THE EFFECT OF HCl CONCENTRATION IN THE HYDROLYSIS PROCESS OF NATA DE CASSAVA ON THE CHARACTERISTICS OF MICROCRYSTALLINE CELLULOSE

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ABSTRACT

Aim: Nata is a fermented food product which produced from bacterium *Acetobacter xylinum*. One type of nata is Nata de Cassava. This can be obtained from the utilization of tapioca flour industrial waste. Cellulose contents in nata is 82.37%. This has the potential to become a raw material for microcrystalline cellulose. The purity level of microcrystalline cellulose is based on the alpha cellulose contents. This is influenced by the process of hydrolysis with acidic solutions. The aims of this study was to determine the effect of variations in HCl concentration on the characteristics of microcrystalline cellulose from Nata de Cassava compared with Avicel PH 102.

Methods: The method of produced microcrystalline cellulose from Nata de Cassava was carried out by hydrolysis using HCl at various concentrations (0.5N; 1.5N; 2.5N; 3.5N and 4.5N). The examined parameters were organoleptic, flowability properties, Carr's index, alpha cellulose contents, XRD and SEM.

Results: The research concluded that the variation of HCl concentration in the hydrolysis process had a significant effect on alpha cellulose contents. However, it did not have a significant effect on organoleptic, flowability properties, Carr's index, XRD and SEM analysis.

Conclusion: The best characteristics of microcrystalline cellulose were obtained in concentration of HCl 4.5N.

Keywords: microcrystalline cellulose, Nata de Cassava, Hydrolysis, HCl

INTRODUCTION

Indonesia has abundant food products, one of them is cassava. Cassava is widely used as tapioca flour by the tapioca industry. Tapioca industry is one of the industries that produces abundant liquid and solid wastes which almost 20 m^3/ton of tapioca or 5 m^3/ton cassava. The reuse of tapioca liquid waste is one way to overcome environmental pollution.

The tapioca liquid waste can be processed into Nata de cassava which is source of cellulose. Nata de cassava contains 82.37% cellulose. The advantages cellulose derived from Nata de cassava has higher purity level, high crystallinity degree, and higher tensile strength or more elastic [1-2]

Cellulose has been widely developed into microcrystal cellulose. In the pharmaceutical field, microcrystal cellulose can be used as filler, binder, and crusher component in the tablet formulations. Microcrystal cellulose is obtained from hydrolysis process using acid. HCl is one kind of acid that can be used in the hydrolysis process. Hydrolysis and HCl will produce better crystallinity cellulose than sulfuric acid [3]

One factor that influences hydrolysis process is the concentration of acid solution. Previously, several studies have been conducted on the effect of HCl concentration to the hydrolysis process of microcrystal cellulose. According to Deviana (2017), the higher HCl concentration used in the Cladophora sp. hydrolysis process will obtain higher level of alpha cellulose and lower gamma and beta cellulose. The highest HCl concentration was 2.4 N. Nosya (2016) said that the hydrolysis process of empty bunches oil palm produced microcrystal cellulose with the highest crystallinity level of 61.6% with HCl concentration of 3 N.

Based on above, it is necessary to conduct a research in the effect of HCl concentration (0.5 N; 1.5 N; 2.5 N; 3.5 N; and 4.5 N) in the hydrolysis process to the characteristic of microcrystal cellulose of Nata de cassava.

MATERIALS AND METHODS

Materials

Materials that are needed in this research such as tapioca liquid waste derived from cassava which obtained in Bangli Regency of Bali, sucrose, ammonium sulfate (pa), acetic acid glacial *Acetobacter xylinum*, HCl (pa) and distilled water.

Methods

A. Nata de cassava

The tapioca liquid waste is filtered and taken about 5000 mL. Then, 500 g of sugar and 25 g of ammonium sulfate were added. The solution is heated while stirring. Next, the solution is poured into a pan which has been sterilized. The pH of the solution is set to 4. Then, the solution was cooled and added with 15% *Acetobacter xylinum* (v/v) aseptically. It incubated at 27^{o}C for 14 days until Nata de cassava is formed.

B. Nata de cassava Powder

Nata de cassava which has been washed and removed from its outer layer is heated. Then, Nata de cassava is cut and pressed to remove the water content. Next, it powdered and dried using oven.

C. Purification

Nata de cassava powder is heated to boiling. Then, the residual result is heated using 2% of NaOH. The residual content is washed with distilled water to pH 7.

D. Hydrolysis Process

Nata de cassava powder that has been purified then hydrolyzed with HCl solution with various concentration (0.5 N; 1.5 N; 2.5 N; 3.5 N; and 4.5 N). Next, it is heated until 92^{o}C while stirring with magnetic strirrer. After it has reached 92ºC, it is left overnight at a room temperature. Then, the residual content is neutralized using distilled water. The residual content then dried in an oven. After drying the sample, it is sieved with 60 mesh siever until the result are obtained in form of microcrystalline cellulose.

E. Evaluation of Microcrystalline Cellulose

1. Organoleptic
   Observation on shape, color and odor are carried out.
2. Flowability properties
   It can be done by measuring the flow time and angle of repose.
3. Carr's index
   The Carr's index shows the ability of compressibility of a powder. This is calculated based on the difference between bulk density to tapped density

$$\text{Carr's Index} = \frac{\rho_T - \rho_B}{\rho_T} \times 100\ \%$$

Which are :
ρ_B : bulk density
ρ_T : tapped density

4. Alpha cellulose contents
Alpha cellulose contents from microcrystalline cellulose were determined based on the method of "Alpha, Beta and Gamma Cellulose Content Tests" listed in the Indonesian National Standard (2009). Determination of alpha cellulose content is calculated based on the following equation:

$$x = 100 - \frac{6{,}25\ (V1 - V2) \times N \times 20}{A \times W}$$

which is :
x : alpha cellulose content (%)
V1 : the volume of blank titration (mL)
V2 : volume of pulp titration (mL)
N : normality of ferrous ammonium sulfate solution
A : the volume of pulp filtrate analyzed (mL)
W : pulp dry weight (gr)

5. XRD
X-Ray Diffraction (XRD) is used to see crystallinity based on the atomic structure of the material.

6. SEM
The surface morphology of microcrystalline cellulose was observed with a Scanning Electron Microscope and compared to commercial products commonly used in pharmaceutical preparation formulations such as Avicel PH 102.

RESULTS AND DISCUSSION

Table 1 : The results of evaluation of microcrystalline cellulose from Nata de cassava

Evaluation	HCl concentration					Avicel PH 102
	0.5 N	1.5 N	2.5 N	3.5 N	4.5 N	
Organoleptic	Powder, brownish white, odorless	Powder, brownish white, odorless	Powder, brownish white, odorless	Powder, brownish white, odorless	Powder, brownish white, odorless	Powder, white, odorless
Flowability properties (g/s)	NA	NA	NA	NA	NA	NA
Carr's index (%)	36.77±0.27	37.13±0.71	37.42±0.29	36.86±0.56	37.34±0.58	22.50±0.17
Alpha cellulose (%)	88.06±0.11	90.47±0.14	90.78±0.09	91.10±0.31	91.38±0.23	-
Crystanillity Index (%)	86.26±0.05	88.42±0.18	88.62±0.23	89.57±0.22	89.60±0.27	68.02±0.15

1) Organoleptic.
The aims of this evaluation is to determine the form, color, and odor of the microcrystal cellulose produced from Nata de cassava. Every MCC from each HCl concentration has a

same organoleptic such as form, color and odor. Based on the observation, MCC from Nata de cassava is powder with brownish white color and no smell (table 1).

2) Flowability properties.

It intended to determine the flowability of a substance. The uniformity of weight during filling and packaging process was influenced by the flowability properties. Based on the results, all of the MCC could not flow. These results are then compared to the results obtained with the MCC commercial products, Avicel® PH 102. They have no flowability neither MCC from Nata de cassava nor Avicel® PH 102. The flowability characteristic of a material can be influenced by the value of compressibility. It because the value of compressibility is directly proportional to the flowability characteristic. Besides that, it is also caused by the non-spherical powder form. Powder with non-spherical form will cause the powder has low density and has no flowability [6].

3) Carr's index.

Carr's index determine the level of compressibility. It is a measure of the relative volume change of a material as a response to a pressure [8].

The Carr's index of MCC from Nata de cassava is high, in the range between 36 to 37 %. Meanwhile, the Carr's index Avicel PH 102 is 22,50%. It shows that the compressibility of the MCC from Nata de cassava and Avicel PH 102 are poor. Poor compressibility of the material will has an impact on the lack of flowability properties [6, 11].

According to the statistical test, each Carr's index by each MCC from Nata de cassava showed that the variations of HCl concentration did not significantly influence to the result ($p>0,05$). Based on Carr's index results, MCC from Nata de cassava with different HCl concentration are classified as quite poor compressibility. This is because MCC has low density.

4) Alpha cellulose contents.

Alpha cellulose is often used as a parameter of the purity of a cellulose with a degree of polymerization of 600-1500 (SNI, 2009). The higher the rate of alpha cellulose, the better the quality of a material. The alpha cellulose contents of MCC from Nata de cassava is in the range between 88 to 91%. It shows that the MCC has a good purity levels.

The research showed that the HCl concentration would affect the alpha cellulose contents. The occurrence of a significant increase in alpha cellulose contents due to the lower HCl concentrations can only attract cellulose which is not strongly bound to hemicellulose. Whereas when the higher HCl concentrations is use in hydrolysis process, it can attract all parts of the cellulose which are strongly bound to hemicellulose [8].

5) X-Ray Diffraction (XRD).

The aims of this test is to see crystallinity based on the structure of MCC from Nata de cassava with HCl concentration 0.5N; 1.5N; 2,5N; 3.5N and 4.5N in hydrolysis process compred with Avicel PH 102. The diffractogram composed in lines or peaks with different position (degree) and intensity. The obtained information of the peaks is depends on the crystal structure [9]. Cellulose is composed of crystalline and amorphous regions.

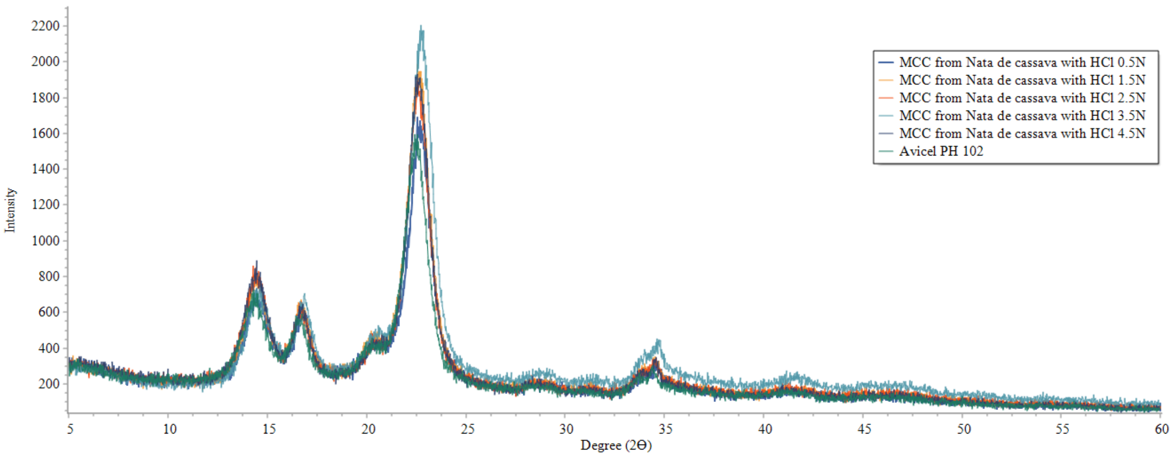
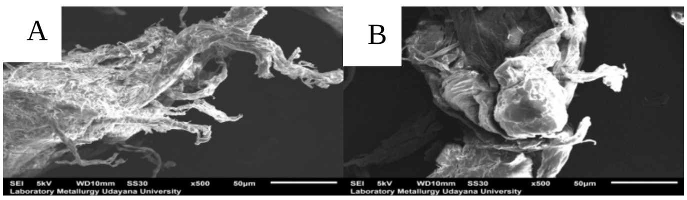


Figure 1 : X-Ray Diffraction results of microcrystalline cellulose from Nata de cassava and Avicel PH 102

Figure 1 shows that the spectrum of MCC from Nata de cassava with variation of HCl concentration and Avicel PH 102 have similar peaks. It shows that there is no difference in the structure of MCC due to different concentration of HCl that is used in the hydrolysis process with Avicel PH 102. Microcrystalline cellulose which was hydrolyzed with various HCl concentration showed the highest peak at an angle of 2Θ respectively 22.58°, 22.78°, 22.59°, 22.78°, and 22.56°. This shows the result obtained in accordance with the library, that is the crystalline area is located between the angle of 2Θ 21-24°. However, there is also a peak in the amorphous region of all MCC from Nata de cassava at the angles of 2Θ 13-16°. This is due to the hydrolysis process has not been able to eliminate all the amorphous parts found in cellulose [10]. Avicel PH 102 has the highest peak at an angle of 2Θ 22.26°.
The crystallinity index of MCC from Nata de cassava with various HCl concentration respectively 86.26±0.05%, 88.42±0.18%, 88.62±0.23%, 89.57±0.22% and 89.60±0.27%. It shows that more higher concentrations of HCl in hydrolysis process, more easily to degrade amorphous structures. It means higher levels of crystallinity will be obtained. The high value of crystalline index showed that alpha cellulose contents in MCC was also high [8]. Meanwhile, the crystalline index of Avicel PH 102 is 68.02±0.15. It lower than all MCC from Nata de cassava.

6) Scanning Electron Microscopy (SEM).
Particle shape measurement can be done with Scanning Electron Microscopy (SEM). In figure 2, showed that MCC from Nata de cassava with 500 times magnification having an irregular shapes and light fibers. This causes a low density that make it has a poor compressibility [6]. Compressibility is directly proportional to the flowability properties. A poor compressibility value will cause the powder's ability to flow was worse [11].



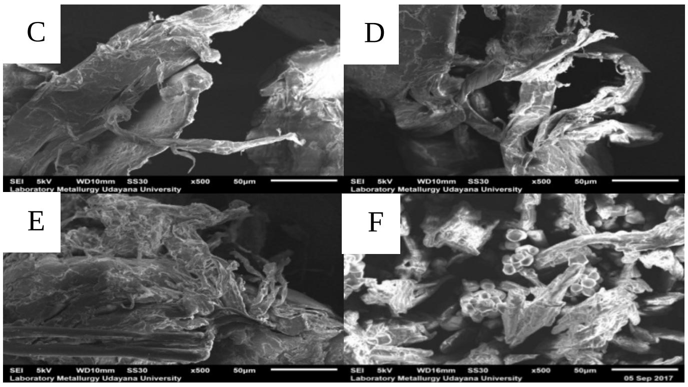


Figure 2 : *Scanning Electron Microscope* with 500 times magnification of MCC from Nata de cassava with concentration of HCl 0,5 N (A); 1,5 N (B); 2,5 N (C); 3,5 N (D); 4,5 N (E) and Avicel PH 102 (F)

CONCLUSION

The variation of HCl concentration in the hydrolysis process gave a significant effect on the alpha cellulose contents of MCC from Nata de cassava. However, it did not have any significant influence to the organoleptic, flowability properties, Carr's index, XRD and SEM. The best characteristics of MCC from Nata de cassava were obtained in concentration of HCl 4.5N.

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