

Pectin and cellulose extraction from passion fruit peel waste

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Abstract:

Pectin and cellulose were successfully extracted from passion fruit peel waste. The maximum pectin yield of the process was 12.60% at pH=2 for 1.5 h. The pure cellulose obtained from the passion fruit peel waste was prepared by refluxing of the passion fruit peel powder with 1 M NaOH and 1.25 M HNO₃ solutions at 90°C for 1 h and 1.5 h, respectively. The passion fruit peel cellulose was converted to carboxymethyl cellulose (CMC) by etherification. The pure cellulose was soaked in a mixed solution of isopropyl alcohol and NaOH for 1.5 h. After that, it was reacted with chloroacetic acid at 70°C for 1.5 h. The optimum conditions for carboxymethylation were 5 g cellulose, 2.0 g chloroacetic acid, and 15 ml 20%w/v NaOH. The optimised product had a degree of substitution (DS) of 0.78 and was used as constituent in a biopolymer.

Keywords: carboxymethyl cellulose, cellulose, passion fruit peel waste, pectin.

Classification number: 2.2

Introduction

Vietnam is one of the world's largest producers, consumers, and exporters of fresh and produced passion fruit. Passion fruit peel accounts for 80% of the fruit's weight, resulting in a very large amount of waste. Instead of recovering the waste, most companies choose to throw them away, causing environment pollution that subsequently has an adverse effect on human health. Passion fruit peels contain about 42% dry weight of cellulose and 18% pectin [1, 2]. Therefore, the potential of cellulose and pectin recovery from this waste is very high and quite feasible. Recovering value from fruit processing waste provides an alternative way to reduce the cost of biological waste disposal whilst creating added value for the fruit juice processing industry. However, only a few works have been found that mention the problem of pectin recovery [3-6] from passion fruit by-products. Furthermore, to the best knowledge of the authors of this work, no research on the simultaneous extraction of pectin and cellulose from passion fruit peel has been published. Therefore, studies on the recovery of cellulose and pectin from this waste is essential and important. The purpose of this work is to confirm the potential of Vietnamese passion fruit peels as a raw material for industrial pectin extraction and CMC production.

Experimental

Materials and passion fruit peel source

The main chemicals used in this study include monochloroacetic (MCA) (UK), acetic acid, nitric acid, and sodium hydroxyl (Merck). The solvents include methanol, ethanol, isopropanol, and acetone (Merck).

Preparation methods

Pectin extraction from passion fruit peels:

The extraction procedure was done according to the Kulkarni method [7] with a slight modification. Ten grams of dried passion fruit peel powder were mixed with 150 ml of diluted HNO₃ at 70°C. The dark slurry obtained was filtered by using a centrifuge machine. The residual solid part was put into a clean Erlenmeyer flask (volume of 250 ml) for the separation of the cellulose. The resulting slurries were cooled to 4°C and centrifuged for 30 min at 5000 rpm. The supernatant was precipitated with ethanol under an ethanol/concentrate ratio (v/v) of 1.5 at 4°C for 30 min in order to achieve pectin flotation. The floating pectin was separated by filter paper and rinsed with 96% ethanol. At the end of the process, the pectin precipitate was washed with acetone and dried in an oven at 65°C for 24 h. The

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dried pectin was ground into a fine powder. The yield of the pectin was gravimetrically determined and expressed as a weight of the extracted dried pectin to 100 g of the dried peel used for extraction. This process was carried out at different pH values (pH = 1, 2, 3, 4) and different periods of time (t = 10, 30, 60, 90, 120 min). The treatments were carried out in duplicate.

The yield of the pectin was determined by using the below equation:

$$H_p(\%) = \frac{m_p}{m_0} \times 100$$

where H_p is the yield of pectin, m_p is the weight of obtained pectin, and m_0 is the weight of initial dried passion fruit peel powder.

Cellulose recovery from passion fruit peels:

Determination of optimum NaOH concentration: the solid residual part from the above process was treated with 50 ml of x M NaOH (x = 0.25 M, 0.50 M, 1.00 M, 1.25 M, 1.50 M) and cooked at 90°C for 60 min under continuous stirring. The dark slurry obtained was filtered and washed with 1 l of distilled water to the recover solid part. After that, this solid was treated with 50 ml of 1.25 M HNO₃ and cooked at 90°C for 90 min. This mixture was then filtered and washed with cold distilled water until the indicator paper did not change colour. The residue was dried in an oven at 60°C overnight until constant weight. Finally, the dried cellulose was ground and kept in a polyethylene bag for the cellulose modification in the next process.

Determination of optimum HNO₃ concentration: the solid residual part from the above process was treated with 50 ml of 1.0 M NaOH and cooked at 90°C for 60 min under continuous stirring. After that, this solid was treated with 50 ml of y M HNO₃ (y = 0.3 M, 0.75 M, 1.25 M, 1.75 M) and cooked at 90°C for 90 min. Then, the cellulose recovery procedure was repeated as above.

The yield of the cellulose extraction was determined by using the below equation:

$$H_c(\%) = \frac{m_c}{m_0} \times 100$$

where H_c is the yield of the cellulose extraction, m_c is the weight of the obtained cellulose, and m_0 is the weight of the initial dried passion fruit peel powder.

Synthesis of CMC:

Five grams of cellulose extraction obtained from passion fruit peel powder was added to 50 ml of isopropanol under

continuous stirring for 30 min. Then, 15 ml of (10%, 15%, 20%, 25% w/v) NaOH was added dropwise into the mixture and further stirred for 1 h at room temperature. The carboxymethylation was started when y gram of MCA (y = 1.0 g, 1.5 g, 2.0 g, 2.5 g) was added under continuous stirring for another 90 min at 70°C. The solid part was neutralized with acetic acid to pH=7 and washed three times by soaking in 20 ml of ethanol for 10 min to remove undesirable by-products. The obtained CMC was filtered and dried at 60°C until constant weight and kept in a dry place.

The yield of the CMC was determined by using the below equation:

$$H_{CMC} = \frac{m_{CMC} - m_c}{m_c} \times 100$$

where H_{CMC} is the yield of the CMC, m_{CMC} is the weight of the obtained CMC, and m_c is the weight of the cellulose used to synthesis CMC.

Solubility of CMC: m_0 g of CMC were dissolved in 100 ml H₂O, at 50°C, for 30 min. After that, these were filtrated by filter paper. The solubility of the CMC was determined by using the below equation:

$$S = m_0 - m$$

where m_0 is the initial CMC weight and m is the excess CMC weight.

Research methods

Infrared spectroscopy (FTIR):

FTIR spectra were recorded on a FT/IR-6300 spectrometer, 32 times of scan, with a resolution of 4 cm⁻¹, in the wavenumber range of 600-4000 cm⁻¹.

The degree of substitution, DS_{rel} , of the carboxyl group in CMC can be determined by FTIR spectra by means of taking the ratio of the absorption spectra, as shown in the below equation [8]:

$$DS_{rel} = \frac{A_{1614} - B}{A_{2912}}$$

where is A_{1614} is the absorbance at 1614 cm⁻¹, which is assigned to the stretching vibration of the carboxyl group (COO⁻), A_{2912} is the absorbance at 2912 cm⁻¹, which is assigned to the stretching vibration of methine (C-H), and B is a numerical constant correspondent to the A_{1614}/A_{2912} ratio of the cellulose, which was found to be zero. A linear relationship between the absolute and relative value of the degree of substitution was proved by Pushpamalar [9] as shown in the below equation:

$$DS_{abs} = 0.4523DS_{rel}$$

Viscosity measurement method:

The average molecular weight (M) of the polymers was determined by viscometry according to the Mark and Houwink-Sakurada equation:

$$[\eta] = K.M^\alpha$$

where $[\eta]$ (dl.g⁻¹) is the intrinsic viscosity, M is the average molecular weight of the polymers, K and α are the characteristic constants for the used polymer-solvent systems. At room temperature (25°C) for pectin, K and α are 1.4x10⁻⁶ and 1.43 [10], respectively. At room temperature (25°C) for CMC (CMC was dissolved in 0.1 M NaCl solution), K and α are 7.3x10⁻³ and 0.93, respectively [8].

Intrinsic viscosity measurements were carried out using an Ubbelohde capillary viscometer having an internal diameter of 0.5 mm and a length of 10 cm. The flow times were recorded using a stopwatch.

Results and discussion

Pectin extraction

Effect of pH on the yield of pectin extraction:

The results presented in Fig. 1A indicated that the pH of the diluted HNO₃ solution had a significant impact on the pectin yield from passion fruit peel waste. The maximum yield of pectin was obtained at an extraction pH of 2.0. This is in agreement with the findings of other researchers who

found that the highest yield of pectin was achieved from passion fruit peel at pH 2 [3, 7, 10-12]. This may be due to increasing acid strength (pH<2) that could hydrolyse and degrade pectin to uncollected small pectin particles resulting in an increased pectin-particle solubility and, consequently, pectin precipitation with alcohol is hindered [13]. On the other hand, at lower acid strength (pH>2), pectin molecules can be partially solubilized without degradation leading to difficulty in extraction of some pectin fractions due to attachment to other cell wall components [14].

Effect of treatment time on the yield of pectin extraction:

The yield of pectin extraction at different extraction times is shown in Fig. 1B. The extraction time was maintained at 10, 30, 60, 90 and 120 min for each extraction. The other extraction conditions, such as the ratio of water to peels, extraction temperature, and pH of the extracting medium were maintained at 15:1, 70°C, and 2, respectively. The results shown that the yield of pectin increased with extraction time up until 90 min. However, after further increase of the extraction time to 120 min, the pectin yield reduced. This could be due to the partial degradation of pectin. These results are in agreement with Xue [15]. It can be seen that, when reaction time was increased from 60 to 90 min, the pectin yield showed no significant increase, with pectin yield of 12.3 and 12.6%, respectively. Thus, the optimum time of extraction for the maximum yield of pectin was found to be 90 min. But, in case of prioritizing economic efficiency, a reaction time of 60 min is reasonable.

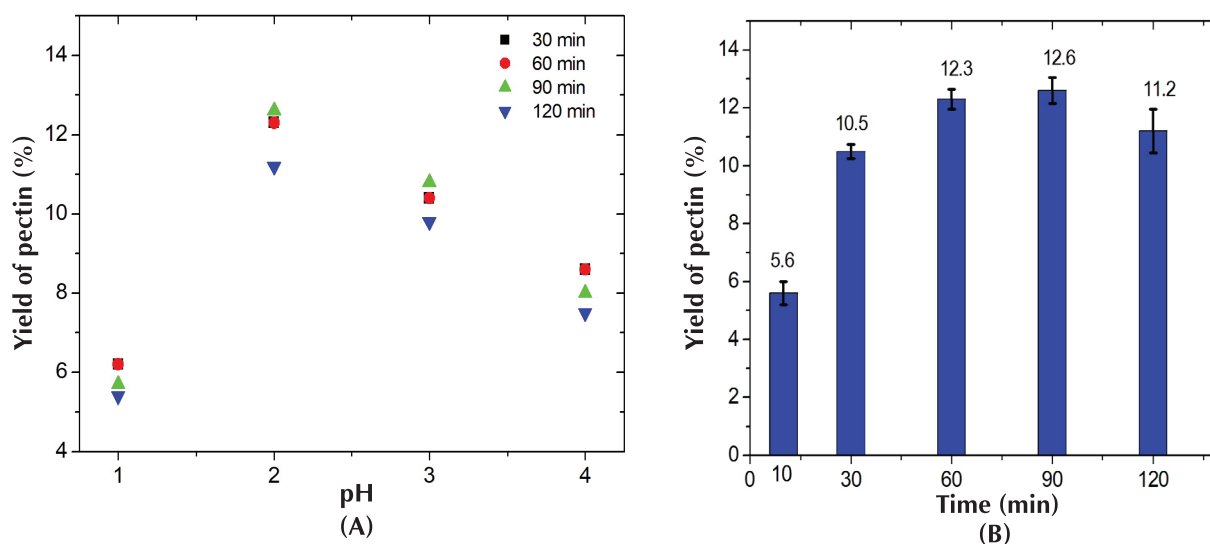


Fig. 1. Effect of (A) pH and (B) treatment time at pH 2 on yield of pectin extraction.

Characterization of obtained pectin:

The average molecular weight of pectin is $M = 12532.82$ (g/mol). The obtained pectin was characterized by FTIR spectroscopy and the result is shown in Fig. 2.

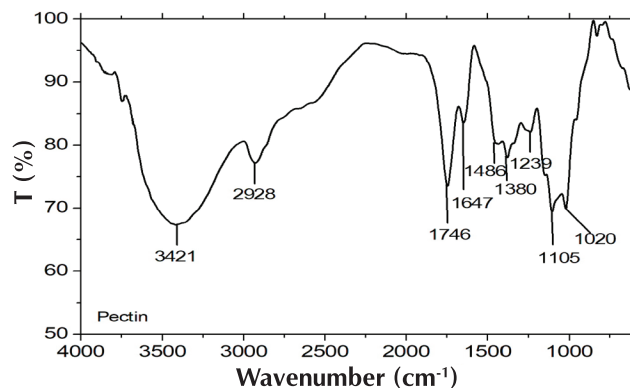


Fig. 2. FTIR spectroscopy of pectin.

The band at 3421 cm^{-1} can be assigned to the OH stretching mode, while the signal observed at 2928 cm^{-1} is attributed to the stretching vibrations of the C-H groups. The distinct band registered at 1746 cm^{-1} corresponds to asymmetric C=O stretching vibrations present in carboxyl or COOCH_3 groups. The band at 1647 and 1486 cm^{-1} may be assigned to COO-symmetrical and asymmetrical stretching vibrations of polygalacturonic acid, respectively. The occurrence of the band at 1380 cm^{-1} likely comes from the bending vibrations of C-H or O-H groups, while the presence of distinct bands observed together in the range of $1239\text{--}1020\text{ cm}^{-1}$ are associated with C-O-C stretching vibrations. The signal at 1105 cm^{-1} implies overlapping of the bands coming from C-O stretching and OH bending vibrations found in CH-OH groups. The band centered at 1746 cm^{-1} has been utilized to probe the degree of esterification (DE) in pectin. The FTIR spectroscopy results are entirely similar to other authors' results [16]. Therefore, the pectin extracted from passion fruit peel waste is of high purity.

Cellulose extraction

The process of cellulose recovery was conducted at various concentrations of NaOH and HNO_3 to determine the optimum treatment conditions. The results are listed in Table 1.

Table 1. Cellulose yield with various NaOH concentrations and HNO_3 concentrations.

Yield of cellulose	C_{NaOH} M, (HNO_3 1.25 M)				
		0.25	0.50	1.00	1.25
H_c (%)	21.70	28.50	32.13	29.60	26.8
Yield of cellulose	C_{HNO_3} %, (NaOH 1 M)				
	0.30	0.75	1.25	1.75	
H_c (%)	23.50	28.50	32.13	26.20	

From Table 1 we see that the yield of cellulose varies depending on the change of NaOH concentration. The maximum yield of cellulose extraction was obtained at NaOH 1 M, and upon further increasing the NaOH concentration, the cellulose yield reduced.

In this experiment, HNO_3 was used to treat excess hemicellulose in the previous stage and the yield of cellulose reached the best result at HNO_3 1.25 M. After that, when the concentration of HNO_3 increases, the yield of pectin decreases (as shown in Table 1). This might be due to the destruction of the cellulose structure at high concentrations of HNO_3 . In brief, the highest yield of the cellulose extraction is 32.13% at a NaOH concentration of 1.00 M and HNO_3 of 1.25 M.

Characterizations of cellulose by FTIR spectroscopy: FTIR spectroscopy of cellulose is displayed in Fig. 3. The band at 3350 cm^{-1} can be assigned to the OH stretching mode, while the signal observed at 2912 cm^{-1} is attributed to the stretching vibrations of the C-H groups of molecular cellulose. The C-C ring breathing band appeared at 1159 cm^{-1} . Besides, the vibration peaks at 1365 cm^{-1} and 1425 cm^{-1} are assigned to the CH and C-O bending vibration in the polysaccharide aromatic rings, respectively. Lastly, the wavenumber range of about $895\text{--}1053\text{ cm}^{-1}$ is associated with the β -(4, 17)-glycosidic linkages between the glucose units in cellulose [8]. FTIR spectroscopy of the cellulose extracted from passion fruit peel waste is similar to the result of Hong who recovered cellulose from sugarcane bagasse [8]. In addition, the absence of peaks at $1600\text{--}1800\text{ cm}^{-1}$, characterized for the functional groups C=O and the aromatic ring of hemicellulose and lignin molecules [17, 18], proved that hemicellulose and lignin were completely removed. This means that the recovered cellulose is of high purity. This pure cellulose was then used for CMC synthesis.

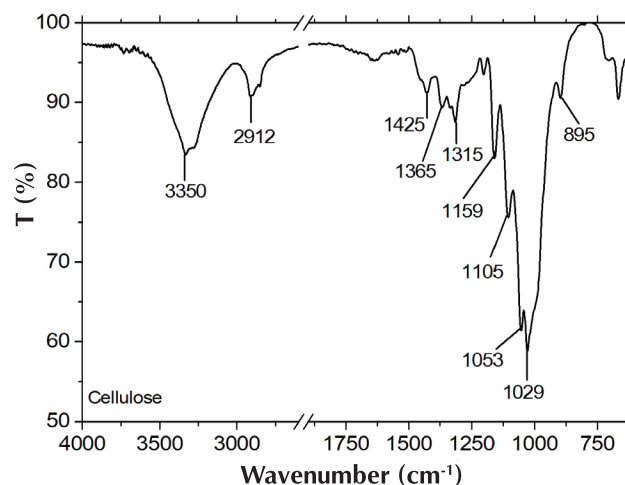


Fig. 3. FTIR spectroscopy of extracted cellulose.

CMC synthesis

Effect of NaOH concentration on DS and yield of CMC:

NaOH was used as an alkaline reagent to swell cellulose chains, which provides the ability of substitution by sodium carboxymethyl groups in cellulose units. The DS of the CMC obtained with different concentrations of sodium hydroxide are shown in Table 2.

Table 2. The yield and DS of synthesized CMC with various NaOH concentrations.

	NaOH, %wt			
	10	15	20	25
H _{CMC} , %	32.1	52.3	63.8	56.6
DS	0.48	0.57	0.78	0.61

As shown in Table 2, the DS of the CMC increased with NaOH concentration and attained the highest DS of 0.78 at a NaOH concentration of 20% (w/v). However, upon further increase of the NaOH concentration, a reduction in DS value was observed. This can be explained by the degradation effect of high concentrations of alkali reagent on CMC polymer chains. These results are similar to that of Hong [8] and Sunardi [12]. Table 2 also shows the CMC yields from different experiments, which had similar trends as the DS results.

Effect of MCA weight on DS and yield of CMC:

The effect of MCA weight on the DS value was determined by changing the amount of MCA from 1.0 g to 2.5 g. The result is shown as in Table 3, where the DS of the CMC increased with an increasing amount of MCA in a range of 1.0-2.0 g and then decreased slightly with further increase of the MCA amount. The highest DS value was observed at MCA weight of 2.0 g. The reason for this observation is that an undesired side reaction occurred that dominated CMC production with the greater availability of the MCA molecules. This range of DS value (from 0.48-0.78) is similar to another author's report [8] for passage waste. Table 3 also shows that the trend in the change of CMC yield is similar to that of the DS.

Table 3. The yield and DS of CMC synthesized with various amount of MCA.

	Amount of MCA			
	1.0	1.5	2.0	2.5
H _{CMC} , %	43.7	63.8	79.5	72.2
DS	0.58	0.71	0.78	0.77

The optimum condition for carboxymethylation was 5 g cellulose, 2.0 g chloroacetic acid, and 15 ml of 20% w/v NaOH solution. The obtained CMC had a DS of 0.78 and yield of 79.5%.

Solubility of CMC

Solubility of CMC in water is one of the important properties that determine CMC applications, and depend on the DS of CMC. The relationship between DS and solubility can be plotted as a linear equation, and this result shows that the solubility of CMC in water increases linearly with the increasing DS. Surely, this was due to the greater substitution of carboxymethyl groups for the hydroxyl groups of the cellulose polymers. These carboxymethyl groups act as hydrophilic groups, so the increase of the DS will improve the CMC's ability to immobilize water in the system (Fig. 4).

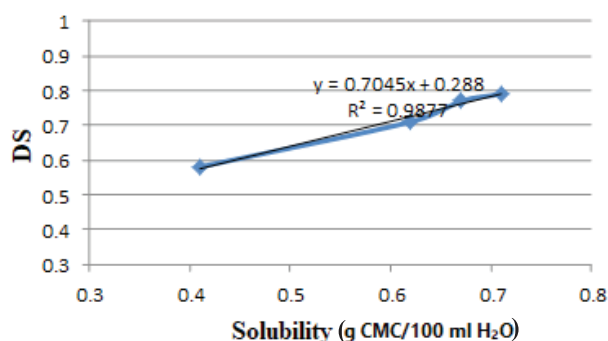


Fig. 4. The relationship between DS and solubility of CMC.

Thus, it can be concluded that the solubility of CMC depends on DS. The solubility of CMC with a given DS can be extrapolated from the linear equation.

Characterizations of CMC by FTIR spectroscopy: the FTIR spectroscopy of the synthesized CMC is shown in Fig. 5.

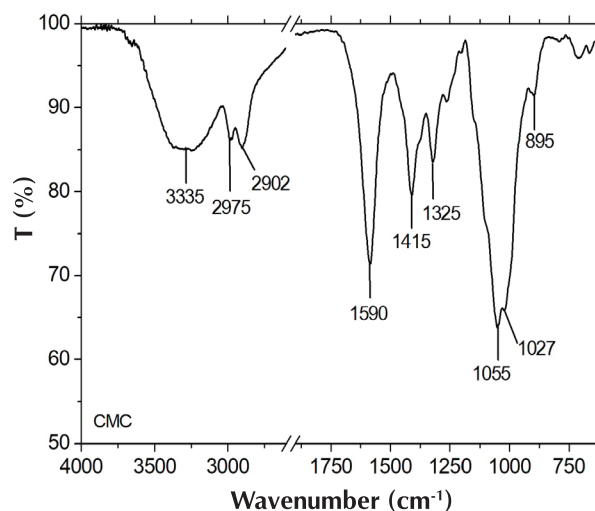


Fig. 5. FTIR spectroscopy of CMC from passion fruit peel.

The absorption peak at 3335 cm^{-1} appearing in the spectra indicates the free OH stretching vibration as well as inter-and intramolecular hydrogen bonds in the cellulose molecules. The bands at 2902 cm^{-1} and 1415 cm^{-1} are attributed to the stretching and scissoring vibrations of the C-H groups. The bands at 1055 cm^{-1} and 1027 cm^{-1} are relevant to the β -(4, 17)-glycosidic linkages between the glucose units in cellulose [8, 9, 19]. The presence of a strong absorption band at 1590 cm^{-1} was attributed to C=O stretching, confirming the presence of the -COO and -COONa groups, indicating the successful etherification of cellulose. This peak does not exist in the FTIR spectroscopy of cellulose (Fig. 2). The above analysis results are similar to those of earlier publications of Hong [8] for passage waste and Sunardi [12] for purun tikus.

Conclusions

Pectin was successfully extracted from passion fruit peel waste with a maximum pectin extraction yield of 12.60 % at pH=2 for 90 min in a diluted HNO_3 solution. The obtained pectin was confirmed by FTIR spectra with the appearance of peaks at 1746 cm^{-1} and 1647 cm^{-1} related to methoxylated and free carboxyl groups, respectively. Cellulose was successfully recovered from passion fruit peel waste with yield of 32.13% at NaOH concentration of 1 M and HNO_3 of 1.25 M. CMC has been obtained by etherifying cellulose with monochloroacetic acid. The optimal condition for carboxymethylation was 5 g cellulose, 2.0 g chloroacetic acid, and 15 ml of 20% w/v NaOH solution. The optimised CMC products have a DS of 0.78 and yield of 79.5%. The chemical structure of the CMC was confirmed by FTIR spectra, indicating the C=O group at 1590 cm^{-1} . These results show that the separation of cellulose and pectin from passion fruit peel waste has great potential and feasibility.

The authors declare that there is no conflict of interest regarding the publication of this article.

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