Optimization of Sulfuric Acid Concentration and Hydrolysis Time on Crystallinity of Nanocrystalline Cellulose: A Response Surface Methodology Study

D Sartika, K Syamsu, E Warsiki, F Fahma

1Department of Agroindustrial Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, Bogor 16680, West Java, Indonesia.
2Faculty of Agriculture, Muhammadiyah University of Makassar, Makassar 90221, South Sulawesi, Indonesia

Email: khaswars@yahoo.com

Abstract. Kapok is a source of natural fiber in addition to cotton, which availability is abundant and contain high cellulose but not been utilized optimally. This study aimed to determine the optimal conditions of sulfuric acid concentration and hydrolysis time in the production process of nanocrystalline cellulose (NCC) from kapok fiber. The research was conducted in several stages, namely the delignification with alkali hydrothermal, bleaching with alkaline hydrogen peroxide agents, isolation with sulfuric acid solutions, and NCC characterization. The optimization used Response Surface Methodology (RSM) with Central Composite Design (CCD), which consisted of two factors, namely H₂SO₄ concentration and hydrolysis time. The optimized parameter was the crystallinity degree of the NCC. NCC characterization included functional group analysis with Fourier Transform Infrared Spectroscopy (FTIR), surface profiles and dimensions by Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM). The results showed that the optimum condition of NCC isolation from kapok fiber was obtained at 54.46% (b/b) H₂SO₄ concentration and hydrolysis time of 48.96 minutes, resulting in a maximum degree of crystallinity of 71.8%. The hydrolysis process with H₂SO₄ caused a change in the NCC functional group. NCC had a diameter of about 11.2 ± 2.63 nm and high thermal stability that potential for various composite materials.

1. Introduction
Cellulose is one source of natural fiber that has a crystalline structure. This structure can improve mechanical properties so that researched widely and used as reinforcing materials in various composites. Kapok is a type of cellulose source. Until today, kapok is used only as a filler for mattresses, pillows, and dolls. Kapok
fiber contains cellulose ranging from 53.4-64.0% [1,2]. Research and application of cellulose in the form of nanomaterials have been developed rapidly. Nanocellulose is known to have various advantages including high surface area, low density, high tensile strength, and high modulus of elasticity [3]. The superiority causes nanocellulose to be widely used as a reinforcing agent in the polymer composite matrix to produce new composites with superior characteristics. Nanocellulose has been widely used as a composite material in construction, packaging, transportation, electronic devices, and biomedicine [4,5].

The chemical isolation with acid hydrolysis is a widely used method for nanocrystalline cellulose production [6]. One of the advantages of acid isolation is the nanocellulose with abundant hydroxyl groups. It causes nanocellulose to be highly reactive and hydrophilic so as facilitating the mixing and dispersing in the composite matrix [7]. However, acid hydrolysis also has disadvantages, especially in the conditions of the isolation process. Various factors become very sensitive to consider in the isolation process including type and concentration of acid, temperature, and reaction time. Extreme reaction conditions will cause uncontrolled degradation. Cellulose will be hydrolyzed into unwanted products such as sugar. [8]. The improper isolation process has an impact on the yield, crystallinity, and dimensions of the nanocellulose [9,10].

Optimization is usually applied as a method to determine ideal conditions in a production process to produce the best response. Response surface methodology (RSM) has been widely applied to modelling, simulation, and optimization of complex processes based on statistical design and analysis. RSM not only efficiently achieves optimal conditions at low cost and without conducting extensive experimental tests but also can easily find out the relationship between variables or several response variables with visualization of surface responses [11]. There is limited information about the optimal conditions of sulfuric acid concentration and hydrolysis time in the isolation of nanocrystalline cellulose from kapok fiber. This study will determine the optimum conditions of these two factors.

2. Materials and Methods

2.1 Tools and materials
Kapok fiber from Javanese varieties was obtained from Badung Regency, Bali-Indonesia. Chemicals used with pro analytic grade (PA) and tools for analysis.

2.2 Experimental optimization and design
Optimization of the isolation process of nanocrystalline cellulose (NCC) from kapok fiber using the RSM-Central Composite Design (CCD) method with H2SO4 concentration (X1) and hydrolysis time (X2) as independent variables (table 1). The research response data were analyzed by Design-Expert software version 7.0 to create mathematical models, graphs, and analyze optimal conditions of NCC isolation response data.

| Table 1. Design of Central Composite Design |
|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Treatment | Code | Factor | |
| (X1) | (X2) | (X3) | (X4) |
| 1 | -1 | -1 | 56 | 40 |
| 2 | -1 | 1 | 64 | 40 |
| 3 | 1 | 1 | 56 | 60 |
| 4 | 1 | -1 | 64 | 60 |
| 5 | 1.414 | 0 | 54.34 | 50 |
| 6 | -1.414 | 0 | 65.65 | 50 |
| 7 | 0 | 1.414 | 60 | 35.85 |
| 8 | 0 | -1.414 | 60 | 64.14 |
| 9 | 0 | 0 | 60 | 50 |
| 10 | 0 | 0 | 60 | 50 |
| 11 | 0 | 0 | 60 | 50 |
| 12 | 0 | 0 | 60 | 50 |
2.3 Isolation of nanocrystalline cellulose

The isolation process of kapok cellulose was carried out chemically through acid hydrolysis using H₂SO₄ concentration and time hydrolysis according to the CCD design in Table 1. Hydrolysis was carried out at 50°C, the ratio between the cellulose fiber weight and H₂SO₄ concentration at 1: 80 (w/w), and stirring at 600 rpm with a hotplate stirrer. Hydrolysis was stopped by adding aquades (8-10°C) 4-5 times when the processing time has been reached. Furthermore, the formed suspension was centrifuged at 11,000 rpm, for 20 minutes, at -4°C (repeated 5-6 times) to obtain a neutral pH nanocrystalline cellulose supernatant.

2.4 Characterization of nanocrystalline cellulose

Characterization of nanocrystalline cellulose included measuring the crystallinity degree with the Segal method [13]; the value of d-spacing with the equation Bragg’s; crystallite size with the Scherrer’s equation [14]; morphology, size, and distribution of particles with Scanning Electron Microscope (SEM); and thermal stability with Thermogravimetry Analysis (TGA).

3. Results and Discussions

3.1 Optimization model of isolation factors for crystallinity degree

In general, the p-value<0.05 indicates that independent factors have a statistically significant effect on the response measured [15]. A good model must meet several criteria, namely the significance of the model, significance of lack of fit, coefficient of determination (R²), coefficient of variation (CV), standard deviation (SD), and adequate precision of the model. The significance of the model from the p-value was 0.0003 or smaller than the significance level (p-value <0.05). It shows that the suitability analysis of the second order model in quadratic form has a very significant effect on the value of kapok NCC crystallinity. The lack of fit value of the quadratic model was 0.5599 (p-value > 0.05). It shows that the value is relatively insignificant. A good model has insignificant lack of fit and has a relatively insignificant lack of fit to pure error [16]. This shows that the obtained model is in accordance with existing data. The R² model value was 0.9652. It shows that the factor of H₂SO₄ concentration and hydrolysis time have a significant effect on the degree of crystallinity of 96.52%. Adjusted R-square value (R² adj) and predicted R-square (R² pred) showed the variation of data around the average data described by the model. R² adj value of 0.9363 and R² pred value of 0.8542 were very close to the value of R², indicating the data in reasonable agreement which means the value of the predictive response corresponds to the actual response value so that the model obtained can model the data properly. Coefficient of variation (CV) shows the coefficient degree of confidence in the conclusions/results of an experiment. The lower the coefficient of variation, the more accurate the estimation is [17]. The reproducible model has a CV value of less than 10% [15,18]. The optimization model in this study had a CV value of 5.34%, which shows the high reliability of the study. SD value of 3.11 indicates that the experimental model has satisfying precision, reliability, and reproducibility. Adequate precision value (Adeq pre) is used to measure the ratio between signal and noise, which is to compare the range of predictive values at the design point of the model with an average prediction error. A ratio greater than 4 indicates adequate model discrimination [19]. Adequate precision values was 14,219 indicating that the model has a sufficient signal. Variance analysis of regression model of the crystallinity degree is presented at Table 2.

Multiple regression analysis of the experimental data relationship between the independent variable (X) to the dependent variable or response (Y) produced the quadratic polynomial equation as in Eq. 1.

\[ Y = -3664.91 + 109.36X_1 + 19.26X_2 - 0.25 X_1X_2 - 0.82 X_1^2 - 0.04 X_2^2 \] (1)

The positive regression coefficient indicates that there is a synergistic effect, while the negative coefficient value indicates the effect of an inverse relationship [20]. In the regression equation, it can be seen that all linear factors (X₁, X₂) show a positive correlation. It indicates that the increase in sulfuric acid concentration and hydrolysis time causes an increase in the degree of crystallinity and vice versa. However, the regression coefficients which was in the form of interactions of factors (X₁X₂) and quadratic of the concentration factor...
(X1²), and the hydrolysis time (X2²) had a negative value. This shows that an increase in the interaction conditions and squares between each factor causes a decrease in the degree of crystallinity and vice versa.

Table 2. Variance Analysis of the order II regression model of the crystallinity degree

| Source of heterogeneity | JK  | db  | KT  | F-count | p-value Prob > F |
|-------------------------|-----|-----|-----|---------|------------------|
| Model                   | 1610.50 | 5   | 322.10 | 33.31 | <0.0003*         |
| X1                      | 104.27  | 1   | 104.27 | 10.78 | <0.0167*         |
| X2                      | 0.67    | 1   | 0.67  | 0.070  | 0.8007           |
| X1X2                    | 396.01  | 1   | 396.01 | 40.96 | 0.0007*          |
| X1²                     | 1088.89 | 1   | 1088.89 | 112.62 | <0.0001*         |
| X2²                     | 122.15  | 1   | 122.15 | 12.63 | 0.0120*          |
| Residual                | 58.01   | 6   | 9.67  |        |                  |
| Lack of Fit             | 26.27   | 3   | 8.76  | 0.83   | 0.5599           |
| Pure Error              | 31.74   | 3   | 10.58 |        |                  |
| Cor Total               | 1668.51 | 11  |       |        |                  |

| Std. Dev.               | 3.11    |      |       | R²     | 0.9652           |
| Mean                    | 58.19   |      |       | R² adj | 0.9363           |
| C.V. %                  | 5.34    |      |       | R² pred | 0.8542          |
| Adeq Pre.               |   |      |       |        | 14.219           |

*p-value<0.05 show a significant effect.

Figure 1. The contour plot and the three-dimensional surface response from the interaction between the concentration of sulfuric acid and the hydrolysis time.

The concentration factor of sulfuric acid (X1) in both linear and quadratic forms had the highest regression coefficient. It shows that sulfuric acid concentration has the highest and most significant effect on changes in the crystallinity degree (p-value <0.05) (table 2). Meanwhile, the quadratic form of the time
factor (X2) does not have a significant effect on the changes in the crystallinity degree (p-value> 0.05), but in the form of interaction and quadratic shows a significant influence in the crystallinity degree.

3.2 Effects of isolation factors to crystallinity degree
The three-dimensional surface response plot and contour plot in figure 1 shows that the crystallinity degree increased rapidly if the acid concentration and temperature were increased from 54.34-59.11% and 40-52.20°C, respectively. However, an increase in the concentration of sulfuric acid and temperatures of more than 59.11% and 52.20°C caused a decrease in the crystallinity degree.

3.3 Optimum condition of isolation process and model validation
Optimization process in NCC isolation produced optimal conditions of 59.11% H2SO4 concentration, hydrolysis time of 52.19 minutes with the result of approximately 70.06% crystallinity degree. Model validation uses t-test analysis by comparing two groups of mean data, namely data on the crystallinity degree of experimental results with predictions. The model is indicated valid if there is no significant difference between the mean values of the experiment and the prediction of crystallinity degree (p-value>0.05). The insignificant difference between the value of experimental and predictions data is obtained if the t-test value is in the range of values of $-t_{table}(1-0.5\alpha) < t_{test} < t_{table}(1-0.5\alpha)$. From the t-test analysis obtained t-test 0.47 was located between the ranges of -2.45 <0.47 <2.45 or (p-value> 0.05) indicating that the crystallinity degree generated from experimental data is not significantly different from the model prediction data (table 3). It can be concluded that the resulting model shows reasonable predictability and adequate accuracy to optimize the NCC in experimental conditions.

Table 3. The results of the t-test analysis of experimental data with model predictions for model validation

| Concentration H2SO4 (% b/b) | Time (minute) | Crystallinity degree (%) | Experiment | Prediction |
|-----------------------------|---------------|--------------------------|------------|------------|
| 59.11                       | 52.19         | 73.75                    | 70.06      |            |
| 59.11                       | 52.19         | 76.15                    | 70.06      |            |
| 59.11                       | 52.19         | 73.48                    | 70.06      |            |
| 59.11                       | 52.19         | 73.04                    | 70.06      |            |
| 60.00                       | 60.00         | 60.70                    | 68.30      |            |
| 60.00                       | 50.00         | 71.00                    | 69.70      |            |
| 60.00                       | 40.00         | 59.20                    | 63.10      |            |
| Mean                        |               | 69.62                    | 68.76      |            |

3.4 Characteristic of nanocrystalline cellulose
The morphology of KF, BKF, and nanocrystal cellulose shows that KF had a smooth and sleek fiber surface with a fiber diameter of 19.9 μm. After the delignification and bleaching process (BKF), the fiber surface became wrinkled, flat with a diameter of 12.57 μm. Changes in fiber morphology and diameter indicate that the delignification and bleaching can degrade lignin and hemicellulose resulting in smaller fiber diameters [21]. The NCC isolation process using sulfuric acid produced needle-shaped fibers with a diameter of 14.38 nm. Acid hydrolysis is not only able to break down the fiber, but also promotes the defibrillation fiber to the nanoscale [22]. The process of delignification, bleaching, and isolation of NCC caused an increase in the crystallinity degree and crystallite size. The higher H2SO4 concentration are easier to penetrate and degrade the amorphous fraction of cellulose so that they are more sensitive to glycosidic bonds cleavage and produce nanocellulose structures with higher crystallinity [23]. Meanwhile, the crystallite size of KF, BKF, and NCC were 0.58 nm, 1.94 nm, and 2.58 nm, respectively. Increased crystallite size is caused by
the recrystallization process in nanocellulose crystal structures [24]. The thermal decomposition of KF occurred at temperatures around 64°C, 260°C, and 350°C, BKF at 57°C, 209°C, and 348°C, and NCC at 70°C, 272°C, and 368°C. Decomposition mechanism for each ingredient consists of 3 stages. The first stage decomposition shows the evaporation of water bound to the material [25], the second stage decomposition shows the depolymerization process from the cellulose and non-cellulose fractions [26], and the third stage decomposition shows the occurrence of carbonation to produce residues [27].

4. Conclusion
Optimization of NCC isolation with RSM showed that the interaction between H$_2$SO$_4$ concentration and hydrolysis time had a significant effect on the crystallinity degree with a determination coefficient of 0.9652. The optimal condition of kapok NCC isolation process was obtained at 59.11% H$_2$SO$_4$ concentration and hydrolysis time for 52.19 min with the degree of crystallinity approximately 70.06%. The NCC isolation process causes a decrease in fiber diameter from raw materials of 19.90 μm to NCC of 14.38 nm and changes in fiber functional groups. The NCC had a d-spacing value of approximately 0.39-0.58 nm and the crystallite size was approximately 2.51 nm. The $T_{onset}$ value of 271.53°C and $T_{max}$ 298.69°C shows that NCC has high thermal stability so that it has the potential to be used as a reinforcing material or filler in the manufacture of polymer composite materials.

5. Acknowledgments
The author would like to acknowledge Directorate General of Higher Education, BUDI-DN scholarship 2016 for funding this research

References

[1] Liu Y, Wang J, Zheng Y and Wang A, Adsorption of methylene blue by kapok fiber treated by sodium chloride optimized with response surface methodology Chem. Eng. J. 184 (2012) 248–255 doi:10.1016/j.cej.2012.01.049

[2] Draman S F S, Daik R, Latif F A and El-Sheikh S M Characterization and thermal decomposition kinetics of kapok (Ceiba pentandra L.-based cellulose BioResources 9 (2014) 8–23 doi:10.15376/biores.9.1.8-23

[3] Sanjay M R, Arpitha G R, Naik L L, Gopalakrishna K and Yogesha B Applications of Natural Fibers and Its Composites: An Overview Nat. Resour. Res. 07 (2016) 108–114. doi:10.4236/nr.2016.73011

[4] Bharimalla A K, Deshmukh S P, Vigneshwaran N, Patil P and Prasad V Nanocellulose Based Polymer Composites for Applications in Food Packaging: Future Prospects and Challenges J. Polym. Technol. Eng. 56 (2017) 805–823 doi:https://doi.org/10.1080/03602559.2016.1233281

[5] Tayeb A H, Amini E, Ghasemi S and Tajvidi M Cellulose nanomaterials-binding properties and applications: A review Molecules 23 (2018) 1–24. doi:10.3390/molecules23102684

[6] Li W, Yue J and Liu S Preparation of nanocrystalline cellulose via ultrasound and its reinforcement capability for poly(vinyl alcohol) composites Ultrason. Sonochem. 19 (2012) 479–485 doi:10.1016/j.ultsonch.2011.11.007

[7] Grishkewich N, Mohammed N, Tang J and Tam K C, Recent advances in the application of cellulose nanocrystals Curr. Opin. Colloid Interface Sci. 29 (2017) 32–45 doi:10.1016/j.cocis.2017.01.005

[8] Lee H V, Hamid S B A and Zain S K Conversion of lignocellulosic biomass to nanocellulose: Structure and chemical process Sci. World J. 2014 (2014). doi:11.155/2014/631013

[9] Chen Y W, Tan T H, Lee H V and Hamid S B AEasy fabrication of highly thermal-stable cellulose nanocrystals using Cr(NO3)3-catalytic hydrolysis system: A feasibility study from macroto nano-dimensions Materials (Basel) 10 (2017). doi:10.3390/ma10010042

[10] Fahma F, Iwamoto S, Hori N, Iwata T and Takemura A Isolation, preparation, and characterization
of nanofibers from oil palm empty-fruit-bunch (OPEFB) Cellulose 17 (2010) 977–985 doi:10.1007/s10570-010-9436-4

[11] Bezerra M A, Santelli R E, Oliveira E P, Villar L S and Escaleira L A Response surface methodology (RSM) as a tool for optimization in analytical chemistry Talanta 76 (2008) 965–77 doi:10.1016/j.talanta.2008.05.019

[12] Montgomery D C Design and Analysis of Experiments, 5th Edition, 5th ed., John Wiley & Sons, Inc., New York, 2000

[13] Segal L, Creely J J, Martin A E and Conrad C M An Empirical Method for Estimating the Degree of Crystallinity of Native Cellulose Using the X-Ray Diffractometer Text. Res. J. 29 (1959) 786–794 doi:10.1177/004051755902901003

[14] Fahma F, Iwamoto S, Hori N, Iwata T and Takemura A, Effect of pre-acid-hydrolysis treatment on morphology and properties of cellulose nanowhiskers from coconut husk Cellulose. 18 (2011) 443–450. doi:10.1007/s10570-010-9480-0

[15] Chen Y W, Lee H V and Abd Hamid S B, Investigation of optimal conditions for production of highly crystalline nanocellulose with increased yield via novel Cr(III)-catalyzed hydrolysis: Response surface methodology Carbohydr. Polym. 178 (2017) 57–68 doi:10.1016/j.carbpol.2017.09.029

[16] Shabbiri K, Adnan A, Jamil S, Ahmad W, Noor B and Rafique H M Medium optimization of protease production by (2012) 1051–1061

[17] Mohan SK, Viruthagiri T and Arunkumar C Statistical optimization of process parameters for the production of tannase by Aspergillus flavus under submerged fermentation 3 Biotech. 4 (2013) 159–166. doi:10.1007/s13205-013-0139-z

[18] Masoumi H R F, Kassim A, Basri M and Abdullah D K Determining optimum conditions for lipase-catalyzed synthesis of triethanolamine (TEA)-based esterquat cationic surfactant by a Taguchi robust design method Molecules. 16 (2011) 4672–4680 doi:10.3390/molecules16064672

[19] Adalarasan R and Santhanakumar M Response Surface Methodology and Desirability Analysis for 7 (2015) 2625–2631

[20] Kara Ali M, Outili N, Ait Kaki A, Cherfia R, Benhassine S, Benaissa A and Kacem Chaouche N Optimization of Baker’s Yeast Production on Date Extract Using Response Surface Methodology (RSM) Foods. 6 (2017) 64 doi:10.3390/foods6080064

[21] Kumar A, Singh Negi Y, Choudhary V and Bhardwaj N K Characterization of Cellulose Nanocrystals Produced by Acid-Hydrolysis from Sugarcane Bagasse as Agro-Waste &quot;Characterization of Cellulose Nanocrystals Produced by Acid-Hydrolysis from Sugarcane Bagasse as Agro-Waste J. Mater. Phys. Chem. 2 (2014) 1–8 doi:10.12691/jmpe-2-1-1

[22] Kargarzadeh H, Ahmad I, Abdullah I, Dufresne A, Zainudin S Y and Sheltami R M Effects of hydrolysis conditions on the morphology, crystallinity and thermal stability of cellulose nanocrystals extracted from kenaf bast fibers Cellulose. 19 (2012) 855–866 doi:10.1007/s10570-012-9684-6

[23] Prado K S and Spinacé M A S Isolation and characterization of cellulose nanocrystals from pineapple crown waste and their potential uses Int. J. Biol. Macromol. 122 (2019) 410–416 doi:10.1016/j.ijbiomac.2018.10.187

[24] Sèbe G, Ham-Pichavant F, Ibarboure E, Koffi A L C and Tingaut P Nanowhiskers Produced by Acid Hydrolysis of Cellulose I Substrates Biomacromolecules. 13 (2012) 570–578

[25] Mahardika M, Abral H, Kasim A, Arief S and Astrofi M Production of Nanocellulose from Pineapple Leaf Fibers via High-Shear Homogenization and Ultrasonication Fibers. 6 (2018) 28 doi:10.3390/fib6020028

[26] Chandra J, George C S, Narayanankutty N, S K Isolation and characterization of cellulose nanofibrils from arecanut husk fibre Carbohydr. Polym. 142 (2016) 158–166
[27] Chen X, Yu J, Zhang Z and Lu C Study on structure and thermal stability properties of cellulose fibers from rice straw *Carbohydr. Polym.* **85** (2011) 245–250 doi:10.1016/j.carbpol.2011.02.022.