Optimization of NaOH concentration and trichloroacetic acid in bacterial carboxymethylation cellulose

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Abstract

The aims of this study were to obtain the optimal concentration of NaOH and trichloroacetic acid in the process of making CMC from bacterial cellulose. The experimental design used was response surface method. In the present study, NaOH as the variable $X_1$ is concentration with a minimum limit (-1) of 30%, optimal limit (0) of 35% while the maximum limit (+1) of 40%. Variable $X_2$ is the trichloroacetic acid concentration with a minimum limit (-1) of 25%, an optimal limit (0) of 30% and a maximum limit (+1) of 35%. Based on calculations using the program, expert design combination of treatments was obtained by 13 samples. The selection of the treatment above is based on the fact that in making CMC the alkalization process and carboxymethylation play an important role. The results showed variable optimum points of NaOH and Trichloroacetic Acid concentrations were 35.95% and 30.87%. The optimum point of the independent variable, the optimum response value of the substitution degree obtained was 0.897, water content of 20.08%, pH of 10.04 and viscosity of 17.26%. In conclusion, the best treatment of NaOH concentration on 35.95%, trichloroacetic acid on 30.87%, substitution degrees on 0.93, water content on 19.86%, pH on 10.17, and viscosity on 17.39 cPs.

1. Introduction

Nata de coco is a layer with a thickness of approximately 2 cm in white and elastic formed as a result of the activity of the bacterium *Acetobacter xylinum* in coconut water media through fermentation (Setiaji *et al.*, 2002; Martins *et al.*, 2009; Lin *et al.*, 2013). Inside of it, the nata contains cellulose known as bacterial cellulose (Hirayama *et al.*, 2013). The advantages of this bacterial cellulose are very good for digestive health (Hidayat *et al.*, 2006; Manoi, 2007; Fifendy *et al.*, 2011).

Nurhayati (2006) suggests that the water content of nata de coco was around 90%. High water content may cause this product to be easily damaged and transportation is limited. So far, to prevent damage to the nata de coco is done by soaking the water must be replaced every day (Rizal *et al.*, 2013). This will cause nata de coco difficult to be carried everywhere. So, it is necessary to find a solution to overcome this problem by making it into powder. According to Winarno (2007), the advantages of materials that have been dried into powder have more durability in nature, their storage time is long, volume and weight are reduced so that it is easier in packing, transporting and transportation which may reduce production costs.

The main requirement for good powder making is that the powder must be soluble in water. Nata de coco contains a water-insoluble substance, the bacterial cellulose. Bacterial cellulose according to Phillips and Williams (2009) is insoluble in water. Efforts to increase the solubility of bacterial cellulose in water are carried out through derivatization which is made into cellulose derivatives. Cellulose derivative in question is in the form of carboxymethylcellulose (CMC) made by alkalization and carboxymethylation (Nisa and Putri, 2013). The carboxymethylcellulose (CMC) produced can function as a binder, coating, microemulsion (Sugita *et al.*, 2010; Ramli *et al.*, 2015).

The most influential factor in the derivation of bacterial cellulose into CMC is alkalization and carboxymethylation. Alkalization carried out using NaOH aims to reactivate OH groups in the structure of cellulose. The calibration also functions to make the cellulose structure expand. The structure of the cellulose that expands makes the pores widen so as to facilitate
Carboxymethylation reagents to penetrate and diffuse inside (Candido and Gonçalves, 2016).

Carboxymethylation is basically an etherification process, carried out using trichloroacetic acid reagent. This process involves the attachment of the carboxylic group to the cellulose structure. The carboxylic group in question comes from trichloroacetic acid. This process is important to control because it is to see how much influence the carboxylic group can substitute with hydroxyl glucose in the cellulose structure (Bono et al., 2009). First, cellulose is converted to alkali cellulose in the presence of sodium hydroxide, followed by a reaction of the alkali cellulose with monochloroacetic acid to form CMC sodium salt (Joshi et al., 2015). The study aimed to obtain the optimal concentration of NaOH and trichloroacetic acid in the process of making CMC from bacterial cellulose.

2. Materials and methods

2.1 The materials and research equipment

Materials used in this study were bacterial cellulose nata de coco. Nata de coco was made using coconut water from sawarna variety (DSA) coconuts of old age, obtained from Dampit Village, Malang Regency. The old-age physical characteristics of coconut fruit are inter Alia dark brown color, the thickness of the fruit is 1.5 cm and the coconut milk can be taken. A. xylinum was obtained from the Microbiology Laboratory of Universitas Brawijaya, sucrose, glucose, yeast extract, peptone bacto, glacial acetic acid, NaH2PO4, MgSO4·7H2O, agar, H2SO4, NaOH, Aquadest, K2SO4 and CaCO3 which all of these chemicals were obtained from the Makmur Sejati Chemical Store in Malang City.

2.2 Cultivation of Acetobacter xylinum in starter

Starters, namely the strains of A. xylinum which were cultured into the media. The making process of a starter was conducted by growing pure strains into coconut water from Sawarna variety (DSA) coconut which is enriched using nutrients in the form of sucrose and ammonium sulfate and the pH of the media were made into 4 by adding glacial acetic acid. The number of A. xylinum cells to be inoculated into fermentation media was uniformed, i.e. 2 x 10⁷ cells/mL. To reach the number of cells, it was calculated directly using the haemocytometer method (Oliveira et al., 2015; Sulistyani et al., 2016)

2.3 Making nata de coco

Coconut water from sawarna variety (DSA) coconut fruit was boiled at 100°C for 15 mins. Next, the nutrients in the form of 2% sucrose and 0.06% ammonium sulfate were then boiled again. After that, the media were poured into a fermentation tub and covered with parchment paper and filter cloth then tied using rubber bands. Media were left for the cooling process for 12 hrs after which the pH of the media was made into 4 by adding glacial acetic acid to the media as much as 20 ml. Inoculation of the starter, A. xylinum, into the media was then incubated to ferment for 14 days.

2.4 Making of natrium carboxymethyl cellulose (Na-CMC)

Nata de coco fermented coconut water from DSA coconut was dried using a cabinet dryer for 12 hrs at 70°C, after drying, it is smoothed into smaller sizes to pass the 100-mesh sieve. Bacterial cellulose was then made into Na-CMC with an alkalinization, carboxymethylation and neutralization process.

Alkalization was carried out by eight grams (8 g) dry weight of bacterial cellulose powder put into 500 mL Erlenmeyer which was placed on a hotplate stirrer then added 200 mL isopropanol, 25 mL ethanol, 25 mL distilled water and stirred for 10 mins. Then NaOH solution was added in accordance with the treatment (30%, 35%, 40%) drop by 20 mL. This alkalinization process place at a temperature of 24°C for 1 hr. Carboxymethylation was still carried out on top of the hotplate stirrer by adding trichloroacetic acid according to treatment (25%, 30%, 35%) as much as 20 mL little by little, this carboxymethylation last for 1 hr at 75°C, during the alkalinization process and the carboxymethylation continues. spinning around.

Neutralization was carried out after alkalinization and carboxymethylation had been completed by pouring product into a glass beaker and left to cool. PH was measured then glacial acetic acid was added drop by drop until pH was neutral and decanted. The resulting residue was added with 100 mL of ethanol and left for 1 hr while stirring occasionally. The mixture was then wrapped using aluminum foil and dried using an oven at 60°C for 6 hrs.

2.5 Experimental design

Experimental design used was Response Surface Method. In this study as variable X1 is NaOH concentration with a minimum limit (-1) of 30%, optimal limit (0) of 35% while the maximum limit (+1) of 40%. Variable X2 is the trichloroacetic acid concentration with minimum limit (-1) of 25%, optimal limit (0) of 30% and maximum limit (+1) of 35%. Based on calculations using the expert design program, a combination of treatments was obtained by thirteen samples.
2.6 Observation of Na-CMC

Na-CMC from bacterial cellulose which was finished processing was then analyzed for its characteristics with observational parameters, namely the degree of substitution (Varma et al., 2014), gravimetric method moisture content (AOAC, 2002), pH Meter method (Brooks et al., 2013), viscosity of the viscosity method (Sompie et al., 2015).

2.7 Data analysis

The data of research results were analyzed using DX software design expert. 7.0.0 with Central Composite Design Response Surface Methodology. Determination of the optimum response point is done by verifying between the optimum points of the research results with the optimum point of the calculation using the software design expert (Montgomery, 2017).

3. Results and discussion

3.1 Optimization of NaOH and trichloroacetic acid concentration on substitution degrees water content, pH and viscosity from bacterial cellulose

The results of observation of the substitution degree, water content, pH and viscosity of Bacterial cellulose are presented in Table 1.

The lowest result of degrees substitution response with a value of 0.58, was in the treatment of NaOH with concentration of 27.93% and TCA of 30%. The results of the highest substitution degree response with a value of 0.93 were found in the treatment of 35% NaOH and 30% TCA. Based on research conducted by Candido and Gonçalves (2016), it was shown that the degree of CMC substitution made from sugarcane waste was influenced by the concentration of cellulose acid which caused the degree of substitution to be low. The results of BC-CMC showed that NaOH and TCA concentrations affected the degree of substitution.

The lowest water content response was 16.85%, which was treated with NaOH concentration of 27.93% and TCA of 30%. The highest water content response results were 19.86% obtained at 35% NaOH treatment and 30% TCA. The results of a study by Nisa and Putri (2013) showed that the concentration of TCA did not affect the CMC water content of cocoa pods. In the study of BC-CMC, it was shown that NaOH and TCA concentrations affect the water content. The highest pH value with a value of 10.17 was found in the treatment of 35% NaOH concentration and 30% TCA while the lowest pH with a value of 5.94 was found in the treatment of NaOH concentration of 27.93% and 30% TCA. Research conducted by Coniwanti et al. (2015) also showed that the treatment of NaOH concentration of 20% gave a pH of 6.5 on Na-CMC from peanut shell waste.

The highest viscosity was seen in the treatment of 35% NaOH concentration and 30% TCA with a value of 17.39 cP and the lowest value in the treatment of NaOH concentration was 27.93% and 30% TCA with a value of 12.78 cP. This shows that NaOH and TCA concentrations affect BC-CMC viscosity. The study conducted by Coniwanti et al. (2015) also showed that Na-CMC viscosity from peanut shell waste was also influenced by NaOH concentration.

3.2 Degree of substitution

Three selection processes of models were conducted and the best model for response surface was quadratic, then a variety analysis was performed. DeRousseau et al. (2018) state that the main criteria for accuracy are based on testing the Lack of Fit. The model is considered appropriate if during the test the model's inaccuracy is statistically insignificant and is considered inappropriate

Table 1. Data of substitution degree response, water content, pH and viscosity

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<th>No</th>
<th>Treatment of NaOH (%)</th>
<th>TCA (%)</th>
<th>X1</th>
<th>X2</th>
<th>DS</th>
<th>Water Content (%)</th>
<th>pH</th>
<th>Viscosity (cP)</th>
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to explain a problem from an analysis examined if the
inaccuracy of the model is significant. The quadratic
model regression equation of the response $Y_1$
(substitution degree) which is influenced by the
concentration of NaOH ($X_1$) and the concentration of
trichloroacetic acid ($X_2$) is as follows:

$$Y_1 = -8.25852 + 0.33155X_1 + 0.2067X_2 + 0.000000X_1X_2 - 4.62500E-003X_1 - 3.32500E-003X_2$$

This equation is the actual equation needed to
respond to the degree of substitution that will be
obtained if the value of the variable is treated differently.
In this equation, the coefficients of $X_1$ equation and $X_2$ are negative, which means that there is a maximum
stationary point and the surface of the response obtained.
This can also be used in predicting possible responses with various levels of proportions. In this equation, the variable mostly affecting the response of substitution
degrees is the NaOH concentration variable with the
highest coefficient at the $X_1$ coefficient of 0.33.

The equation above shows the response of the degree
of substitution increases with increasing concentrations of NaOH and trichloroacetic acid indicated by positive
coefficient values. The role of NaOH activates OH
groups in cellulose and makes the cellulose structure expand, this process facilitates trichloroacetic acid
(TCA) diffuse into the cellulose structure. The higher
congestion of NaOH added causes the OH group to
become more active and the structure of cellulose expands which results in more trichloroacetic acid
diffusing into it as the concentration of trichloroacetic acid increases. This process makes the substitution of
anhydroglucose units in the cellulose structure higher (Candido and Goncalves, 2016). High concentrations of
NaOH and trichloroacetic acid make it easy for cellulose to be converted to alkaline cellulose (Coniwanti et al.,
2015). The degree of substitution plays an important role in making bacterial cellulose Na-CMC powder because
the higher the degree of substitution the more trichloroacetic acid is attached to the cellulose structure so that more cellulose is converted to Na-CMC. The value of the degree of substitution indicates the success of the alkalization process in providing opportunities for trichloroacetic acid to substitute into the cellulose structure which can ultimately convert cellulose to Na-CMC. Increasing the concentration of NaOH and trichloroacetic acid (TCA) will have an impact on the number of side products in the form of sodium glycolic and sodium chloride, thus reducing the rate of substitution response. The by-product formed is thought to be excess NaOH reacting with trichloroacetic acid to form sodium glycolic and sodium chloride, this causes the ability to convert cellulose to Na-CMC to decrease (Nisa and Putri, 2013).

3.3 Water content

Three selection process models were selected and the
best model for response surface was quadratic, then a
variety analysis is performed. Bezerra et al. (2008) stated that the main criteria for accuracy were based on testing
the Lack of Fit. The model was considered appropriate if
during the test the model's inaccuracy was statistically insignificantly and considered inappropriate to explain a
problem from an analysis examined if the inaccuracy of
the model is significant. The quadratic model regression
equation of the response $Y_1$ (moisture content) which is
influenced by the concentration of NaOH ($X_1$) and the
concentration of TCA ($X_2$) is as follows:

$$Y_1 = -8.25852 + 0.33155X_1 + 0.2067X_2 + 0.000000X_1X_2 - 4.62500E-003X_1 - 3.32500E-003X_2$$

This equation is the actual equation needed to determine the water content response that will be
obtained if the value of the variable is treated differently.
In this equation, the coefficients $X_1$ equation and $X_2$
are negative, which means that there is a maximum
stationary point and the surface of the response obtained.
This can also be used in predicting possible responses with various levels of proportions. In the equation, the
most influential variable on the response of the water
content is variable concentrations of NaOH with the
highest coefficient in the coefficient $X_1$ equal to 2.28.

Increased water content is directly proportional to
the increasing concentration of NaOH and trichloroacetic
acid (TCA). The high concentration of NaOH causes the
bonding of bacterial cellulose to widen so that more
trichloroacetic acid (TCA) that comes in contact with the
cellulose structure will further substitution the hydroxyl
group into a carboxyl group. The increased water content
will cause an increase in the degree of substitution which
impacts the ease of Na-CMC in binding water (hygroscopic) (Biswas et al., 2014). Water-binding compounds and water trapped in the material are
different. In this case, CMC has water trapped inside its
structure so that this water cannot enter or leave the
material. If the agitation is given longer and the degree
of substitution produced is higher, it will cause more and
more water contained in the CMC (Estiasih, 2006).

The greater the value of the degree of substitution,
the quality of CMC will be better and the solubility in
water will increase the greater (Wijayanti et al., 2005).
The solubility index in waterfalls because it depends on
the degree of substitution of CMC which also decreases
this is due to the substituted groups also decreasing with
the duration of agitation. The longer the agitation will
cause the cellulose structure to expand and increase the
distance between one group and another group which
makes it more difficult to break bonds and replace the group (Nisa and Putri, 2013).

3.4 pH

Three model selection processes were carried out and the best model for response surface was quadratic by which variety analysis was performed. Kadrigama et al. (2008) suggest that the main criteria for accuracy were based on testing the Lack of Fit. The model was considered appropriate in case after the test, the model's inaccuracy was statistically insignificant and considered inappropriate to explain a problem from an analysis examined if the inaccuracy of the model was real (significant). The quadratic model regression equation of response \( Y_1 \) (pH) which is influenced by the concentration of NaOH (\( X_1 \)) and the concentration of TCA (\( X_2 \)).

\[
Y_1 = -108.47213 + 4.45626 X_1 + 2.55297 X_2 + 4.50000E-003 X_1X_2 - 0.063785 X_1^2 - 0.045185 X_2^2
\]

This equation is the actual equation needed to determine the pH response that will be obtained if the variable value is treated differently. In this equation, the coefficients \( X_1 \) and \( X_2 \) are negative, which means that there is a maximum stationary point and the response surface obtained. This equation can also be used in predicting possible responses with various levels of proportion. In this equation, the variable that most influences the pH response is the NaOH concentration variable with the highest coefficient at the \( X_1 \) coefficient of 4.46.

pH increases with increasing concentrations of NaOH and trichloroacetic acid. This situation is caused when there is a substitution between cellulose and Na +. After the alcalization process is continued with the carboxymethylation stage using trichloroacetic acid, increasing the concentration of trichloroacetic acid is given, the more NaCl salt formed so that the resulting pH will increase. But if the concentration of trichloroacetic acid given increases, the pH level will drop to acid (Candido and Gonçalves, 2016).

3.5 Viscosity

Three process selection models have been carried out and the best model for response surface is quadratic, then a variety analysis is performed. Shayanfar et al. (2017) state the main criteria for accuracy were based on testing the Lack of Fit. The model was considered appropriate if, after the test, the model's inaccuracy was statistically insignificant and considered inappropriate to explain a problem from an analysis examined if the inaccuracy of the model is significant. The quadratic model regression equation of the response \( Y_1 \) (viscosity) which is influenced by the concentration of NaOH (\( X_1 \)) and TCA concentration (\( X_2 \)) is as follows:

\[
Y_1 = -95.52951 + 4.27098 X_1 + 2.27975 X_2 - 4.0000E-004 X_1 X_2 - 0.058970 X_1^2 - 0.036170 X_2^2
\]

This equation is the actual equation needed to determine the viscosity response that will be obtained if the value of the variable is treated differently. In this equation, the coefficients of \( X_1 \) equation and \( X_2 \) are negative, which means that there is a maximum stationary point and the surface of the response obtained. This can also be used in predicting possible responses with various levels of proportions. In this equation, the variable that most influences the viscosity response is the NaOH variable with the high coefficient at the \( X \) coefficient of 4.27.

The viscosity level will decrease at a certain point along with the addition of interaction of NaOH concentration and trichloroacetic acid, NaOH interaction and trichloroacetic acid interaction. This is indicated by the negative coefficient. This is due to the increasing number of trichloroacetic acid (TCA) which binds to the cellulose structure and has a high degree of substitution. The high degree of substitution is due to the increasing cellulose conversion into Na-CMC. This process can run optimally because of the role of NaOH which can activate OH groups and make the cellulose structure expand so that it facilitates trichloroacetic acid binds to the cellulose structure (Biswas et al., 2014).

The viscosity of Na-CMC depends on its ability to bind water so that it can produce a solution with a certain viscosity. The ability of Na-CMC to bind water is strongly influenced by the degree of substitution. The higher the degree of substitution the greater the ability of Na-CMC in binding water to produce the desired viscosity. The optimum NaOH concentration will produce the best conditions for widening cellulose fibers so that trichloroacetic acid will increasingly substitute with cellulose structure so that the degree of substitution increases, which means that viscosity will also increase (Candido and Goncalves, 2016).

Graph Variable Interaction of NaOH and TCA concentrations in response to substitution degree (A), water content (B), pH (C) and viscosity (D) are shown in Figure 1.

3.6 Optimum of substitution degree response, water content, pH and viscosity

This study aims to obtain the best substitution degree, water content, pH and viscosity in the process of making CMC from bacterial cellulose. Figure 2 shows the surface curve of the optimum response point of the
NaOH and TCA concentration variables in response to substitution degrees, moisture content, pH and viscosity.

Figure 2 shows a contour that shows the accuracy of the results of the optimization called desirability contours. The flag image in the middle of this contour shows an explanation of the optimal point located on the point (node) on the flag. Optimization of the program is based on variable input data and response measurement data. The output from the optimization phase is in the form of recommendations for an optimal new formula according to the program. Formulas with maximum desirability are the most optimal formulas. Zhao et al. (2017) stated desirability value range of 0 - 1 which means the approaching 1 show program capability to result the desired product is getting valid. Step optimization is not intended to seek the value of desirability 1 but to transform and get the best conditions of all functions.

The solutions obtained from the Design Expert program calculation system, the variable optimum points of NaOH and Trichloroacetic Acid concentrations were 35.95% and 30.87%. The optimum point of the independent variable, the optimum response value of the substitution degree obtained was 0.897, water content of 20.08%, pH of 10.04 and viscosity of 17.26%.

4. Conclusion

Based on the results of the study, it can be concluded that the best treatment of NaOH concentration was 35.95% and the concentration of trichloroacetic acid was 30.87%. The highest response to substitution degrees is 0.93. The highest response to water content is 19.86%. The highest pH response is 10.17. The highest viscosity response was 17.39 cps. Based on the three criteria for model selection analysis, inter alia Sequential Model Sum of Squares, Lack of Fit and Model Summary Statistic in response to substitution degrees, moisture content, pH and viscosity.

Figure 1. Interaction graph of variable NaOH and TCA concentration in substitution degrees response (A), water content (B), pH (C) and viscosity (D)

Figure 2. Surface curve response optimum point in 2 dimensions (a) and 3 dimensions (b)
content, pH and viscosity were chosen quadratic models. From the four equations, it appears that the NaOH concentration variable is the variable that most influences the response of substitution degrees, moisture content, pH and viscosity.

Conflict of Interest
Authors declare no conflict of interest.

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References


lama fermentasi terhadap kualitas nata de soya. 

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