

**O-YS-11**

**The Partial Purification of Water-Soluble Cellulose  
from Banana Peel Extraction**

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**DOI:**

**ABSTRACT**

Banana peel is one of the lignocellulosic wastes from food processing and often used as a raw material for cellulose extraction. To obtain water-soluble cellulose, the extracted cellulose was hydrolysed by cellulase. The purification was needed since the process of hydrolysis provided mixed products of glucose, cello-oligosaccharides, as well as soluble cellulose. Thus, the aim of this study was to partially purify the soluble cellulose from the enzymatic hydrolysate. Initially, cellulose fiber was extracted from banana peel by alkaline and bleaching treatment. Cellulose extracted was then hydrolyzed by commercial cellulase. The hydrolysate was purified by using ethanol precipitation. The purification was investigated by thin layer chromatography and glucose kit assay. The results revealed that the ethanol could precipitate the hydrolysate into 2 phases, the supernatant and the ethanol insoluble pellet. The thin layer chromatography depicted that glucose, cellobiose, cellotriose, and cellotetraose were detected in the supernatant. The ethanol insoluble pellet was water-soluble cellulose. Moreover, the glucose kit assay could not detect any glucose in the pellet. Therefore, this convenient purification could be effectively used for water-soluble cellulose production. This soluble fiber would be further studied as a food additive.

**Keywords:** Cellulose, Enzymatic hydrolysis, Ethanol precipitation

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## INTRODUCTION

Banana production is the popular industrial fruits in the world especially the tropical region. Kluai Namwa (*Musa ABB*) production has been accounted for 75% of all banana production in Thailand. The banana peel is dominant waste that have been accounted for 35% of the whole fruit (Vu, Scarlett, & Vuong, 2016). From the industrial food processing, it has left many banana peels waste day by day (FAOSTAT., 2016). To add the value of this waste, the peel has been used as material of dietary fiber extraction due to it has remarkable characteristics including softness, non-toxicity as well as odorless. Dietary fiber is non-digestible food ingredients that are mostly found in plant components such as cellulose, hemicellulose, and lignin. Dietary fiber is divided into 2 groups including water-soluble fiber and water-insoluble fiber. The adequate consumption of dietary fiber could normalize the bowel movements, increase the volume of feces, and lose weight, relying on its solubility (Prasad et al., 2017). In addition, the supplementation of soluble dietary fiber in food could improve the mouthfeel as well as the texture of products since it provided the viscosity, improved emulsion, and foam (Dhingra, Michael, Rajput, & Patil, 2012).

Cellulose is the most natural dietary fiber production in the world that has been abandoned. Its structure consists of crystalline region and amorphous region. The existing of hydrogen bond network in the cellulose results in the insolubility in all organic and inorganic solvent (Pang et al., 2016). To improve the solubility, cellulose must be modified by many chemical treatments to become cellulose derivatives (Pang et al., 2015). For the native water-soluble cellulose, it might be included the short chain of cellulose and cello-oligosaccharides (COSs). These types of cellulose are considered as a water-soluble dietary fiber. Structurally, they are a short chain polymer of cellulose that consists of many repeated glucose units linked by a  $\beta$  - 1,4 glycosidic bond. They are generally an intermediate products from cellulose degradation such as acid hydrolysis and enzymatic hydrolysis. Nevertheless, the uses of an acid chemical are not applicable to food products since the strong treatment condition provides some toxic by-products such as furfural and hydroxymethylfurfural (Tyagi, Lee, Mulla, Garg, & Chae, 2019). Thus, the methods that unrequired the extreme condition are more suitable for dietary fiber production.

The biological enzymatic hydrolysis is one of the principal procedures of cellulosic material degradation with the mild condition requirement. However, the final products of every cellulose-rich material degradation contain many types of compounds including glucose, cellobiose, cellotriose, short chain of cellulose, as well as undigested cellulose. In order to get only the water-soluble cellulose, the prior purification needs to be conducted. There are many purification methods that have been carried out such as solvent and salt precipitation, column affinity, and chromatography. Ethanol precipitation is a convenient procedure due to it is easy to experience and it is a basic chemical in laboratory uses (Zhang, Ran, Zheng, Ding, & Chen, 2018).

Therefore, the objective of this study was to partial purify the water-soluble cellulose fiber from banana peel hydrolysate by using ethanol precipitation.

## MATERIAL AND METHODS

### Material

The completely ripe banana peel “Kluai Namwa” was collected from the local market in Khon Kaen Province, Thailand. The banana peel was dried at 80 °C. The dried peel was milled and then sieved to get fine powder. The banana peel powder was stored at 4 °C in the plastic jar for the further analysis.

### Cellulose Hydrolysate Preparation

To obtain the cellulose fiber, banana peel powder was extracted by using alkaline and bleaching treatments with some modification by Pelissari, Sobral, & Menegalli (2014). To prepare the cellulose hydrolysate, the banana peel cellulose was soaked in distilled water and then added Celluclast<sup>®</sup> 1.5L (C2730, Sigma-Aldrich) into the swelled cellulose. The sample was incubated at 50 °C, for 12 h. The hydrolysate was boiled immediately to stop the enzyme reaction. Cellulose hydrolysate was kept at -20 °C for the further analysis.

### Purification of Cellulose Hydrolysate

The partial purification of enzymatic hydrolysate was conducted by using ethanol precipitation. The absolute ethanol (Merck, USA) was added into the cellulose hydrolysate at a ratio of 3 : 1 (v/v). To completely precipitate, the supernatant was kept at 4 °C for 12 h. After that, the sample was centrifuged to gain the insoluble pellet. The insoluble pellet was washed

95% ethanol several times. Samples were kept at -20 °C for the further analysis.

## **Analytical methods**

### **Thin-layer Chromatography**

To evaluate the sugar type in the supernatant and pellet, the samples and standards were spotted on TLC plate using silica gel 60 F254 (Merck, USA). The mobile phase was the mix solution by butanol: isopropanol: ethanol: deionized water at a ratio of 2: 3: 3: 2, respectively. The color development reagent was 2% (w/v) orcinol in 1% (v/v) sulfuric in ethanol. (Apiraksakorn, Nitisinprasert, & Levin, 2008).

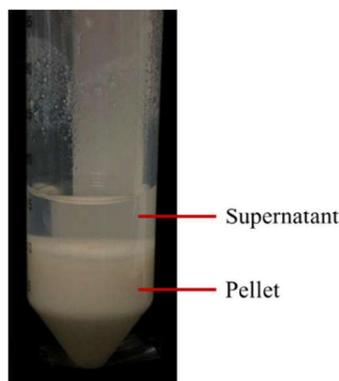
### **Glucose Assay Kit**

To determine the glucose remaining in the sample. Sample were incubated with glucose enzyme kit (Glucose Liquicolor, HUMAN) at 37 °C for 15 min. The color of samples was analyzed at A<sub>500</sub> by using the spectrophotometer (Beckman Coulterm, Germany).

## **RESULTS**

### **The Partial Purification of the Cellulose Hydrolysate**

After adding ethanol into the hydrolysate, the supernatant and pellet were observed as shown in Fig. 1. The color of ethanol-precipitated pellet was similar to banana peel cellulose since it came from the enzymatic hydrolysis of banana peel cellulose. Additionally, the ethanol insoluble pellet could completely re-soluble in water that was unlike to a native cellulose. Thus, the ethanol-precipitate pellet of cellulose hydrolysate was high solubility in water.



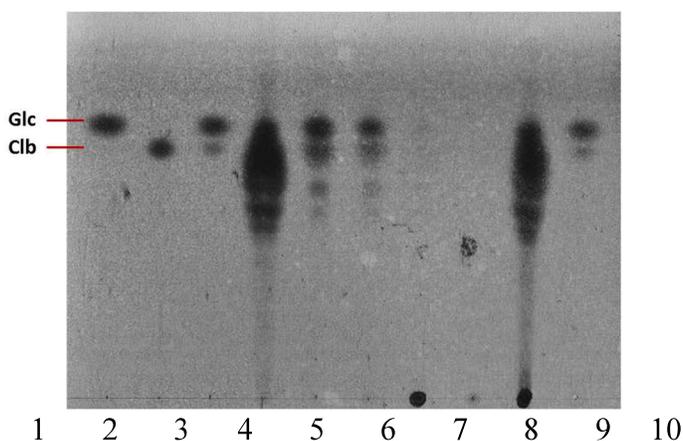
**Figure 1.** The Precipitation of Extracted Cellulose Hydrolysate

### **The Investigation of the Sugar Content in Samples**

To recover the water-soluble cellulose without any contaminated sugars, the ethanol insoluble pellet had to be washed with 95% ethanol for several times. The thin-layer chromatogram showed that the enzymatic hydrolysate consisted of glucose, cello-oligosaccharides, and water-soluble cellulose as shown in Fig.2. The spot at starting line might be the soluble cellulose since it was a big molecule. The purification by using ethanol could separate the sample into 2 phases including the supernatant as well as the pellet.

The supernatant consisted of glucose and cellobiose. The supernatant that was diluted ten times show that the mixed compounds composed of cello-oligosaccharides which were longer than cellobiose and it might be cellotriose, and cellotetraose. The undiluted supernatant unclosed that the hydrolysate might consist with another more that was cellopentaose. Due to this study lacked of the exact standards that could be used to confirm about the type of compound.

The pellet that was obtained from the ethanol precipitation was then spotted onto the TLC plate. The result showed that it could not be moved by the mobile phase since it was the big molecule of cellulose. No glucose and any cello-oligosaccharides were detected in the pellet by TLC and glucose kit assay. Thus, the thin-layer chromatogram showed that the ethanol precipitation could partial purify the water-soluble cellulose from the banana peel cellulose hydrolysate.



**Figure 2.** Thin-layer chromatogram of enzymatic hydrolysate and the sample after purification with ethanol precipitation

- |                               |                               |                |                |
|-------------------------------|-------------------------------|----------------|----------------|
| 1. Glucose (Glc)              | 2. Cellobiose (Clb)           | 3. Glc + Clb   | 4. Supernatant |
| 5. Diluted 10x of Supernatant | 6. Diluted 20x of Supernatant |                |                |
| 7. Pellet                     | 8. Diluted Pellet             | 9. Hydrolysate | 10. Glc + Clb  |

## DISCUSSION

Ethanol is the basic solvent that can be used for purification. The ethanol can dehydrate the polymer molecule especially cellulose structure and decrease the dielectric constant of hydrolysate solution (Liu et al., 2016, Lubambo et al., 2013). Since the water molecule in the hydrolysate preferentially interact with ethanol than those polysaccharides; as a consequence, the dehydrated cellulose fibers re-interacted themselves and then coagulated. The digested cellulose that was obtained from the hydrolysis and precipitation method had a high water solubility unlike a banana peel cellulose that was water-insoluble fiber. As enzymatic hydrolysis affected to the solubility of cellulose. The cellulose fiber was reduced its size by cellulase hydrolysis, so it could be water soluble molecule. According to Gan et al. (2015) reported that the solubility of cellulose relies on its average molecular weight. After the hydrolysis, the cellulose hydrolysate consisted mainly amorphous part of cellulose and short chain of cellulose; resulting in the fiber had more water penetration. Moreover, the accessibility of the cellulose structure could be improved by the rearrangement of cellulose especially the

pore size and accessible area (Ruiz et al. 2013). However, this water-soluble cellulose was still might long enough to re-interact with each other after decreasing the polarity of ethanol adding.

## CONCLUSION

The ethanol purification could separate the water-soluble cellulose from the enzymatic hydrolysate that consisted of many sugar compounds including glucose as well as cello-oligosaccharides. The water-soluble cellulose could be the potential fiber that are consumed as soluble dietary fiber, food additive, and prebiotic fiber. It is superb interesting since the unique property on the water solubility. Basically, to achieve the completely water-soluble cellulose, the cellulose must be modified by chemical treatments with high knowledge requirement. For food supplement, it is necessary to reduce the use of harmful chemical. Not only the food quality but waste treatment is also the important factor that needs to be prioritized for an industrial scale. Therefore, this procedure provided the water-soluble cellulose by without any chemical medication but biological degradation and ethanol precipitation. However, this water-cellulose fiber would be investigated more on their properties and functions for industrial food adaptability.

## ACKNOWLEDGEMENTS

The current study was funded by National Research Council of Thailand (NRCT) (I62-00-26-01). The authors were thankful for Research Fund for Supporting Lecturer to Admit High Potential Student to Study and Research on His Expert Program Year 2018 from Graduate school of Khon Kaen University (611JT104).

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