7 Characterization of antimicrobial and antioxidant of whey protein isolate film containing RPEs

2.7.1 Color

The film color was measured using a colorimeter (Lovibond AT100, USA) and the CIELAB color system parameters of lightness (L*), redness/greenness, (a*) and yellowness/blueness (b*). The total color difference (ΔE*) was also calculated using Equation (2):

$$(\Delta E^*) = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2} \quad (2)$$

2.7.2 Antimicrobial activity

The antimicrobial activity of films was determined using the agar disc diffusion method, which was described in section 2.4.1. Briefly, WPI films were used as the specimens instead of the sterile paper discs.

2.7.3 Antioxidant activity

The antioxidant activities of WPI film and the one containing RPE were determined using DPPH assay following the modified method of Noronha, de Carvalho, Lino, and Barreto (2014). Approximately 100 mg of film samples were cut into small pieces and placed into a tube containing 5 mL of ethanol and stirred for 3 min. Diluted ethanol solution (0.1 mL) was mixed with 2.9 mL of 0.1 mM DPPH in ethanol. The mixture was stirred and kept in the dark at room temperature for 30 min and absorbance was measured at 517 nm. The antioxidant capacity of film was expressed as the IC_{50}, which was determined from the plot of % DPPH activity and calculated using Equation (1).

2.8. Statistical analyses

All experiments were performed at least triplicates. A one-way analysis of variance (ANOVA) was used for comparing mean values with statistical significance at $p < 0.05$. Duncan's multiple range test (DMRT) was used for comparing group means. Analysis was performed using the SPSS 17.0 for Windows software (SPSS Inc., Chicago, IL, U.S.A.).

3. Results and Discussion

3.1 Rambutan peel extract

3.1.1 Bioactive compounds

The main bioactive components of rambutan peel extract (RPE) were analyzed using HPLC. The HPLC chromatogram (Figure 1.) indicates that the main components of RPE were corilagin, ellagic acid, geraniin and gallic acid. This result corresponded to the study of Thitilertdecha *et al.*, (2010). It should be noted that the main bioactive components of RPE were categorized in the group of phenolic compounds, which exhibited antioxidant and antimicrobial activities (Thi tilertdecha *et al.*, 2010; Thitilertdecha & Rakariyatham, 2011).

3.1.2 Antimicrobial activities

1) Antimicrobial activity using agar disc diffusion assay

The antimicrobial activity of RPE against nine strains of foodborne pathogen was evaluated using the agar disc diffusion method. The inhibitory growth of bacteria was shown in Table 1 and pictures of the clear zone for some foodborne pathogens are shown in Figure 2. RPE exhibited potential activity against four strains of bacteria (*S. Typhimurium, M. luteus, Bacillus sp. and S. aureus*). This might be due to the main components such as ellagic acid, corilagin, and geraniin found in the RPE showing the antimicrobial activity. Moreover, RPE had more effective antimicrobial activity against Gram-positive than Gram-negative bacteria because the latter have a complicated cell wall (Tajkarimi, Ibrahim, & Cliver, 2010). However, the extracts were not effective against *E. faecalis*, *E. coli*, *Leuconostoc* sp., *P. fluorescens* and *Streptococcus* sp. (Table 1). Thitilertdecha *et al.* (2008) found that RPE exhibited antimicrobial activity against 5 pathogenic bacteria; however, the most sensitive strain inhibited by the methanolic extract (MIC = 2.0 mg/mL) was *Staphylococcus epidermidis*. The inhibition effect of rambutan extracts against *S. aureus* MRSA and *S. mutans* was also reported by Tadtong *et al.* (2011); however, the efficiency against *E. coli* was not demonstrated.

3.2.2 Minimum inhibitory concentration and minimum bactericide concentration

The minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) were determined to compare the potential of RPE against nine strains of foodborne pathogen and the data are shown in Table 2. From the broth micro dilution assay, MIC levels of RPE against the nine strains of bacteria were in the range 1.60–12.80 mg/mL, while their MBCs were in the range 1.60–25.60 mg/mL (Table 2). The MIC and MBC results could be divided into two categories, with the MBC values generally higher than those for MIC, except for *M. luteus* and *S. typhimurium* where the MIC and MBC values were similar. The RPEs had a

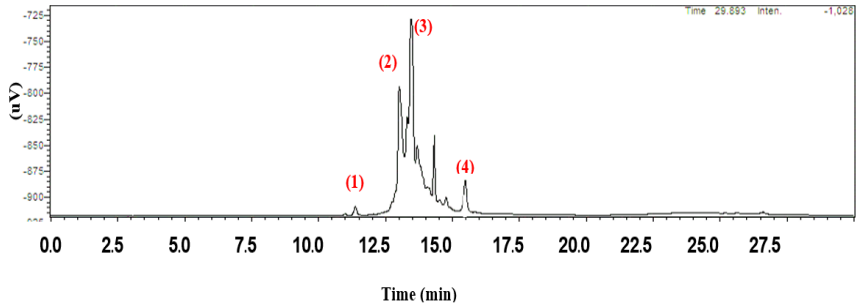

Figure 1. HPLC chromatogram of ethanolic rambutan peel extract; (1) gallic acid, (2) geraniin, (3) corilagin, and (4) ellagic acid.

Table 1. Diameter of inhibition zone representing antimicrobial activity of rambutan peel extract against nine strains of food-borne pathogens.

Bacterial strain	Inhibition zone diameter (mm)
Gram-negative bacteria	
Escherichia coli	ND
Pseudomonas fluorescens	ND
Salmonella typhimurium	8.67±0.29^{c}
Gram-positive bacteria	
Enterococcus faecalis	ND
Leuconostoc sp.	ND
Streptococcus sp.	ND
Micrococcus luteus	8.83±0.58^{b}
Bacillus sp.	9.00±0.50^{b}
Staphylococcus aureus	11.17±0.58^{a}

Different letters (a, b, c) in the same row indicate the results are significantly different at p ≤0.05 using Duncan's multiple-range test. ND: not detected.

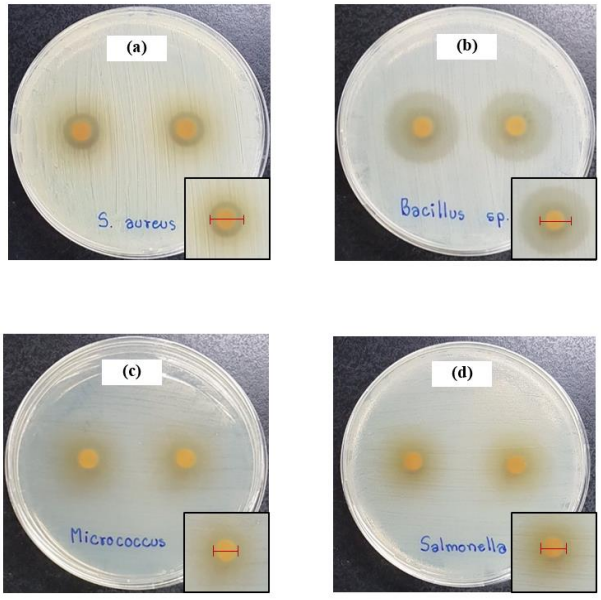

Figure 2. Zone of bacterial inhibition of rambutan peel extract against (a) Staphylococcus aureus, (b) Bacillus sp., (c) Micrococcus luteus and (d) Salmonella typhimurium. Bar line in the small picture represents the clear zone.

Table 2. MIC and MBC values of rambutan peel extract.

Bacterial strain	MIC (mg/mL)	MBC (mg/mL)
Gram-negative bacteria		
Escherichia coli	6.40	12.80
Pseudomonas fluorescens	6.40	12.80
Salmonella typhimurium	12.80	12.80
Gram-positive bacteria		
Enterococcus faecalis	3.20	25.60
Leuconostoc sp.	12.80	25.60
Streptococcus sp.	12.80	25.60
Micrococcus luteus	1.60	1.60
Bacillus sp.	1.60	3.20
Staphylococcus aureus	1.60	25.60

descending order of potency of *M. luteus* > *Bacillus* sp.> *E. coli*, *P. fluorescens*, *S. typhimurium* > *S. aureus*, *E. faecalis*, *Leuconostoc* sp. and *Streptococcus* sp.

Although, the RPE had the highest inhibitory efficiency against *S. aureus* (lowest MIC values), their bactericidal efficacy was lowest (MBC = 25.60 mg/mL). Therefore, in the next section we used the MBC of RPE, 25.60 mg/mL or 3% w/v of film solution, to prepare bioactive WPI/CNC film incorporating RPE.

3.3 Antioxidant activity

3.3.1 DPPH radical scavenging activity

The IC_{50} of RPE in this study indicated that the antioxidant activity of the RPE was higher than those antioxidant agents. Moreover, the antioxidant activity of the RPE was lower than that of vitamin C. These results agreed with previous published research, which reported that the DPPH activity of RPE was better than the activity of BHT (Sukatta *et al.*, 2010), while being comparable to vitamin C (Palanisamy *et al.*, 2008).

3.3.2 ABTS radical scavenging activity

The ABTS activity of ethanolic RPE was higher than that of BHT compounds, whereas the ABTS activity of the extract was significantly lower than that of vitamin C. These results corresponded to the results from DPPH assay. This might have been due to the higher number of hydroxyl functional groups of the phenolic compounds in the RPE

providing higher antioxidant activity (Naczk & Shahidi, 2004). It should be pointed out that RPE had better anti-oxidant activity than the other standards (BHT and α-tocopherol).

3.3.3 Ferric reducing antioxidant power activity

The FRAP value of ethanolic RPE was significantly higher than that of BHT, but lower than those of vitamin C and α-tocopherol (Table 3). These results indicated that RPE had higher redox potential or reducing power than BHT, whilst being lower than those of vitamin C and α-tocopherol. Khonkarn, Okonogi, Ampasavate, and Anuchapreeda (2010) found that ethanolic RPE exhibited higher antioxidant activity than BHT; however the reducing power of their extract was similar as for α-tocopherol.

3.3.4 Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of RPE (IC_{50} = 1.432 mg/mL) was lower than for BHA (IC_{50} = 1.613 mg/mL) as shown in Table 4, indicating better activity. However, the $OH^{\cdot}$ antioxidant activity of the extracts was lower than that of gallic acid.

3.3.5 Superoxide anion radical scavenging activity

The RPE had an IC_{50} value of 0.006 μg/mL, which indicated greater antioxidant activity than BHA, Trolox and vitamin C, while not being significantly different from that of gallic acid (Table 4). These results suggested that RPE could act as an antioxidant agent to inhibit intermediate substances involved in oxidative stress.

3.4. Properties of whey protein film with antioxidant extract

The color of the whey protein film is summarized in Table 5; the values of L*, a*, and b* are not significantly different between the samples with and without CNCs. The film was dark-brown, which is appropriate for a food packaging application with UV light sensitivity.

To verify the antioxidant and antimicrobial activity of the extract in WPI film with 5% CNC without RPE was formed. It was found that this film showed the DPPH activity of 44.57±0.37 μg/mL but no inhibition against *S. aureus.* However, the incorporation of 3% RPE in both whey protein films with and without 5% CNC exhibited higher antioxidant activity than film without RPE (Table 6). Moreover, films with 3% RPE inhibited *S. aureus.* However, incorporating the CNCs did not significantly affect the antimicrobial activity against *S. aureus* (Table 6). The antimicrobial activity was due to main components such as gallic acid and corilagin compounds found in WPI film (Table 7).

4. Conclusions

The main bioactive components of ethanolic RPE

Table 3. Antioxidant activity of rambutan peel extract using DPPH, ABTS and FRAP assays.

Compound	DPPH scavenging activity (IC_{50} μg/mL)	ABTS scavenging activity (IC_{50} mg/mL)	FRAP value (mmol Fe(II)/g sample)
Rambutan peel extract	8.668±0.473^{a}	0.411±0.056^{b}	5.768±0.230^{c}
Vitamin C	5.133±0.107^{a}	0.201±0.002^{a}	11.111±0.170^{a}
α-Tocohpherol	12.800±0.274^{a}	0.475±0.012^{c}	7.482±0.049^{b}
BHT	160.199±9.338^{b}	0.446±0.007^{bc}	4.044±0.040^{d}

Different letters (a, b, c, d) in the same row indicate the results are significantly different at $p \leq 0.05$ using Duncan's multiple-range test.

Table 4. Antioxidant activity of rambutan peel extract, BHA, gallic acid, Vitamin C and Trolox using hydroxyl scavenging activity and super oxide scavenging activity assays.

Compound	Hydroxyl scavenging activity (IC_{50} mg/mL)	Super oxide scavenging activity (IC_{50} μg/mL)
Rambutan peel extract	1.432±0.018^{b}	0.006±0.001^{a}
BHA	1.613±0.076^{c}	11.678±0.419^{d}
Gallic acid	1.339±0.060^{a}	0.007±0.001^{a}
Vitamin C	NA	0.187±0.011^{b}
Trolox	NA	0.629±0.045^{c}

Different letters (a, b, c, d) in the same row indicate the results are significantly different at $p \leq 0.05$ using Duncan's multiple-range test. NA: no analysis.

Table 5. Color parameters (L*, a*, b*, and ΔE*) of whey protein isolate (WPI) films containing 3% rambutan peel extracts at 0 and 5% cellulose nanocrystal (CNC).

CNC content (%)	Color parameter^{ns}			
	L*	a*	b*	ΔE*
0	46.92±4.09	9.60±0.88	7.30±2.35	-
5	49.90±1.12	8.62±0.56	6.16±0.92	5.67

ns = Non-significant difference between two samples ($p \leq 0.05$).

Table 7. Main active compounds of whey protein isolate (WPI) films containing rambutan peel extract.

CNC content (%)	Rambutan peel extract (%)	Active compounds (µg/g film)			
		gallic acid	geraniin	corilagin	ellagic acid
0	0	0.00	0.00	0.00	0.00
0	3	45.31	0.00	102.22	0.00
5	3	33.18	0.00	113.72	0.00

Table 6. Antioxidant activity (DPPH scavenging activity in terms of IC_{50}) and antimicrobial activity (inhibition zone) against *S. aureus* of whey protein isolate (WPI) films containing 3% rambutan peel extract at 0 and 5% cellulose nanocrystal (CNC).

CNC content (%)	Antioxidant activity	Antimicrobial activity
	DPPH scavenging activity IC_{50} (µg/mL)	Inhibition zone (mm)
0	34.40 ± 0.36^{b}	15.83 ± 0.58^{ns}
5	30.59 ± 0.12^{a}	15.67 ± 0.76^{ns}

Different letters in the superscript mean the significant difference between two samples ($p \leq 0.05$).

were corilagin, ellagic acid, geraniin and gallic acid. The extract could inhibit the growth of nine species of pathogenic and food spoilage bacteria. The best inhibitory effect was against *M. luteus* followed by *Bacillus* sp. However, the extracts had the same potential inhibition against five species of bacteria (*E. coli*, *P. fluorescens*, *S. typhimurium*, *S. aureus* and *E. faecalis*). The extract was less effective at inhibiting *Leuconostoc* sp. and *Streptococcus* sp., respectively. The antioxidant activity of RPE had better antioxidant activity among other standard substances such as BHT, trolox, vitamin C, α-tocopherol depending on the method of radical scavenging activity assay. These results showed that RPE had highly potent antioxidant activity and the extract could be incorporated as a bioactive compound for film packaging. Thus, RPEs were applied in whey protein isolate/cellulose nanocrystal film, producing a film that was dark-brown, which is appropriate for food packaging applications involving light sensitive products. However, the film did not exhibit antimicrobial activity against *S. aureus*. Synergism with substances from natural extracts, such as essential oil that possess high antimicrobial activity potential could improve the optical property and antimicrobial activity of the film. Nevertheless, the amount of RPE in practical use should be concerned because it affects the visibility of the product inside as well as the stickiness of film. The formula of film-forming solution should be modified to be suitable for film application and this will be presented in the further study.

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References

Chingsuwanrote, P., Muangnoi, C., Parengam, K., & Tuntipopipat, S. (2016). Antioxidant and anti-inflammatory activities of durian and rambutan pulp extract. *International Food Research Journal, 23*(3), 939-947.

Clinical and Laboratory Standards Institute. (2006). Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; Approved guideline. M45-A. Wayne, PA: Author.

Clinical and Laboratory Standards Institute. (2012). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-ninth edition. M07-A9. Wayne, PA: Author.

Khonkarn, R., Okonogi, S., Ampasavate, C., & Anuchapreeda, S. (2010). Investigation of fruit peel extracts as sources for compounds with antioxidant and antiproliferative activities against human cell lines. *Food and Chemical Toxicology, 48*, 2122–2129.

Kumar, S., Kumar, D., Jusha, M., Saroha, K., Singh, N., & Vashishta, B. (2008). Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. methanolic fruit extract. *Acta Pharmaceutica, 58*(2), 215-220.

Lam, N. T., Chollakup, R., Smitthipong, W., Nimchua, T., & Sukyai, P. (2017). Characterization of cellulose nanocrystals extracted from sugarcane bagasse for potential biomedical materials. *Sugar Tech, 19*(5), 539-552.

Lee, S. E., Han, J. H., & Han, J. (2016). Development and evaluation of apple peel- and Carboxymethylcellulose-based biodegradable films with antioxidant and antimicrobial properties. *Journal of Food Safety, 36*(3), 317-324.

Lim, T. K. (2013). *Nephelium lappaceum*. In T. K. Lim (Ed.), *Edible medicinal and non-medicinal plants: Volume 6, Fruits* (pp. 62-71). Dordrecht: Springer, the Netherlands.

Ma, Q., Guo, Y., Sun, L., & Zhuang, Y. (2017). Anti-diabetic effects of phenolic extract from rambutan peels (*Nephelium lappaceum*) in high-fat diet and streptozotocin-induced diabetic mice. *Nutrients, 9*(8), 801.

Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A, 1054*(1-2), 95-111.

Nanthakumar, R., Udhayasankar, M., Ashadevi, V., Arumugasamy, K., & Shalimol, A. (2014). In vitro antimicrobial activity of aqueous and ethanol extracts of *Rhinacanthus nasutus* - a medical plant. *International Journal of Phamaceutical, Chemical and Biological Sciences, 4*, 164-166.

Noronha, C. M., de Carvalho, S. M., Lino, R. C., & Barreto, P. L. M. (2014). Characterization of antioxidant methylcellulose film incorporated with α-tocopherol nanocapsules. *Food Chemistry, 159*, 529-535.

Özyürek, M., Bektaşoğlu, B., Güçlü, K., & Apak, R. (2008). Hydroxyl radical scavenging assay of phenolics and flavonoids with a modified cupric reducing antioxidant capacity (CUPRAC) method using catalase for hydrogen peroxide degradation. *Analytica Chimica Acta, 616*(2), 196-206.

Palanisamy, U., Cheng, H. M., Masilamani, T., Subramaniam, T., Ling, L. T., & Radhakrishnan, A. K. (2008). Rind of the rambutan, *Nephelium lappaceum*, a potential source of natural antioxidants. *Food Chemistry, 109*(1), 54-63.

Pereira, R. C., de Deus Souza Carneiro, J., Borges, S. V., Assis, O. B. G., & Alvarenga, G. L. (2016). Preparation and characterization of nanocomposites from whey protein concentrate activated with lycopene. *Journal of Food Science, 81*(3), 637-642.

Perera, A., Appleton, D., Ying, L. H., Elendran, S., & Palanisamy, U. D. (2012). Large scale purification of geraniin from *Nephelium lappaceum* rind waste using reverse-phase chromatography. *Separation and Purification Technology, 98*, 145-149.

Qazanfarzadeh, Z., & Kadivar, M. (2016). Properties of whey protein isolate nanocomposite films reinforced with nanocellulose isolated from oat husk. *International Journal of Biological Macromolecules, 91*, 1134-1140.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9), 1231-1237.

Rohman, A. (2017). Physico-chemical properties and biological activities of rambutan (*Nephelium lappaceum* L.) fruit. *Research Journal of Phytochemistry, 11*(2), 66-73.

Saelee, K., Yingkamhaeng, N., Nimchua, T., & Sukyai, P. (2016). An environmentally friendly xylanase-assisted pretreatment for cellulose nanofibrils Isolation from sugarcane bagasse by high-pressure homogenization. *Industrial Crops and Products, 82*, 149-160.

Sekar, M., Jaffar, F. N. A., Zahari1, N. H., Mokhtar, N., Zulkifli, N. A., Kamaruzaman, R. A., & Abdullah, S. (2014). Comparative evaluation of antimicrobial properties of red and yellow rambutan fruit peel extracts. *Annual Research and Review in Biology, 4*(24), 3869-3874.

Sukatta, U., Rugthaworn, P., Leebunyanon, M., & Pitpiang chan, P. (2010). Bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of some fruit peels extract. *Proceedings of 48th Kasetsart University Annual Conference: Agro-Industry*. 3-5 March, 2010, Kasetsart University, Bangkok, Thailand.

Sukatta, U., Rughtaworn, P., Tuntawiroon, O., & Meaktrong, W. (2011). Physico-chemical properties, chemical composition and in vitro antimicrobial and free radical-scavenging capacity of tea tree essential oil in Thailand. *Kasetsart Journal (Natural Science), 45*(3): 473-480.

Sukyai, P., Anongjanya, P., Bunyahwuthakul, N., Kongsin, K., Harnkarnsujarit, N., Sukatta, U., Sothornvit, R., & Chollakup, R. (2018). Effect of cellulose nanocrystals from sugarcane bagasse on whey protein isolate-based films. *Food Research International, 107*, 528-535.

Tadtong, V., Sirivan, A., Pichsinee, W., Pimpa, C., Pichai, C., & Sareedenchai, V. (2011). Antibacterial activities of rambutan peel extract. *Journal of Health Research. 25*(1), 35-37.

Tajkarimi, M. M., Ibrahim, S. A., & Cliver, D. O. (2010). Antimicrobial herb and spice compounds in food. *Food Control, 21*(9), 1199-1218.

Thitilertdecha, N., Teerawutgulrag, A., & Rakariyatham, N. (2008). Antioxidant and antibacterial activities of *Nephelium lappaceum* L. extracts. *LWT - Food Science and Technology, 41*(10), 2029-2035.

Thitilertdecha, N., Teerawutgulrag, A., Kilburn, J. D., & Rakariyatham, N. (2010). Identification of major phenolic compounds from *Nephelium lappaceum* L. and their antioxidant activities. *Molecules (Basel, Switzerland), 15*(3), 1453-1465.

Thitilertdecha, N., & Rakariyatham, N. (2011). Phenolic content and free radical scavenging activities in rambutan during fruit maturation. *Scientia Horticulturae, 129*(2), 247-252.

Zhu, K., Zhou, H., & Qian, H. (2006). Antioxidant and free radical-scavenging activities of wheat germ protein hydrolysates (WGPH) prepared with alcalase. *Process Biochemistry, 41*(6), 1296-1302.