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Preparation and Characterization of Nanocrystalline Cellulose from Cassava Stem Waste by Electromagnetic Induction

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Abstract

Cassava stems were one of the largest agricultural byproducts in Indonesia, especially in Lampung Province. It is known that cassava stems consist of lignocellulose content, especially cellulose which reaches 39.29%. The cellullose value of cassava stems has a great potential to be used as raw material for nanocrystalline cellulose (NCC) production. The preparation of NCC consists of four main stages, namely: pre-hydrolysis, delignification, bleaching, and acid hydrolysis. The pre-hydrolysis stage was carried out by boiling a solution of CH_3COOH and cassava stem powder for 60 minutes at a temperature of 105°C. Cassava stem powder was then delignified using NaOH 25% solution heated to a temperature of 105°C for 1 hour. NaOCl 3.5% solution was used in the bleaching stage and take place for 60 minutes at temperature of 50°C. The last step was acid hydrolysis using 2.5 N HCl solution for 15 minutes at a temperature of 105°C, then the electromagnetic induction treatment was at various temperatures of 30°C, 50°C, and 70°C for 60 minutes. The prepared nanocrystalline cellulose was tested its lignocellulose content, and with x-ray difrraction, and particle size analyzer. The results showed that the percentage of cellulose reached 62.93%, the high index of crystallinity 90.68%, and an average particle size of 18.04 µm with some nanometer-sized particles. It was concluded that electromagnetic induction has increased crystallinity and decreased the size of nanocrystalline cellulose.

Keywords: acid hydrolysis, cassava stems, electromagnetic induction, nanocrystalline cellulose, α-cellulose

1. Introduction

Nanotechnology is currently an interesting topic for academicians, industrialists, and researchers as a science of waste management, especially the use of biomass waste. Biomass waste contains natural polymer fibers in the form of crystals (microcrystals or nanocrystals) called cellulose. With nanotechnology, nanocrystals in cellulose have the potential to be used as fillers, binders, and disintegrants in tablets and capsules, as well as being useful as viscosity enhancers (Rowe et al., 2009). In general, nanocrystals are nanocellulose with a diameter of 5–20 nm (Samir et al., 2005) with a length of hundreds of nanometers, which consists of many crystalline parts.

The main substances for obtaining nanocrystalline cellulose were plants and their waste, but the use of plant waste maximizes the utilization of these plants and has a good impact on the environment. One of the plant wastes that is abundant in Indonesia and has a high cellulose content is cassava stem. Based on data from the Central Statistics Agency (BPS, 2018) the total production of cassava in Indonesia is 19.341.233 tons. Meanwhile, production in Lampung Province reached 6.683.758 tons. Cassava stems contain 39.29% cellulose, 24.34% hemicellulose, and 13.42% lignin (Lismeri et al., 2016). In another study, Sumada et al. (2011) obtained that cassava stems contain 56.82% α-cellulose, 21.72% lignin, and 21.45% acid detergent fiber (ADF).

Nanocrystalline cellulose in cassava stems could be obtained through hydrolysis of α-cellulose using strong acids, such as HCl and H_2SO_4. There have been many studies on isolation of nanocrystalline cellulose using various methods. Investigation of nanocrystalline cellulose from cabbage fiber has been done by Arjuna et al. (2018). The best results were obtained with a crystallinity index of 78.01% as a crystal size of 58.91 nm. Another isolation of nanocrystalline cellulose was found from wood sawdust (Sumiati et al. 2016). The best crystallinity degree of 74% was found at 8.75 ml/g concentration of HCl. Furthermore, synthesis and characterization of microcrystalline cellulose from cassava stem waste has been carried out by Lismeri

et al. (2020). The maximum purity of α-cellulose was 99.68%, but the microcrystalline was still quite brown in color.

Compared to commercial microcrystalline cellulose (MCC), MCC produced by Lismeri et al. (2020) still has lower quality of brightness, low crystalline index 78.12% and quite large in particle size. Commercial MCC has a white color, crystallinity of 84.31% and a size of 57.66 μm. Based on the study, the authors used electromagnetic tools to improve the MCC product with higher brightness and crystallinity, as well as nanometer-sized particle. Reducing the size of the raw material from 16 mesh to 120–200 mesh and implementing the electromagnetic induction to the hydrolysis process was proposed in this research. Electromagnetic is a tool that uses a changing of electric field into a magnetic field to enable its use in the manufacture of nanocrystalline cellulose. The presence of a magnetic field results in an increase in molecular activity, causing the groups of molecules to split (Fuhaid et al., 2011). It is hoped that the electromagnetic can weaken the bonds of these molecules to enable them break more easily so that it will reduce the energy used.

2. Methodology

2.1. Materials

The materials used in this study were cassava stems, distilled water, acetic acid (99.9%, Darmstadt, Germany), sodium hydroxide (caustic soda > 99%, Thailand), sodium hyphoclorite (12%, India), hydrochloric acid (37%, Darmstadt, Germany), ethyl ether (99,7%, Darmstadt, Germany), ethanol (96%, Darmstadt, Germany), and iodin (99,8%, Darmstadt, Germany).

2.2. Electromagnetic Specification

The electromagnet used in this study had 800 turns of wire, wire diameter of 0.7 cm, socket height of 7 cm, socket diameter of 15 cm, current capacity of 10 Amperes, additional metal core of 0.32 cm, and magnetic induction of 2.6674×10^{-5} Wb/m^2.

2.3. Pre-hydrolysis Process

Pre-hydrolysis was carried out by boiling cassava stem powder using 0.1 N acetic acid with a sample to solvent ratio of 1:20 for 60 minutes at 105^{o}C (Haafiz et al., 2013). After that, the sample was separated from the solvent by filtering and squeezing it, and it was rinsed repeatedly until the pH was neutral (Umar, 2011). Then it was dried in an oven.

2.4. Delignification Process

The isolation stage was continued with alkaline heating using 25% w/v sodium hydroxide (NaOH) at 105^{o}C, and boiled for 60 minutes with a sample/NaOH ratio of 1:20 (Haafiz et al., 2013). In this process a brown pulp (α-cellulose) was formed which was isolated as a residue. The pulp was then rinsed to be neutral and dried.

2.5. Bleaching Process

This process was carried out by immersing plant fibers in a 3.5% NaOCl solution with a sample and solvent ratio of 1:20 for 60 minutes (repeated up to two times). After that, the solution was filtered, and the residue obtained was rinsed repeatedly using distilled water until the pH was neutral. Furthermore, the pulp was dried using an oven at 50^{o}C for ± 6 hours. The dry pulp obtained was referred to as α-cellulose (Widia and Wathono, 2017).

2.6. Hydrolysis Process

α-cellulose obtained was further hydrolyzed. α-cellulose was then added with 2.5 N HCl solution with a ratio of 1:20, and treated using an electromagnetic induction device with a time of 60 minutes and various temperatures of 30, 50, 70^{o}C. Then, the mixture was boiled at 105^{o}C for 15 minutes. The solution was filtered and the residue was rinsed repeatedly until the pH was neutral. The results obtained were then stored in the freezer for further drying using a freeze dryer. The result was cellulose nanocrystal.

2.7. Characterization

Characterization of nanocrystal cellulose products obtained were then performed including tests of organoleptic, solubility, starch, ph, moisture content, lignocellulosic, degree of crystallinity, and particle size.

Organoleptic Test
The sample is placed on a white base, then its shape, color, taste, and smell are observed (Indonesian Pharmacopoeia, 1979).

Solubility Test
The sample was taken 5 grams and shook with 80 mL of distilled water for 10 minutes. Then, it was filtered, evaporated on a water bath at a temperature of 100–105^{o}C for one hour. The residual weight should not exceed 12.5 mg (0.25%) (British Pharmacopoeia,

2009). The same way was carried out on 96% ethanol, 2 N HCl, 1 N NaOH, and ether.

Water Content
The water content test was carried out on two grams of the sample with the heating method at a temperature of 105°C until a constant weight was reached. Based on the requirements in the Handbook of Pharmaceutical Excipients, the moisture content of cellulose microcrystals was less than 5%.

Lignocellulosic Analysis
Based on the Chesson Method (Chesson, 1981), one gram of cassava stem sample was refluxed for two hours with 150 mL H_2O at 100°C. Then, it was dried until a constant weight. The sample residue was refluxed with 150 mL 0.5 M H_2SO_4 at 100°C for 2 hours. After that, it was rinsed with distilled water until neutral and placed in an oven until a constant weight was obtained. The dried sample was added with 10 mL of H_2SO_4 72% (v/v) stored at room temperature for four hours, then it was diluted to 0.5 M H_2SO_4 and refluxed again at 100°C for two hours. The dried sample residue was then ashed using a furnace at a temperature of 575°C until the weight was constant.

Degree of Crystallinity
The procedure used the X-Ray Diffraction tool (X'pert PRO PANalytical) tool and based on the Gauss method. The sample to be analyzed is placed on the holder. Then, X-rays are shoot at the sample to get the diffraction spectrum. The results of the XRD analysis are in the form of graphs, numbers 2θ and d-spacing numbers to be calculated using software to obtain the degree of crystallinity and crystal size.

Particle Size
The sample is dispersed into a medium size to avoid the agglomeration of its particle with others. Then, it is entered into a tool (PSA Beckman Coulter LS 13 320) to identify the particles. The result of the sample measurement is a single measure to describe the condition of the sample as a whole.

3. Results and Discussion

3.1. Preparation Results

In this study, the treated raw substance in the process of making nanocrystalline cellulose experienced a color change from brownish-yellow to yellowish-white. The color changes occur as shown in Figure 1. The color change that occurred was caused by lignin compound degradation during the treatment of the nanocrystalline cellulose synthesis. Lignin is a constituent compound to cause cellulose a brownish color; therefore, if lignin is removed, it will cause the color of cellulose brighter.

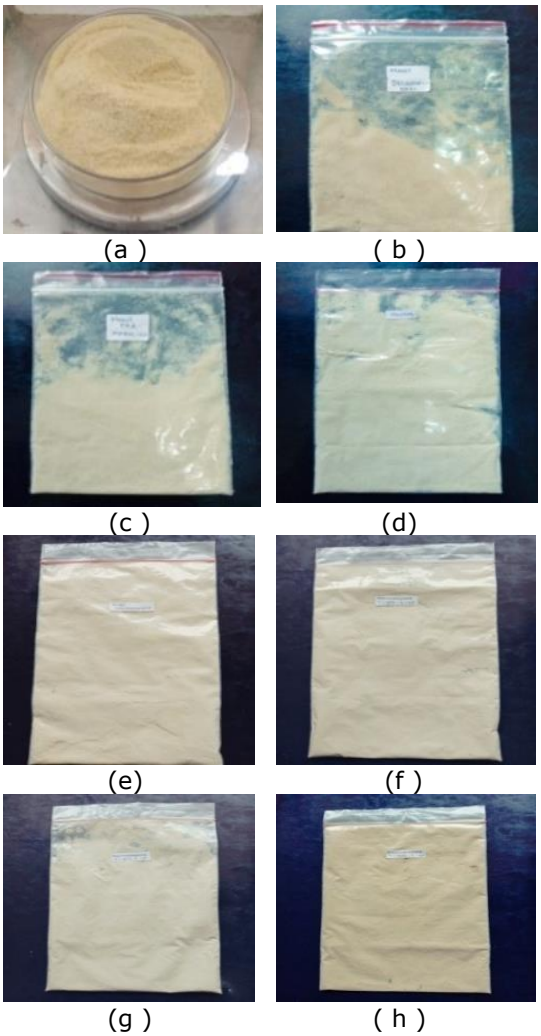

Figure 1. Changes in the color of substances in the nanocrystalline cellulose synthesis. (a) Raw materials; (b) Pre-hydrolysis results; (c) Delignification results; (d) Bleaching results; (e) E0; (f) E30; (g) E50; (h) E70

The effect of prehydrolysis on lignin dissolution was proposed by Wibisono et al. (2011). In this study it was explained that the ability of acetic acid to bind lignin will result in the release of α-cellulose bound to lignin, thus the pulp content with high α-cellulose content is obtained.

Lignin degradation occurred again when the delignification process was carried out. The decrease in lignin during the delignification process is caused by the reaction of breaking the bond between lignin and hemicellulose

because it is degraded by OH^- ions. Na^+ ions cut off with OH- ions will bind to lignin and will be dissolved in water (Nura' et al., 2017). This is corroborated by Hamisan (2009) which states that the dark brown delignification solution is an indication that compounds having chromophore groups with conjugate bonds undergo a dissolution process.

The use of NaOCl in the bleaching process will increase the brightness of the α-cellulose produced. This result is caused by the dissolution of lignin which is still bound to the cellulose after the pre-hydrolysis and delignification. Dissolution of residual lignin during the bleaching process occurs because of the degradation of lignin into short chains as a result of NaOCl solution, thus it is easily dissolved in the solution (Fengel, 1995).

After the pre-hydrolysis, delignification and bleaching processes were carried out, cellulose was obtained and hydrolyzed with various temperatures of electromagnetic induction. Each sample is hydrolyzed without electromagnetic (E0), with electromagnetic 30°C (E30), electromagnetic 50°C (E50), and electromagnetic 70°C (E70). Each of these samples was tested to determine the characteristics of the resulted nanocellulose.

3.2. Characterization of Nanocrystalline Cellulose

The characterization aims to compare the results of the nanocrystalline cellulose obtained from the research to commercial MCC. Based on the tests carried out on each sample of nanocrystalline cellulose and its comparison to commercial MCC sample, the characterization are obtained as in Table 1.

Based on the characteristic test, it was found that all of the test characteristics on the various hydrolyzed nanocrystals complied with the requirements set for MCC. According to the Indonesian Pharmacopoeia Edition III (1979), a good MCC is in the form of a powder, white, tasteless, and odorless. In addition, the water content, solubility in various solutions, pH and starch content in nanocrystalline cellulose fulfil MCC standards. The difference between nanocrystalline cellulose with MCC requirements in characteristic testing is color. The color on the terms of commercial MCC is white, while E0, E30, and E50 have a yellowish white color. While the E70 is brownish yellow.

Nanocrystalline cellulose had a yellowish white to brownish yellow color caused by hydrolysis carried out at high temperatures and a long time. The study of Vanhatalo et al. (2014) which used a hydrolysis temperature in the range of 120–160°C and a time of 0–1440 minutes showed that an increasing hydrolysis temperature would result in a decreasing trend in brightness level.

Therefore, it can be concluded that studies with a hydrolysis temperature lower than 120°C or more than 160°C will have similar trend. Browning of nanocrystalline cellulose is caused by glucose undergoing dehydration and condensation reactions (caramelization) in highly temperature acid solutions (Lichtenthaler, 2011).

Table 1. Characteristic results

Characteristic	Standard	MCC	E0	E30	E50	E70
Form	Powder	✓	✓	✓	✓	✓
Odor	Odorless	✓	✓	✓	✓	✓
Color	White	✓	×	×	×	×
Water	Insoluble	✓	✓	✓	✓	✓
Alcohol 96%	Insoluble	✓	✓	✓	✓	✓
HCl 2N	Insoluble	✓	✓	✓	✓	✓
NaOH 1N	Insoluble	✓	✓	✓	✓	✓
Eter	Insoluble	✓	✓	✓	✓	✓
pH	5,5-7,5	✓	✓	✓	✓	✓
Starch	None	✓	✓	✓	✓	✓
Water Content	<5%	✓	✓	✓	✓	✓

Table 2. Lignocellulosic results

No	Substance	Concentration (%)				
		Hemi	Cell	Lig	Wat	Ash
1	α Selulosa	20.39	33.59	42.42	3.18	0.40
2	E0	16.87	47.10	32.42	2.19	1.39
3	E70	14.35	62.93	20.73	1.52	0.44

Based on the research results, the brightness level of nanocrystalline cellulose is only affected by the parameters of temperature and hydrolysis time, while electromagnetic induction has no significant effect on the brightness level of nanocrystalline cellulose. This is based on the comparison of the brightness level of nanocrystalline cellulose E0 with E30. Based on observations, the brightness level of E0 nanocrystalline cellulose has no difference compared to E30 nanocrystalline cellulose. Therefore, it can be concluded that electromagnetic induction has no significant effect on the brightness level of the nanocrystalline cellulose.

3.3. Lignocellulosic Analysis

Lignocellulose testing on nanocrystalline cellulose aimed to determine the effect of the hydrolysis process and electromagnetic induction on the levels of hemicellulose, cellulose, and lignin contained in nanocrystalline cellulose. Tests were carried out on three samples of materials, namely alpha cellulose from bleaching (α-cellulose), E0, and E70. Based on the tests that have been carried out, the percentage results for each test material are attached in Table 2.

The results of the lignocellulose test showed that the hydrolysis treatment would increase the cellulose content and decrease the hemicellulose and lignin contents. In accordance with research conducted by Sun (2002), the use of an acid solution in hydrolysis will cause damage to the lignin and hemicellulose bonds, then the lignin and hemicellulose are dissolved in the hydrolyzed acid solution. In the hydrolysis process, electromagnetic induction will accelerate the process of breaking lignin and hemicellulose, thus it will increase the cellulose content. This is evidenced by the lignocellulosic test on the variation of E70 cellulose nanocrystal that obtains the highest cellulose value of 62.93%.

3.4. X-Ray Diffraction

XRD testing aims to determine the crystallinity of a sample or material. Based on the XRD test results carried out on the sample, the XRD data is then processed using the Origin Pro application.

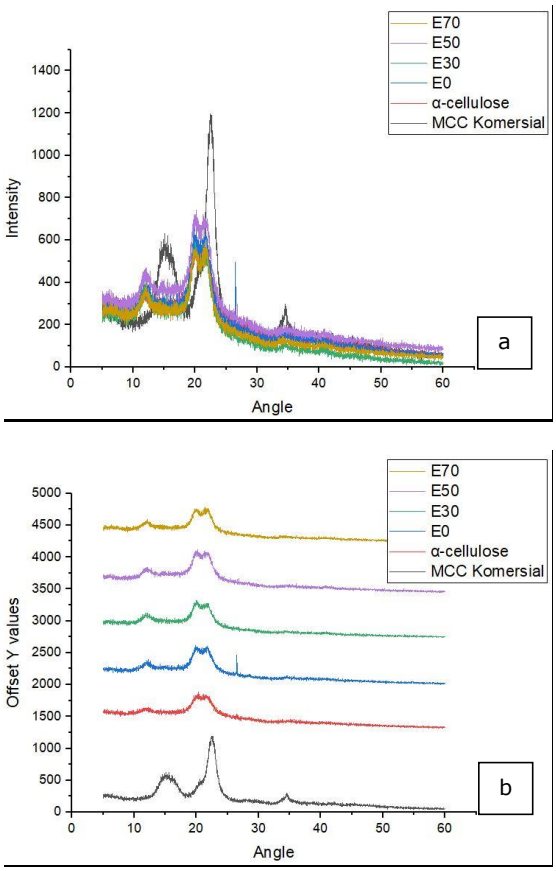

Figure 2. X-ray diffraction results. (a) Projection in terms of angle and intensity; (b) Contour comparison in angle projection

The XRD graph displayed a pattern of fluctuating lines resulted from the refraction of the X-ray by crystals on the particles of the test material (Figure 2). The more and higher the line of refraction, the higher the crystallinity of the material. Based on the calculations carried out using the value of the peak position, the area of the crystalline, amorphous fractions, and the FWHM obtained from the XRD test graph of each test material, the crystallinity is listed in Table 3.

Table 3. Crystalinity

Sample	Crystalinity
α-selulosa	48.99%
E0	74.75%
E30	76.40%
E50	79.23%
E70	90.68%
MCC Commercial	84.31%

Based on the results of calculations, cellulose from bleaching initially only has a crystallinity of 48.99%, then increase to 74.75% after being hydrolized. This proves that hydrolysis affects the crystallinity of nanocrystalline cellulose. The increase in crystallinity occurs because the hydrolysis process will break the long chains of cellulose, thus the amorphous part of the cellulose microfibril is cut off, and leaves the crystalline part (Ma et al., 2016). Thus, the less amorphous part will increases the crystallinity level.

Based on Table 3, high temperatures will increase the level of crystallinity of nanocrystalline cellulose. This is in accordance with Nura's research (2017), hydrolysis with the same time of 75 minutes at different temperatures produces different levels of crystallinity. At a temperature of 120^{o}C, crystallinity of 74.3% is obtained, while at a temperature of 160^{o}C, it is 79.1%. In this study, the best crystallinity value was obtained by hydrolysis treatment with electromagnetic 70^{o}C, where the crystallinity value was 90.68%. Compared with the same treatment at a lower temperature of 50^{o}C, the crystallinity value increased by 14.38%. The higher the hydrolysis temperature used, the more effective the hydrolysis process that occurs, because hydrolysis requires high temperatures to occur optimally.

The effect of electromagnetic on crystallinity can be seen from the comparison of the results of the cellulose nanocrystal test at variations of E0 and E30. At a temperature of 30^{o}C (room temperature) hydrolysis occurs at a small rate, because a large number of hydrolysis reactions occur when the solution temperature reaches 70^{o}C. Thus, in the E30 treatment, the dominant parameter that affects the decrease in size and increase in crystallinity is electromagnetic waves. Judging from the test results, the test material in the E30 treatment had a crystallinity of 76.40%. This result is better than the E0 hydrolysis treatment (room temperature) that is 74.75%. Therefore, it can be concluded that the electromagnetic effect on the increase in crystallinity. The effect of electromagnetic induction is stated by Siregar (2007) where the particles affected by magnetic induction will undergo splitting into smaller parts.

3.5. PSA (Particle Size Analyzer)

PSA testing aims to determine the crystal size of the cellulose produced in the study. Figure 3 is a graph of the results of the PSA test on the material of this study. Similar to the XRD test chart, the PSA chart also displays an up and down line patterns. The line pattern shows the distribution of the cellulose crystal size of the test material in the ratio of volume (%) with a range of 0–100% and diameter in the range of 0.375-2000 μm. The higher the graph, the higher the volume of cellulose crystals at a certain size. If the graph is getting to the right thus the size of the cellulose crystals will be getting bigger. Based on the PSA test results, the average size is shown in Table 4.

Table 4. Particle size

Sample	Size
Cassava Stem Powder	166.2 μm
α-selulosa	83.78 μm
MCC Commercial	57.66 μm
E0	22.22 μm
E30	49.33 μm
E50	46.65 μm
E70	18.04 μm

Based on the data from the particle size test of cellulose crystals obtained in this study, the various hydrolysis with or without electromagnetic resulted in a decrease in the particle size of cellulose crystals. Hydrolysis treatment without electromagnetic can reduce the particle size of alpha cellulose from 83.78 μm to 22.22 μm, or 73.47%. According to Ma et al. (2008), the decrease in cellulose particle size was caused by the erosion of the amorphous part of the microfibrils, thus the more amorphous part eroded, the smaller the size of the cellulose crystals of the test material. The effect of hydrolysis in decreasing the crystal size of cellulose was also proven in a study conducted by Zhang et al. (2007). In this study, pure cellulose with a size of 0.465 mm was hydrolyzed with an acid solution. After hydrolysis, the average particle size was 560 nm. Therefore, it can be concluded that hydrolysis can reduce the size of cellulose particles.

The Particle Size Analyzer (PSA) test results showed that an increase in temperature would tend to reduce the particle size of cellulose crystals. In the E30 variation, the particle size of cellulose crystals was 49.33 µm, E50 results in the particle size of cellulose crystals of 46.65 µm, whereas when using E70, the particle size of cellulose crystals was smaller, namely 18.04 µm. The decrease in particle size resulted from the hydrolysis process was also obtained in a study conducted by Lismeri et al. (2018).

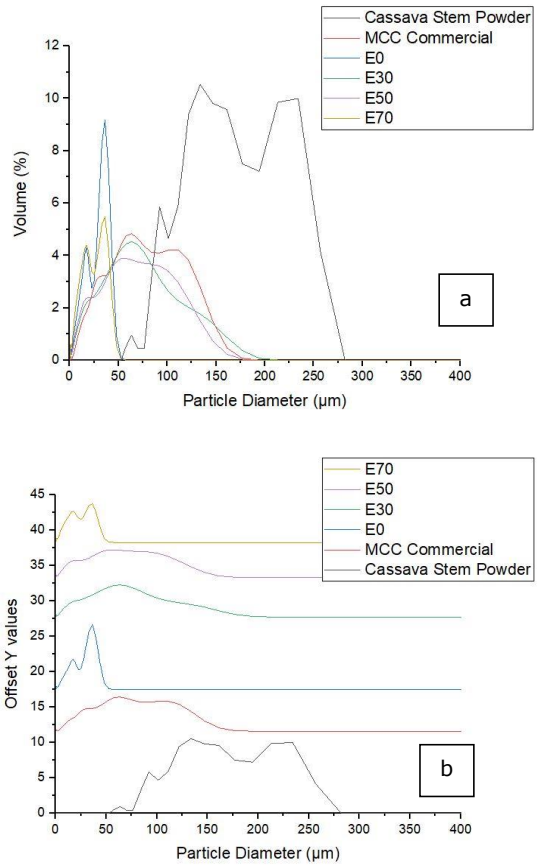

Figure 3. Particle size analyzer results. (a) Projection in terms of particle diameter and volume; (b) Contour comparison in particle size

In this study, particle size of the hydrolysis material with a time of 90 minutes and a temperature of 70°C is 228.8 nm, while at the same time, but at a temperature of 50°C is larger than 400 nm. This happens because at high temperatures the hydrolysis will be more optimal, thus the amorphous degradation process will be higher (Nura' et al., 2017). The electromagnetic effect on this research can be seen from the comparison of the results of the cellulose crystal testing on the E0 and E70 treatments. Based on the test results, E70 has the smallest average size of 18.04 µm. While the hydrolysis treatment without electromagnetic has a size of 22.22 µm. These results indicate that there is an electromagnetic effect on the decrease in the crystal size of cellulose. However, the hydrolysis with electromagnetic at temperatures below 70°C has a larger cellulose crystal size than that without electromagnetic. This is possibly caused by the electromagnetic at certain parameters tends to change some structures to become amorphous. This phenomenon occurs in the study of Sasue et al. (2017) that uses electromagnetic power of 300 watts. After testing, the results indicates that electromagnetic waves will increase the amorphous level in certain areas, thus the size of cellulose crystals becomes larger. Based on this, it can be concluded that the use of electromagnetic induction as a method of reducing the size of cellulose crystals must be regulated by certain parameters.

PSA testing showed that the size of the cellulose crystals produced is still various in the nanometer to micrometer size distribution. The distribution of particles in nanometer size is in the size range of 0.375–0.954 µm or 375–954 nm. The largest percentage of nano-sized crystalline cellulose particles is found in the E70 treatment, namely 4.819%. Threfore, it can be concluded that the hydrolysis treatment with electromagnetic can reduce the size of cellulose crystals into nanoparticles, but in a very small percentage. Based on the test results, it can be concluded that all the results of the various treatments of nanocrystalline cellulose in this study have characteristics close to the standards set for MCC, and the results of research with 70°C electromagnetic hydrolysis treatment have better sizes and crystallinity levels compared to the commercial MCC. The smaller the crystal size, the more and easier the nanocrystalline cellulose are distributed in medicine or food when it used as a filler, thus the product is denser. Meanwhile, the higher the crystallinity of a cellulose nanocrystal used as a medicine or food filler, the stronger and stiffer the product will be.

4. Conclusion

Based on this research, the best cellulose nanocrystal product was obtained, namely at the E70 treatment with a crystallinity degree of 90.68%, and 0.099% of the particle size is 375 nm, where the use of electromagnetic induction in the hydrolysis process has an influence on the size and degree of crystallinity of nanocrystalline cellulose. However, electromagnetic induction did not

affect the brightness of the nanocrystalline cellulose, because the brightness of the nanocrystals was affected by the increase in temperature and the length of time when the hydrolysis process took place.

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