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The effects of cellulose particle sizes on biofilm from longkong peel

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ABSTRACT: The effects of longkong cellulose particle sizes on its biofilm were investigated. The particle sizes were divided into 355, 125, and 75 µm. The X-ray diffraction patterns confirmed the cellulose phase with the secondary phase in some samples. The second phase did not affect biofilm preparation. The FTIR analysis suggested a strong presence of carboxyl and hydroxyl groups in carboxymethylcellulose (CMC) powder, which was synthesized from the cellulose particle size of 75 µm. The SEM and EDS observations revealed that the cellulose particle size of 75 µm produced a smooth surface and homogeneity of biofilm. The decrease in particle size significantly induced the high-water solubility of CMC and high reaction surface, leading to high-quality biofilm. The light absorption of all biofilms was significantly different. Herein, the result suggested that high-quality film from longkong peel could be produced using the cellulose particle size of 75 µm, which may be an alternative bioproduct in the near future.

KEYWORDS: longkong peel, carboxymethylcellulose, biofilm, plasticizer

INTRODUCTION

Biowaste rapidly increases yearly due to large consumption such as in local markets, agriculture, and manufacturing industries [1]. The unsuitable biowaste treatment can impact the environment and human health [2]. Several research studies are concerned about this problem and are trying to develop biomaterials and biofilms from biowaste [3, 4]. Recently, biofilms from biowaste, i.e., fruit peels such as durian peel [5], banana peel [6], and mangosteen peel [7], have been produced. These examples introduce the potential usefulness of biowaste in several industries such as food, packaging, and biomaterial. Therefore, this is our motivation to develop biofilms from fruit peel.

Longkong (*L. domesticum*) is attractive among fruit peels because it is a typical local fruit in Southeast Asia countries such as Indonesia, the Philippines, and Thailand. Longkong is a perishable fruit; thus, it requires processing to prolong its shelf life. Examples are jelly and juice. This preservation makes a large amount of biowaste from longkong peel. Therefore, we are interested in using longkong peel for biofilm fabrication to decrease its biowaste and increase the economic opportunity of longkong peel in bioplastic industries. There are several ways of biofilm preparation from cellulose. One of those is the cellulose derivative, which improved cellulose properties as a constituent of the cell wall by replacing methyl and carboxymethyl groups. It is well known as carboxymethylcellulose (CMC). The CMC is not toxic [8] and has high water solubility; thus, it is widely used for biofilms [5, 6, 9]. However, the solubility of CMC in water significantly affects the physical quality of biofilms. It is well known that small particles have a high surface area; thus, it may improve CMC solubility in water, leading to a high quality of biofilms. With this hypothesis, the influences of cellulose particle sizes on CMC powder and biofilms are attractive.

In previous works, the biofilms from durian peel (100%) had promoted industrial utilization as a film-forming agent, binding agent, sustained release agent, gelling agent, and metal absorber [5]. The biofilms from bananas (100%) promoted a potential edible coating to extend the shelf life of strawberry fruit [6], whereas the biofilm from mangosteen peel still needed using glycerol as a plasticizer in mechanical property improvement [7]. Among these works, they focused on the properties of the biofilms. Unfortunately, the focus on biofilm preparation was scarce, particularly the effects of cellulose particle sizes. This reason became our motivation for investigating the impact of cellulose particle sizes on the biofilm preparation from longkong peel. Moreover, we would like to propose a new biofilm preparation from longkong peel without plasticizer addition such as glycerol and starch. Thus, the effect of cellulose particle sizes on biofilm from longkong peel is examined in this work. The X-ray diffraction (XRD) technique is used to confirm the phase of all samples. The powder morphology and chemical composition of the samples are observed by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS), respectively. Fourier transform infrared (FT-IR) identifies the chemical bonding of all samples. The light absorption of all biofilms is

examined using UV-Vis spectroscopy. The influences of cellulose particle sizes on biofilm fabrication from longkong peel will be discussed.

MATERIALS AND METHODS

Materials

Longkong was obtained from the market (Rayong, Thailand). All chemicals used to extract cellulose and synthesize CMC were sodium hydroxide (NaOH, CAS-No. 1310-73-2) and chloroacetic acid ($C_2H_3ClO_2$; CAS-No. 79-11-8) purchased from Sigma-Aldrich (Missouri, USA). The isopropanol (C_3H_8O; CAS-No. 67-63-0), ethanol (C_2H_5OH; CAS-No. 64-17-5), methanol (CH_3OH; CAS No. 67-56-1), and acetic acid (CH_3COOH; CAS-No. 64-19-7) were purchased from Merck KGaA (Darmstadt, Germany).

Cellulose extraction

The longkong peel was dried by sunlight for 2 days and ground in the first step. The ground powder was boiled in NaOH (1 M) with magnetic stirring for 1 h. Then, the powder was filtered using a 10 µm-polyethylene bag and washed with distilled water. The fibers were bleached with hydrogen peroxide (30%) for 24 h. After that, it was filtered and rewashed. Finally, the powder was dried in the oven at 55 °C for 24 h. After obtaining the cellulose, we used a sieve shaker (Retsch, AS 200, Düsseldorf, Germany) for 1 h to separate the particle sizes of cellulose. This study divided the cellulose particle sizes into 3 dimensions, including 355 µm (mesh No. 50), 125 µm (mesh No. 150), and 75 µm (mesh No. 220).

CMC synthesis

The cellulose powder (7 g), NaOH (24 ml), and isopropanol (165 ml) were mixed in the beaker and were heated at 40 °C for 30 min. Then, the chloroacetic acid (8 g) was added to the solution with magnetic stirring for 1.5 h. After that, the mixture was put in the oven at 55 °C for 2.5 h for a chemical reaction. After taking the mixture from the oven, there were 2 phases: liquid and solid. The liquid phase was discarded, and the solid phase was suspended with methanol (70%) and neutralized using acetic acid. The solid phase suspended in methanol was filtered and washed 3 times with ethanol (70%). The filtration residue was dried in the oven at 55 °C for 24 h.

CMC film preparation

First, the CMC powder (1.2 g) was dissolved in 20 ml of distilled water at 80 °C for 10 min to prepare the solution. Then, the solution was poured into a glass die with a square shape (5 × 5 cm) and then put in the oven at 55 °C for 1 h.

Chemical composition and microstructure characterization

The chemical composition of the longkong peel, cellulose, CMC, and biofilm was confirmed by Fourier transform infrared (FT-IR; FT-NIR PerkinElmer; Massachusetts, USA). The X-ray diffraction techniques (XRD; Smart Lad Rigaku; Tokyo, Japan) were used to study the phase characteristics of the samples. The microstructural observation and chemical composition of samples were investigated using scanning electron microscopy (SEM; SU5000 Hitachi; Tokyo, Japan) and energy dispersive spectroscopy (EDS; SU5000 Hitachi). The light absorbance of all films was examined using a UV-visible (UV-Vis; UV2600 Shimadzu; Tokyo, Japan) spectrophotometer in the wavelength range from 200 to 600 nm.

RESULTS AND DISCUSSION

X-ray diffraction analysis

The crystalline structure of the longkong peel is shown in Fig. 1. The result exhibited the peaks at 22°, corresponding to the (200) diffraction plane. This peak was reported as crystalline cellulose, which agrees with the JCPDS No. 03-0226 standard [10]. Moreover, the (110) and (004) peaks suggested the presence of an amorphous phase of cellulose. This result could confirm that longkong peel was mainly composed of cellulose with a different structure. Regarding the X-ray diffraction patterns of the cellulose with different sizes, it was seen that all celluloses had a similar XRD pattern. The peaks at 16°, 22°, and 35° for cellulose samples corresponded to the (110), (200), and (004) planes, respectively. These peaks indicated the presence of the cellulose phase [11]. The (110) peak was related to the amorphous cellulose phase, whereas the (200) peak was related to crystalline cellulose. Interestingly, the intensity of the (004) cellulose peak seemed to increase compared to that of the longkong peel. This result was attributed to the amorphous lignin or hemicellulose extermination during the bleaching [12].

The NaCl phase was noticeable in the case of CMC powder synthesized from cellulose with different sizes. This phase might have come from NaOH and chloroacetic acid [13]. The effects of the observed phase will be discussed later. Besides the secondary phase, the decrease of (200) peak intensity in all CMC powders could be observed. This decrement resulted from the reduction of cellulose crystallinity due to the hydrogen bonds in the cellulose molecule being broken by NaOH during the carboxymethylation reaction [14]. The XRD patterns of the biofilms did not differ from their CMC powders. The results indicated that the main phase of biofilms was still cellulose with a low crystalline structure, like CMC powder. However, in biofilm, the NaOH phase was present (JCPDS No. 01-075-0642) at 22°, 24°, and 27°. Na^+ ions in a car-

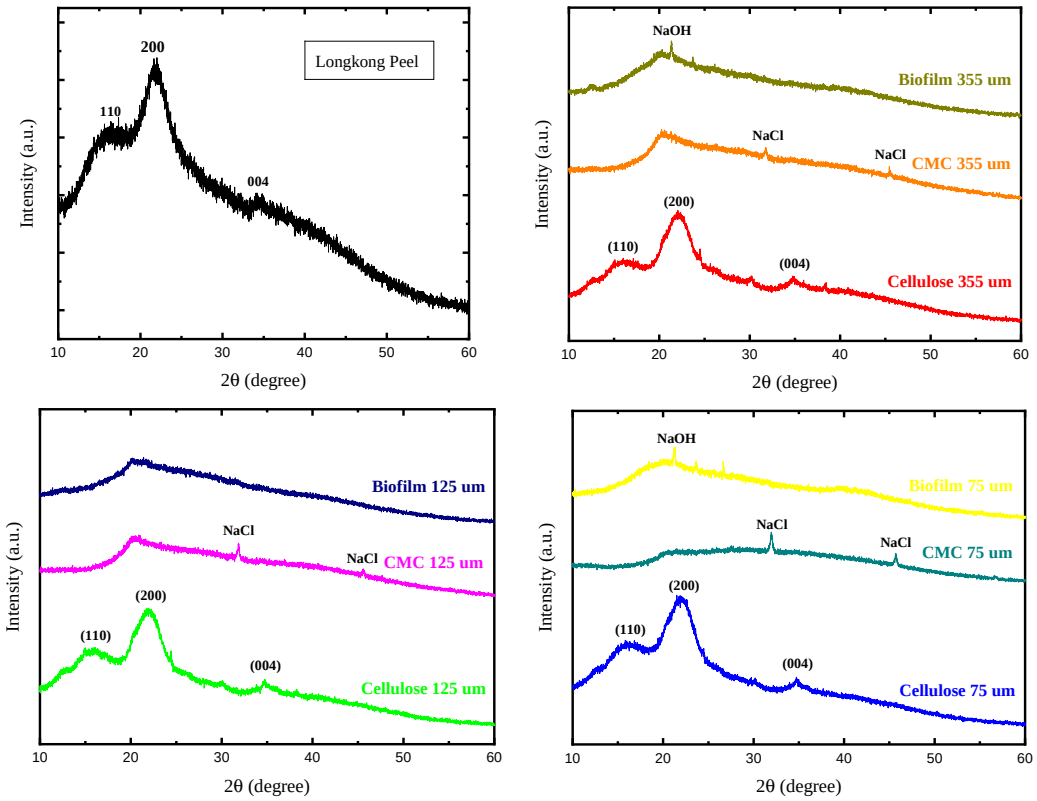

Fig. 1 X-ray diffraction patterns of all samples.

boxylic group of CMC might have reacted with OH^- of water, producing such a phase [15].

FTIR analysis

Fig. 2 illustrates the FTIR spectra of longkong peel, cellulose, and CMC powders. Regarding longkong peel, the FTIR peaks revealed several chemical bonds in these samples. The first peak at 3290 cm^{-1} was associated with a host of hydroxyl group stretching vibrations (—OH stretching); the second peak (at 2927 cm^{-1}) was related to the stretching hydrocarbon group vibrations (C—H stretching). The others were the peaks at 1589 cm^{-1}, 1408 cm^{-1}, and 1102 cm^{-1}, which belonged to the stretching vibration of the carboxylic group (C=O stretching), a $-CH_2$ scissoring peak, and the stretch vibration (—O— stretching) of the ether group, respectively. All observed peaks reflected the chemical bonding in the cellulose structure [16]. This, therefore, confirmed the cellulose phase in the longkong peel. The figure compares FTIR spectra between cellulose and CMC for each particle size. The FTIR peaks of CMC and cellulose of all particle sizes were not significantly different. However, the FTIR absorption intensity of cellulose changed when it became CMC powder; for the particles at 355 µm and 125 µm, the absorption increased slightly compared with their cellulose. Interestingly, the absorption became much better in particle size of 75 µm. This increase was attributed to a strong presence of carboxyl group (1589 cm^{-1}) and hydroxyl group (3290 cm^{-1}) [17], particularly at 75 µm. The result implied the contribution of cellulose particle size to the CMC bonding structure. We also compared FTIR spectra of cellulose and CMC with different sizes, as seen in the figure. It was seen that there was no significant difference in cellulose between all particle sizes. Nevertheless, the difference was evident in CMC powder. The absorption of 355 µm was the lowest, whereas strong absorption was observed in particle size of 75 µm. This result indicated the strong presence of carboxyl and hydroxyl groups in CMC powder synthesized from 75 µm cellulose. These results could conclude that the cellulose particle size of 75 µm significantly enhanced the functional group of cellulose, which might lead to the better water solubility of its CMC powder. This result will be explained in the later section.

SEM-EDS analysis

Fig. 3 shows SEM images of all samples. In the case of the longkong peel, it could be seen that the sample contained fiber and large particles of cellulose (>500 µm). The SEM images confirmed the difference in cellulose

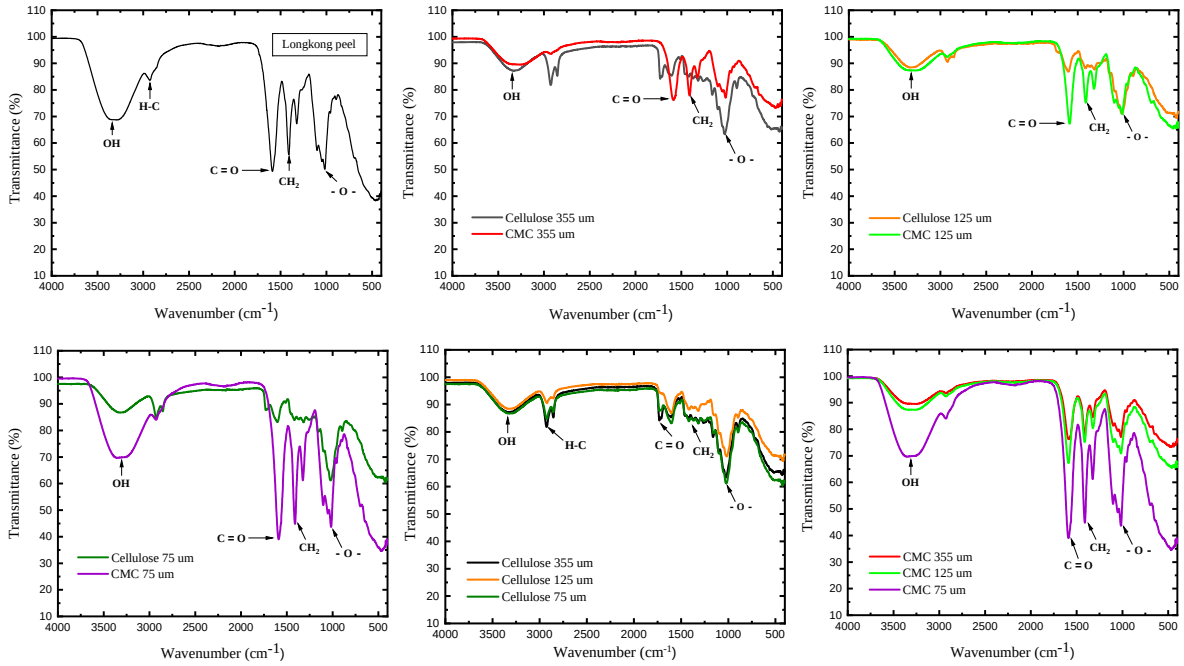

Fig. 2 FTIR spectra of all samples.

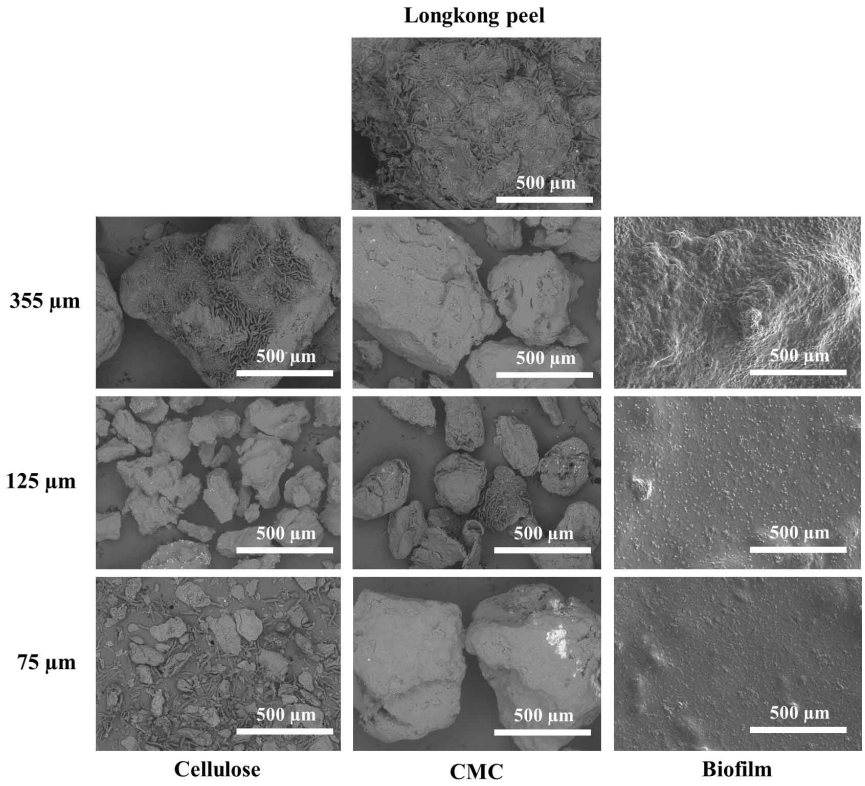

Fig. 3 SEM images of all samples.

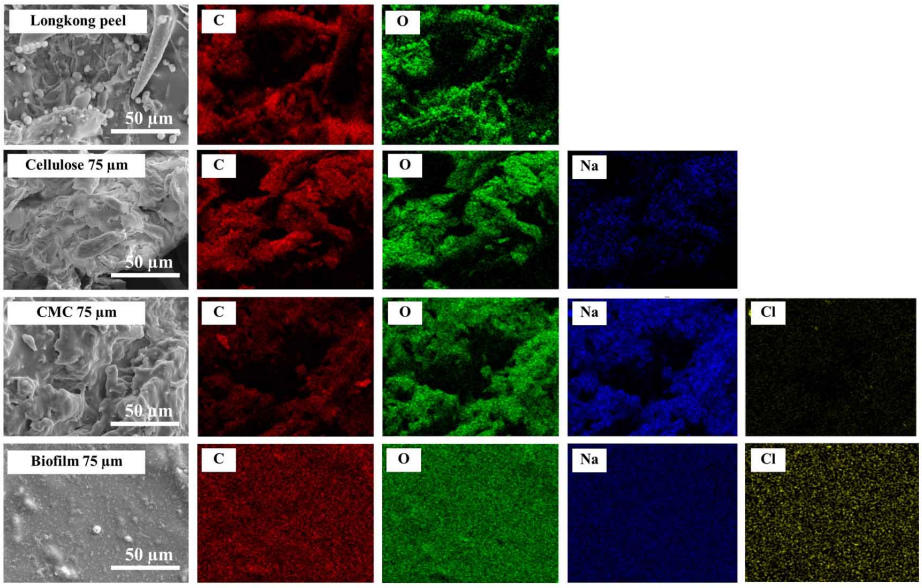

Fig. 4 EDS results of longkong peel, cellulose, CMC, and biofilm from cellulose particles at 75 µm.

particle sizes. When each cellulose was synthesized to CMC powder, it was found that the particle size of CMC increased when compared with their celluloses, particularly at 75 µm. The CMC particle size was about 500 µm or more. This result might be attributed to powder agglomeration because the particle (75 µm) was relatively small compared to others. Thus, it had a higher surface area, leading to better chemical reactions and agglomeration. Therefore, a significant increase in CMC particle size was observed in particle at 75 µm.

It should be noted that, with this result, it was difficult to discuss the apparent effects of cellulose sizes on CMC powders. For the biofilms, it was found that the topography of the biofilm surface was different, depending on the cellulose particle sizes. The obtained biofilm had a rough surface for the cellulose particles at 355 µm, whereas the biofilm surface became better with the decreasing cellulose particle sizes. In general, the water solubility of CMC influences the physical quality of the biofilm; thus, the high solubility of CMC in water causes a smooth and homogeneous surface of the biofilm. In addition, the strong presence of carboxyl and hydroxyl groups in CMC from cellulose particle at 75 µm supported its high-water solubility. As this contribution, the CMC powder synthesized from cellulose particle at 75 µm could produce a smooth surface and better homogeneity of biofilm when compared with others.

The sample prepared from cellulose particles at 75 µm was selected for the study in chemical composition by EDS analysis. The EDS results are shown in Fig. 4. The figure shows that the longkong peel was composed of carbon (C) and oxygen (O). Both elements are compositional, mainly in cellulose. This observation was accompanied well by its XRD and FTIR results. However, when cellulose was extracted from the longkong peel, it was seen that sodium (Na) element was present. This observation did not agree with the XRD and FTIR results of the cellulose. The amount of Na might have been very low (∼1.9 wt%); thus, it was not observed in both techniques. Na in cellulose was expected to come from NaOH used in cellulose extraction. The C and O elements were still found in the sample when the cellulose was synthesized into CMC powder. Interestingly, compared with cellulose, the Na element increased from ∼1.9 wt% to ∼14.3 wt% in CMC powder. With this study, the detected part of Na might be divided into 2 chemical formations. The first should be the NaCl phase, which was observed in XRD patterns (Fig. 1). The chlorine (Cl) element was also detected in its EDS image. The second one should come from the functionalization of cellulose derivatives. This functional group increased the solubility of CMC powder in water. In the case of biofilm, the sample contained C, O, Na, and Cl elements, like CMC powder. From these results, it could be mentioned that the NaOH phase did not affect the biofilm preparation and quality. Hence, we could fabricate a smooth and homogenous surface of biofilm employing the cellulose particle size at 75 µm.

UV-VIS analysis

Fig. 5 presents the light absorption spectra of all biofilms. The figure shows that the biofilms absorbed light from 200 to 300 nm, which belonged to ultraviolet light. Interestingly, 2 absorption peaks existed in this wavelength region. The first had a maximum

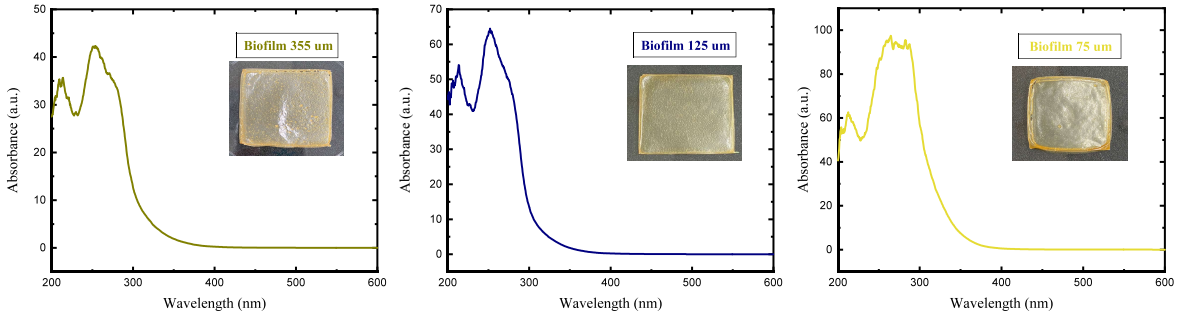

Fig. 5 UV-Vis spectra of all biofilms.

Table 1 The comparison of the components and processing method of biofilms obtained from different agricultural waste.

Agriculture waste	Extracted component	Other component	Biofilm processing method	Ref.
Longkong Peel	Cellulose	–	Casting	This work
Durian peel	Cellulose	–	Casting	[5]
Banana stem	Cellulose	–	Casting	[6]
Mangosteen peel	Cellulose	Cassava starch, glycerol	Casting	[7]
Avocado peel and seed	Pectin, cellulose, starch	Ppolyglycerine-3	Casting	[18]
Carrot	Cellulose, pectin	–	Casting	[19]
Sugarcane bagasse	Cellulose	Glycerol, sorbitol	Casting	[20]
Orange peel	Cellulose, pectin	Glycerol	Casting	[21]
Passion fruit peel	Cellulose	Starch, glycerol	Casting	[22]

absorption peak of around 210 nm; the second had a maximum height of around 260 nm. Moreover, the absorption peak of biofilms prepared from cellulose particles at 355 and 125 µm was quite sharp. On the contrary, the biofilm of cellulose particle at 75 µm showed a broad absorption peak. The quality of the biofilm surface was believed to cause this difference. The images of biofilms are provided as an inset in Fig. 5. It was seen clearly that the biofilms (cellulose particles at 355 and 125 µm) had inhomogeneity and had a large particle of insoluble CMC. These particles might induce those absorption peaks. Whereas the biofilm prepared from cellulose particle at 75 µm had better homogeneity, resulting in better light absorption. Thus, it might absorb the light in a more extensive wavelength range, causing a border peak.

Comparison with the biofilms from different agricultural waste

The comparison of the biofilms from longkong peel and other biofilms from different agricultural waste is listed in Table 1. It should be mentioned first that we fabricated biofilm by casting method; thus, we selected the biofilms from the same method. In the table, several biofilms could be prepared from agricultural waste such as avocado, banana, carrot, sugarcane bagasse, mangosteen, orange, passion fruit, and durian. The most extracted component was cellulose. Some fruits might have other extracted ingredients such as pectin and starch. The examples were the biofilms from avocado peel which needed Ppolyglycerine-3; sugarcane bagasse needed glycerol and sorbitol; mangosteen needed cassava starch and glycerol; orange needed glycerol; passion fruit needed starch and glycerol. Whereas the biofilms from durian, banana, carrot, and longkong in this work could be produced without further addition. The result of this work promoted that the longkong peel could produce biofilm without adding other components. Among these works, most of the study focused on biofilm properties but did not emphasize the cellulose particle sizes. Thus, the result in the effect of cellulose particle sizes on longkong biofilm preparation could be worthwhile for biofilm production.

CONCLUSION

In this work, the biofilms were successfully fabricated from the longkong peel with different sizes of cellulose. The XRD results suggested the cellulose phase in all samples. The presence of secondary phases was attributed to the used starting materials. FTIR analysis revealed a strong presence of hydroxyl and carboxyl groups in CMC powder synthesized by cellulose at 75 µm. The cellulose particle size of 75 µm could form better CMC powder, producing a smooth surface and homogeneity of biofilms. The UV-Vis spectra of all biofilms were not significantly different. The result suggested high physical quality of the biofilm from longkong peel could be produced from the cellulose particle size of 75 µm.

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