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Characterization of gelatin film supplemented with carboxymethyl cellulose from pineapple core

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Abstract

Pineapple (*Ananus comosus*) cores, waste from pineapple processing, contain cellulose and exhibiting a potential for food packaging. The edible film made from gelatin and carboxymethyl cellulose (CMC) synthesized from cellulose extracted from pineapple core showed a light-yellow color and smooth surface. They had the same moisture content, morphology and tensile strength as film from gelatin with commercial CMC. The solubility, thickness, water vapor permeability, and antioxidant activity were also similar. Thus, CMC from pineapple core has the potential for edible film applications which was evaluated compared to commercial CMC.

Keywords: Carboxymethyl cellulose, Film, Gelatin, Pineapple core

1. Introduction

The pineapple (*Ananus comosus*) is the one of economic crop in the world and Thailand is the 4th of pineapple production, which has less productivity than the Costa Rica, Philippines and Brazil. However, the top supplier of processed pineapple products, particularly canned pineapple and pineapple juice has been documented currently to be Thailand [1]. Pineapple plants can be grown throughout Thailand, including the northern, northeastern, central, and southern regions. In order to process, only the flesh (30-60% of whole fruit) has been used for producing the canned pineapple. The other sections are considered to be by-products, which are pineapple corks, stems, peels, and cores, accounting for 2.7-5.9, 2.4-6.8, 29-42, and 9.4-20%, respectively. In Thailand, the 247,089.9 tons of pineapple core (14.7% of whole by-products) have been estimated from canned pineapple industries in 2020 [1]. Such a huge by-product must be managed properly to prevent environmental pollution. Chemical compositions of pineapple core have been reported to contain lignin, cellulose, hemicellulose, and starch [2]. Further utilization of this by-product for being higher value products would be the challenge to maximally utilize the pineapple resources. Extraction of cellulose from pineapple cores is an alternative valorization and has been done. Pineapple core cellulose was a product of alkali extraction with a bleaching process which was 95.2% (dry basis) [3].

Cellulose is an abundant component available in plant cell wall. It is normally conjugated either lignin or hemicellulose. It accounts for approximately 40-55%, depending on plant species. [4]. The application of plant cellulose has been focused on textile and papers industries [5]. Due to less solubility, its utilization in food system is limited. However, modification of cellulose to carboxymethyl cellulose (CMC) improves solubility. In order to prepare, cellulose must be activated by hot alkaline (NaOH) solution in an organic solvent prior to etherifying the active cellulose with monochloroacetic acid. The carboxymethylation process allows the hydroxyl groups to substitute with carboxymethyl group. Production of CMC using cellulose extracted from agricultural waste have been reported in sago [6], durian rind [5], orange peel [7], empty palm bunches [8], papaya peel [9], rice straw [10], cocoa pod husks [11] and young coconut husks [12]. However, CMC synthesis from cellulose extracted from pineapple core has not been reported.

Application of CMC in food industries has been widely employed, including edible film and packages. The development of biodegradable films is currently focused. This would reduce the utilization of commercial plastics, which produce from petrochemical compounds and resistant to decomposes. This accounts for generating pollution and is a severe environmental problem [13]. Therefore, environmentally friendly edible film could help to solve these problems. It can be utilized as an alternative packaging in food applications. An edible casing of sausage is an example of this application. Formation of edible film can be done by mixing film forming solution with plasticizers, particularly glycerol prior to drying the thin layer film forming solution to form a thin film. This film exhibited the flexible property with less water vapor permeability [14] Although a film forming solution could be prepared from several compounds (carbohydrates, proteins, lipids), the final properties will be different. Film forming solution containing gelatin is mostly chosen for producing edible packages for meat products, especially sausages. The gelatin film provides suitable properties by increasing flexibility and reducing tensile strength [15]. On the other hand, the starch increased the tensile strength of film. Incorporating CMC in gelatin film could reduce the vapor transmission rate and improve the film strength. This suggested that CMC synthesized from cellulose extracted from pineapple core has the potential to improve the properties of an edible film from gelatin and should be investigated.

2. Materials and methods

2.1 Raw materials

CMC from pineapple (*Ananus comosus*) core was synthesized according to Seubsunthorn and Jirukkakul [16]. Commercial CMC was purchased from Changzhou Guoyu Environmental S&T Co., Ltd. (Changzhou, China). Sodium hydroxide and glacial acetic acid were purchased from RCI Lab-scan Co., Ltd. (Bangkok, Thailand). Isopropanol, methanol, ethanol, gelatin and cassava starch were purchased from Union Science Co., Ltd. (Khon Kaen, Thailand). Mono chloroacetic acid was purchased from Laba Chemie Pvt., Ltd. (Maharashtra, India) The hydrogen peroxide was produced from QRëC®.

2.2 Formation of CMC film

CMC synthesized from pineapple (P-CMC) or commercial CMC (C-CMC) (1.5 g) was mixed with De ionized (DI) water, DI- water (50 mL) and stirred at 90-95°C for 10 min until completely dissolved. After cooling down the temperature to 60°C, gelatin (1.5 g) or gelatin: starch (1:1) (1.5 g) has been added to the mixture. Subsequently, glycerol (1.5 mL) was added and continued mixing for 15 min to obtain the film forming solution. The film forming solution (50 mL) was poured onto the mold (diameter = 15 cm). Then, it was dried at 40°C and 75%RH for 48 h. Dried film was removed and kept at desiccator for further analysis.

2.3 Determination of physical properties of film

2.3.1 Thickness

The thickness of film was measured with a hand micrometer for three points of each film and the average value was calculated.

2.3.2 Moisture content

The film was cut into 2 x 2 cm and placed in the pre weighed aluminum cup (W_i). The film and aluminum cup were then dried for 24 h at 105 °C [17]. After that, the final weight (W_f) was recorded to calculate the moisture content (MC) as equation:

$$\text{Moisture content (\%)} = \left(\frac{W_i - W_f}{W_i} \right) \times 100 \quad (1)$$

2.3.3 Solubility

Film solubility (FS) was determined according to Oh et al. [18]. Firstly, a 2 x 2 cm sample film was cut and dried at 105 °C for 3 days before being weighed (W_i). Thereafter, the annealed film was placed in an Erlenmeyer flask, filled with 50 mL of DI-water. The flask was then shaken (200 rpm) for 3 h before filtered through filter paper (Whatman No. 4) and dried for 25 min at 105 °C. Weight loss (W_f) or percentage in solubility (%) was calculated as follows equation:

$$\text{Solubility (\%)} = \left(\frac{W_i - W_f}{W_i} \right) \times 100 \quad (2)$$

2.3.4 Water vapor permeability

Water vapor permeability of the CMC films was performed based on modified ASTM E96-87 [19]. According to this test, the films were cut into a circle (with 6 mm diameter). Films were placed on the test cup and sealed the ring lid of the test cup (contain water 30 - 50 mL) prior weighing. Then, sample cups were placed in the hygroscopic bowl and recorded the weigh every 1 h for 8 h. Water vapor transmission rate (WVTR) (g/m².day) was calculated by Equation 3. Finally, water vapor permeability (WVP) was calculated using the following Equation 4.

$$WVTR = \frac{G/t}{A} \quad (3)$$

$$WVP = \left(\frac{WVTR}{(P_{A1} - P_{A2})} \right) \times D \quad (4)$$

Where: G/t is the rate of weigh change per time (g/day), A is the sample surface area (m²), is the pressure difference inside and outside the test cup (kPa) and D is the film thickness (m).

2.3.5 Color

Color of film was measured with a Hunter Lab (MiniScan EZ 4000). The film was placed on the testing chamber prior evaluated the color value (L* a* b* values) and expressed as an average value from 3 measurements.

2.3.6 Tensile strength and elongation

Tensile strength (TS) of films were determined and reported the unit as MPa. In addition, elongation (%) at break (E) was determined using a Universal Testing Machine Model 1000 (H1K-S.UK), according to the procedure of ASTM D882-97 [20]. The film was cut to 12 × 1 cm and then conditioned with 55%RH in a humidity chamber, controlled at 25 °C for 12 h. Samples were clamped between 2 initial grips, which were set at 10 mm. The crosshead speed was controlled at 10 mm/min. Each sample was tested for 5 replications. TS was calculated according to this equation:

$$TS = \frac{F_{max}}{A} \quad (5)$$

Where: F_{max} = Maximum tearing force of the film (N).
A = thickness x width of film (m²)

2.3.7 Antioxidant activity

Antioxidant activity of film was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The film (400 mg) was used to extract antioxidant by soaking in 3 mL of methanol for 48 h [21]. The extract (0.5 mL) was mixed with 1.5 mL of ethyl acetate prior to adding with DPPH solution (1.5 mL). The mixture was incubated in the dark for 30 min before measuring an absorbance at 515 nm (A₅₁₅). This value was reported as A_{sample}. The A_{control} was conducted the same manner without adding the film extract. The DPPH antioxidant activity was calculated as shown in equation 6.

$$AA (\%) = \left(\frac{A_{Control} - A_{Sample}}{A_{Control}} \right) \times 100 \quad (6)$$

Where: AA = antioxidant activity (%)
A_{Control} = A₅₁₅ of DPPH solution without extract
A_{Sample} = A₅₁₅ of DPPH solution with extract

2.3.8 Statistical analysis

The differences between the mean values of the multiple groups were analyzed by one-way analysis of variance (ANOVA) with Duncan's multiple range test. ANOVA data with a *p* < 0.05 was classified as statistically

significant. SPSS 23.0 software and the Microsoft Excel 2010 program were used to analyze and report the data. Mean values from the duplicated experiments were reported.

3. Results and discussions

3.1 Physical appearance and color value of gelatin film supplemented with CMC

A comparison of the physical qualities of gelatin films supplemented with CMC from P-CMC and C-CMC were determined. The appearance of 4 sample films was shown in Figure 1. The physical appearance of films revealed that gelatin -based films produced in the present of C-CMC exhibited the opaque white film (Figure 1(A)), while a reduction of whiteness was observed in the presence of P-CMC (Figure 1(B)). It can be seen that gelatin film incorporated with starch of both C-CMC and P-CMC showed the same appearance that was lower yellowness in color and opaque thin sheet. When CMC, gelatin, and starch were mixed in a 1:1:1 ratio, the smooth surface and soft texture of film were obtained. The results agreed with Uthaiku [22] who studied film containing cellulose from cattail (*Typha angustifolia* L.) in saline soil.

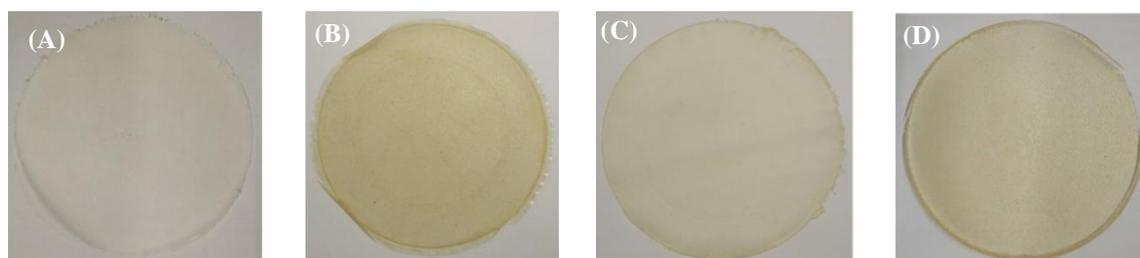


Figure 1 Physical appearance of gelatin films supplemented with CMC from commercial grade (A), pineapple core (B), commercial grade with starch (C) pineapple core with starch (D).

3.1.1 Color of gelatin films

The gelatin films supplemented with P-CMC and gelatin films supplemented with P-CMC with starch appeared as yellow films indicated by the b value (Table 1). This is primarily because of the yellow-brown residues, natural pigments in pineapples, left over after extraction of P-CMC. This is similar to films derived from oil palm lignocellulosic waste, as observed by Song and Othman [23]. They reported that the films appeared brown because of the natural color of the line. In contrast, films produced by C-CMC and C-CMC with starch were clearer and whiter (Table 1). Based on color values, the gelatin film supplemented with P-CMC exhibited higher L* and b* values compared to the others, suggesting the lighter and more yellowness. The clear and yellow appearance was observed in edible film prepared from corn husk waste [24]. The gelatin films supplemented with P-CMC with starch had the highest a* value, suggesting an increase redness up on adding starch in the filming formula. However, a reduction of yellowness was also observed from starch in addition in gelatin film supplemented with P-CMC. Therefore, this film would likely appear as the yellow film as seen in the Figure 1.

Table 1 Color values and mechanical properties of gelatin film supplemented with different CMC with/without starch.

Type of film	Color value			Tensile strength (kg/cm ²)	Elongation (%)
	L*	a*	b*		
Gelatin film with C- CMC	85.56 ± 0.02 ^b	1.04 ± 0.01 ^d	2.00 ± 0.02 ^c	0.24 ± 0.01 ^c	32.40 ± 0.20 ^b
Gelatin film with P-CMC	91.26 ± 0.07 ^a	1.86 ± 0.02 ^b	11.04 ± 0.05 ^a	0.23 ± 0.01 ^c	34.49 ± 0.28 ^a
Gelatin film with C-CMC with starch	86.11 ± 0.01 ^b	1.47 ± 0.01 ^c	0.51 ± 0.02 ^d	0.33 ± 0.01 ^b	26.41 ± 0.11 ^d
Gelatin film with P-CMC with starch	83.19 ± 0.03 ^c	2.15 ± 0.06 ^a	6.12 ± 0.03 ^b	0.37 ± 0.01 ^a	28.43 ± 0.15 ^c

Values are the meaning of three replicates ± standard deviation.

3.1.2 Scanning electron microscope (SEM)

The morphology of the surface of gelatin film with CMC, assed by SEM was shown in Figure 2. The rough surface was found in films supplemented with C-CMC and P-CMC, which is consistent with their physical appearance of films (Figure 2(A), 2(B)). During casting, water is slowly removed from the film forming solution, resulting in the roughness on the surface of the film which may be due to partial phase separation between particle of CMC in the film [25]. However, C-CMC and C-CMC with starch films showed a smoother surface may be because starch provide a better solubility and homogeneous of all filming agent. Starch can be used as a binder to make smooth film [25].

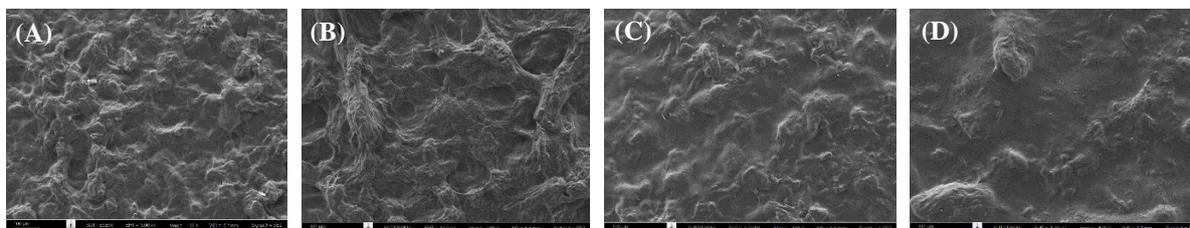


Figure 2 SEM micrographs (x 100) of the gelatin films supplemented with CMC from different sources with and without adding starch. gelatin film with C- CMC (A), gelatin film with P-CMC (B), gelatin film with C-CMC with starch (C), and gelatin film with P-CMC with starch (D).

3.2 Mechanical properties

Tensile strengths (TS) of films supplemented with C-CMC and P-CMC were not different significantly ($p > 0.05$) as shown in Table 1. This indicated that the TS of film was not relied on source of CMC supplemented in the gelatin film. However, the elongation of gelatin film supplemented with P-CMC seemed to show higher value, suggesting the more flexible than did by C-CMC. The addition of starch led to an increase in tensile strength but reduce the elongation regardless the source of CMC incorporated in gelatin films. Uthaiku [22] stated that gelatin film supplemented with different starch provide different TS value. Moreover, higher TS could be found in film supplemented with corn starch when compared to that with tapioca starch. The elongation of gelatin film supplemented with P-CMC seemed to show higher value, suggesting the more flexible than did by C-CMC. However, a reduction of the elongation upon addition of starch was observed regardless the source of CMC incorporated in gelatin films. Films have higher elongation than films made from some kind of synthetic polymers (PLA 4-7%, PS 3%) and gelatin films (0.99-2.37%) [21] because starch is naturally elastic. Therefore, increasing the amount of starch or using appropriate additives can reduce the elongation of the film, as supported by prior research [26].

3.3 Physicochemical properties of gelatin films

3.3.1 Moisture content

The moisture content of the gelatin films supplemented with different CMC in the presence of starch were presented in Table 2. It can be seen that moisture content of film was not different among tested samples. This finding aligns with the research conducted by Suwannarat et al. [27], who studied edible films made from tamarind seed pulp using sorbitol and glycerol as plasticizers.

3.3.2 Solubility

Solubility of gelatin film supplemented with C-CMC showed lowest value compared to others (Table 3), which is different from previous reports [5]. It can be noticed that the lowest value was recorded from gelatin film supplemented with C-CMC. The solubility of the film increased with hydrophilic property of P-CMC, resulting in P-CMC film having a higher solubility than C-CMC film which was consistent with WVP. The degree of substitution of the carboxymethyl groups in the cellulose structure affects solubility of carboxymethyl-cellulose. If the substitution degree is less than 0.3, the CMC could be swollen only but not solubilized in water. However, its solubility could be promoted in alkaline solution. Moreover, CMC can be dissolved in water, if its substitution degree is greater than 0.4 [28]. Addition of starch in this film forming solution resulted in an increase film solubility regardless the CMC sources. However, the addition of starch to the film increased the water solubility as evidenced by an increase solubility of films produced from C-CMC with starch and P-CMC with starch from 37.60 to 40.92% and from 41.77 to 42.32%, respectively (Table 2). This is consistent with the notion that starch

exhibits higher solubility due to its high hygroscopic behavior, forming strong interactions with water and being easily incorporated into hydrogen bond chains [29]. Based on this study, the combination of starch and CMC, improving water-solubility due to highly hydrophilic properties of gelatin-CMC composite film.

3.3.3 Thickness

It was observed that gelatin film supplemented with P-CMC showed higher thickness than that of C-CMC (Table 2). This might be due to higher fiber content in P-CMC compared to C-CMC. These results were in agreement with that reported by Kanchanatecha et al. [30]. It can be seen that addition of starch in filming solution resulted in a reduction of film thickness in case of supplemented with C-CMC. However, the opposite trend was found in the sample supplemented with P-CMC. This could be explained by the fact that the amorphous structure of CMC affects its density and the amount of space between its molecules [28].

3.3.4 Water permeability

The water permeability (WVP) of the gelatin film supplemented with P-CMC had higher WVP than that of C-CMC. This might be due to more hydrophilic compounds presented in P-CMC. This value of C-CMC significantly increased when starch was added but the opposite trend was found in P-CMC. Moreover, WVP may be due to the strong intermolecular hydrogen bonding between the starch matrix and P-CMC, which may lead to cross-linking, which in turn leads to a decrease in free space inside the polymer matrix because of the decreased rate of diffusion of water vapor molecules through the film [31].

3.3.5 Antioxidant activity

Antioxidant activity is of particular importance because free radicals can be harmful to living organisms. The measurement of antioxidant activity was conducted by assessing the decolorization of 2,2-diphenyl-1-picrylhydrazyl (DPPH) when reacting with the film solution, and the results were recorded as a measure of antioxidant activity. Inhibitory concentration values for the free radical scavenging activities of films produced from C-CMC and P-CMC were found to be comparable (Table 2). Mehraliyev et al. [32] indicated that standard C-CMC films have limited free radical scavenging capabilities. Thus, antioxidant activity of film was slightly affected by source of CMC incorporated into the film [32]. It can be noticed that addition of starch into the film recipe resulted in a reduction of antioxidant activity. This finding confirmed that the gelatin film with CMC with starch exhibited a slightly lower antioxidant activity. The presence of carbohydrates as impurities in the starch might have affected the CMC and led to a decrease in its antioxidant properties since the polar groups of the carbohydrate impurities could have formed hydrogen bonds with the phenolic groups of the starch [33].

Table 2 Physicochemical and antioxidant properties of gelatin film supplemented with different CMC with/without starch.

Type of films	Moisture content (%)	Solubility (%)	Thickness (mm)	WVP ($\times 10^{-4}$ g.mm/m ² . day. kPa)	AA (%)
Gelatin film with C- CMC	0.12 \pm 0.01 ^a	37.60 \pm 0.04 ^d	0.24 \pm 0.01 ^c	2.06 \pm 0.07 ^d	24.78 \pm 0.03 ^a
Gelatin film with P-CMC	0.13 \pm 0.01 ^a	41.77 \pm 0.07 ^b	0.32 \pm 0.05 ^b	3.15 \pm 0.19 ^a	23.86 \pm 0.02 ^b
Gelatin film with C-CMC with starch	0.12 \pm 0.01 ^a	40.92 \pm 0.08 ^c	0.23 \pm 0.03 ^d	2.34 \pm 0.51 ^c	18.81 \pm 0.01 ^c
Gelatin film with P-CMC with starch	0.13 \pm 0.01 ^a	42.32 \pm 0.09 ^a	0.36 \pm 0.01 ^a	2.88 \pm 0.11 ^b	16.22 \pm 0.01 ^d

Values are means of three replicates \pm standard deviation.

4. Conclusion

The study investigated the film-forming process of C-CMC and P-CMC. The results showed that gelatin with C-CMC films had excellent formability and were easily removed. The gelatin with C-CMC films exhibited a smooth and milky-white surface, whereas the gelatin with P-CMC films had a light-yellow color and a smooth surface. The gelatin with P-CMC films had the same moisture, SEM, and tensile strength properties as the gelatin with C-CMC films. The solubility, thickness, water vapor permeability, and antioxidant activity were also similar. This shows that the gelatin with P-CMC has the potential to be used to produce films in the same way as C-CMC.

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