Characteristics of Biocellulose from Sago Liquid Waste with Different Ammonium Sulfate Concentration

Nur Arfa Yanti 1*, Sitti Wirdhana Ahmad 1, Nurhayani H. Muhiddin 2

1Jurusan Biologi FMIPA Universitas Hala Oleo, Kampus Hijau Bumi Tridharma, Anduonohu, Kendari Sulawesi Tenggara, 93232
2Jurusan Pendidikan IPA FMIPA Universitas Negeri Makassar, Kampus UNM Parangtambung, Jl. Malengkeri Raya, Makassar Sulawesi Selatan, 90224

Abstract. This research aims to utilize sago liquid waste to produce biocellulose with different concentration of ammonium sulfate and to know characteristics of biocellulose. Production of biocellulose was done in sago liquid waste substrate with different concentration of ammonium sulfate, namely 0.5, 1, 1.5 and 2% (w/v) by using bacteria local strain Acetobacter xylinum LKN6. The observed parameters included thickness, yield, crude fiber and organoleptic. The analysis results showed that ammonium sulfate concentration given a different to the thickness, yield, crude fiber and organoleptic. The best characteristic of biocellulose obtained in the treatment of 1.5% ammonium sulfate concentration. The biocellulose from sago liquid waste potential as dietary fiber food.

Keywords: Biocellulose, characteristic, ammonium sulfate, sago liquid waste

Received 12 November 2018 | Revised 20 December 2018 | Accepted 22 January 2019

1. Introduction

Bacterial cellulose is a metabolic product of acetic acid bacteria, such as the genus Acetobacter, Gluconobacter and Gluconacetobacter (Yamada & Yukphan, 2008; Mamlouk & Gullo, 2013). Cellulose derived from bacterial fermentation is purer than cellulose derived from plants, because it does not mix with lignin and hemicellulose (Esa et al., 2014). Biocellulose production by bacteria, generally using coconut water as its substrate. Coconut water contains glucose, fructose, nitrogen compounds, vitamins, and minerals (Melliawati, 2015), so it is very good to be used as a medium for bacterial growth while producing biocellulose. Along with the development of biotechnology, biocellulose production has been developed from various substrates such as tofu liquid waste (Sulistyo et al., 2007), pineapple waste (Iskandar et al.,...
Biocellulose from bacterial metabolism has specific characteristics which have high molecular weight, high crystalline properties, high degree of polymerization, high mechanical properties (Chawla et al., 2009), and has a high enough fiber content (Esa et al., 2014), so that it can be developed and applied to various industries. Biocellulose has been used in the food industry better known as nata. According to SNI (Indonesian National Standard) in 1996, the characteristics of nata that must be considered are the normal aroma, taste, color, and texture and fiber content. One factor that can influence the characteristics of nata is the concentration of ammonium sulfate as a nitrogen source (Yunianta, 2010; Hamad & Kristiono, 2013; Patria et al., 2013). In this study, biocellulose is produced by local strains of A.xylinum LKN6, using sago liquid waste as a production medium. This study aims to determine the characteristics of biocellulose from sago wastewater produced using different ammonium sulfate concentrations.

2. Materials and Methods

The main materials used in this study were sago liquid waste obtained from the sago flour processing industry in Konawe district, Southeast Sulawesi and local strain bacteria Acetobacter xylinum LKN6 as an inoculum (starter). The chemicals used are ammonium sulfate, sugar, and acetic acid. Biocellulose production was carried out using sago liquid waste as a substrate with the addition of sugar as much as 10% (b / v) and ammonium sulfate according to the treatment, ie concentrations of 0.5, 1, 1.5 and 2% (b / v) with 3 replications. The pH of the production medium is set in the range of 4-5 using glacial acetic acid. A.xylinum bacterial inoculum is added to the production media as a starter. Fermentation is carried out for approximately 14 days using a glass container. The cellulose layer that forms after the end of fermentation, is removed from the fermentation container and washed with water until it is clean. Biocellulose characteristics tested are physical properties including thickness and yield (Goh et al., 2012), chemical properties include fiber content (Sudarmadji et al., 1997) and organoleptic properties include texture, aroma, and taste (Putriana & Aminah, 2013). Organoleptic tests are carried out using a scoring test with criteria that the higher the number the better the quality. The panelists for scoring were semi-trained panelists as many as 21 people from students and lecturers of the Department of Biology, FMIPA UHO.

3. Results And Discussion

a. Physical Characteristics
Biocellulose physical characteristics tested in this study include thickness and yield. The average value of the biocellulose physical characteristics from sago wastewater with different ammonium sulfate concentrations is shown in Table 1.

Table 1. Average value of biocellulose physical test results from sago liquid waste

| Parameters      | Ammonium sulfate concentration (% b/v) |
|-----------------|----------------------------------------|
|                 | 0.5         | 1          | 1.5        | 2          |
| Thickness (mm)  | 9.11        | 13.41      | 19.55      | 15.32      |
| Rendement (%)   | 23.31       | 31.62      | 53.01      | 34.64      |

Biocellulose thickness measurement results in Table 1 show that biocellulose thickness increases with increasing ammonium sulfate concentration in the production medium. However, the thickness decreased in the medium with 2% ammonium sulfate concentration. Several previous studies also reported that the thickness of biocellulose (nata) increased with an increase in the concentration of ammonium sulfate in the production medium (Purwaningsih et al., 2007; Patria et al., 2013). Table 1 also shows that the concentration of 1.5% ammonium sulfate in the sago liquid waste medium is the optimum ammonium sulfate concentration because it produces biocellulose with the highest thickness.

Table 1 shows that biocellulose yield from sago liquid waste has increased with increasing ammonium sulfate concentration with the highest yield reached a concentration of 1.5% (53.01%), and biocellulose yield decreased to 34.64% at 2% ammonium sulfate concentration. The results of the study in Table 1 show that the pattern of biocellulose increase in thickness and yield of sago liquid waste with the same ammonium sulfate concentration treatment, which resulted in the highest biocellulose thickness and yield at 1.5% ammonium sulfate concentration. This indicates that ammonium sulfate affects the physical properties of biocellulose. The results of this study are in accordance with Purwaningsih et al. (2007) which states that the concentration of ammonium sulfate affects physical properties, especially the thickness and rendement of nata Gracilaria sp.

Addition of ammonium sulfate to biocellulose production medium is needed as a nitrogen source. Chawla et al. (2009) stated that nitrogen sources are important nutrients that support microbial activity in the formation of cellulose layers by stimulating bacterial growth and production of cellulose-forming enzymes, thereby increasing production. Based on the results of this study obtained information that the addition of ammonium sulfate in the sago liquid waste medium to obtain the highest thickness and yield, which is as much as 1.5% higher than the need for ammonium sulfate to produce biocellulose from Gracilaria sp. (nata Gracilaria) (Purwaningsih et al., 2007) and nata de soya (Patria et al. 2013), namely 0.75%. This may be...
due to the very small nitrogen content in the sago liquid waste. Based on the results of measurements of nitrogen levels in sago liquid waste it is known that the total N content ranges from 0.02-0.08%, so it does not meet the needs of bacteria to produce biocellulose. Therefore, the addition of nitrogen sources such as ammonium sulfate in the production media can increase biocellulose production from sago liquid waste.

b. Chemical characteristics

Biocellulose chemical characteristics of sago wastewater that analyzed are fiber content. The results of the analysis of biocellulose fiber levels at different ammonium sulfate concentrations are shown in Figure 1.

![Figure 1. Biocellulose fiber content from sago liquid waste with different ammonium sulfate concentrations](image)

Figure 1 shows that the levels of biocellulose fibers from sago wastewater increased as the concentration of ammonium sulfate increased in the production medium with a fiber content range of 2.74-4.53%. The results of this study are consistent with research conducted by Patria et al. (2013) which states that the size of the fiber content is influenced by the nitrogen content in the medium, namely the greater the nitrogen content, the greater the fiber content in biocellulose from tofu liquid waste (nata de soya). Chawla et al. (2009) stated that nitrogen in the medium will be utilized by A. xylinum bacteria for the formation of new cells and more and more bacterial cells cause more cellulose fiber formation.

The results in Figure 1 also show that the levels of biocellulose fiber (nata) of all ammonium sulfate concentration treatments have met the requirements of Indonesian National Standard (SNI) No. 01-4317-1996, where the maximum crude fiber for consumption is 4.5%. Patria et al. (2013) states that crude fiber content exceeds 4.5%, will cause high elasticity so that it is not easily broken when consumed. This indicates that biocellulose from sago liquid waste can be
used as a fiber-rich food (dietary fiber) such as nata de coco. The fiber contained in biocellulose is insoluble dietary fiber and this type of fiber is very good for the health of the digestive tract (Esa et al., 2014; Keshk, 2014).

c. Organoleptic Characteristics

Organoleptic characteristics were tested for biocellulose from sago liquid waste in different ammonium sulfate concentration treatments, including texture, taste, and aroma. These three organoleptic properties are a condition that must be analyzed to utilize biocellulose as a dessert known as nata and regulated in SNI No. 01-4317-1996 namely Nata in packaging.

d. Texture

Good texture for biocellulose as food (nata) is chewy and not hard. The results of the panelists' assessment of the biocellulose texture from sago wastewater are shown in Figure 2.

![Organoleptic texture vs. Concentration of ammonium sulfate (%)](image)

Figure 2. Average panelist's assessment of biocellulose texture from sago wastewater with different ammonium sulfate concentrations

Based on Figure 2, it is known that the average value of the highest assessment of biocellulose texture is found in 1.5% ammonium sulfate concentration, which is 3.82 with the likes of the assessment criteria, while the lowest is 2% ammonium sulfate concentration, which is 2.21. Figure 2 shows that biocellulose is produced with 1 and 1.5% ammonium sulfate concentrations, has favorable assessment criteria (values ranging from 3) while biocellulose produced at concentrations of 0.5 and 2% have less favorable assessment criteria (values range 2). Based on the comments of the panelists it is known that the preferred biocellulose is a product of 1 and 1.5% ammonium sulfate concentration because it has a chewy texture.
Assessment of less like on biocellulose products from 0.5% ammonium sulfate concentration, because in general, the panelists considered biocellulose to be less elastic while for biocellulose from a concentration of 2% it was too chewy so it was difficult to chew. The results of this study are in accordance with the research of Putriana & Aminah (2013) which states that the preferred nata de cassava is nata with a chewy texture.

The results of the assessment of bio cellulose texture (Figure 2) are directly proportional to the results of the measurement of fiber content (Figure 1), namely the higher levels of biocellulose fiber, the biocellulose texture will also be more supple. The results of this study are supported by Putriani & Aminah (2013) and Patria et al. (2007) which states that the texture of biocellulose(nata) is influenced by fiber content, namely nata with high fiber content, the texture will be more supple. Fifendy et al. (2011) also state that high fiber content and tight fiber composition result in supple nata, whereas nata which has low fiber content and has a high wet weight means that the amount of water contained in the cellulose fibril structure is in a high amount so it reduces the elasticity of nata.

e. Taste

A good taste for biocellulose to be consumed is not acidic. The results of the panelist's assessment of the biocellulose flavor from sago liquid waste are shown in Figure 3.

![Figure 3. The average assessment of panelists on biocellulose flavor from sago liquid waste with different ammonium sulfate concentrations](image)

Figure 3 shows that the highest assessment of panelists on biocellulose flavor was obtained from products of 1.5% ammonium sulfate concentration, which was 3.78 (like) and the lowest was 2% ammonium sulfate concentration, which was 2.41 (less like). Figure 3 also shows that
biocellulose produced with a concentration of ammonium sulfate 0.5-1.5% is in the criteria of the likes (value 3) while the product of ammonium sulfate concentration is 2% less preferred (value 2). Based on information from the panelists it is known that biocellulose which is produced with a concentration of 2% is less preferred because it still tastes slightly acidic, while the products of the other 3 treatments do not taste acidic. Biocellulose, when harvested, has a very acidic taste because the biocellulose fermentation conditions by bacteria must be acidic (pH 4-5). Therefore, the treatment of biocellulose products is done to eliminate the sour taste by soaking and washing and boiling at 100°C. This is in accordance with the research of Putriana & Aminah (2013) which states that good taste in nata is not acidic before being flavored or sweetened. Sulistiowati & Suhartiningsih (2016) also stated that good taste of biocellulose(nata) is tasteless.

f. Aroma

The smell of biocellulose(nata) which is good for consumption is not acidic. The results of the panelists' assessment of the biocellulose flavor from sago wastewater are shown in Figure 4.

Figure 4. The average assessment of panelists on the aroma of biocellulose from sago wastewater with different ammonium sulfate concentrations.

Based on Figure 4, it is known that the average panelists' assessment of biocellulose aroma for all ammonium sulfate concentration treatments was preferred, which ranged in value 3. The highest assessment of biocellulose aroma was found in 1.5% (3.48) ammonium sulfate concentration and the lowest at 2% concentration (3.15). The results of the assessment for the aroma of biocellulose belonging to the likes category indicated that the biocellulose produced had a normal aroma and did not smell of acid. The smell of biocellulose from sago liquid waste that does not smell of acid is caused by biocellulose processing through washing and boiling. This is in accordance with the research of Putriana & Aminah (2013) which states that the
The aroma of biocellulose from cassava (nata de cassava) produced is not acidic because, at the time of harvest, nata de cassava is washed and then boiled for 10 minutes at 100 °C so that the aroma of nata de cassava is lost during washing and boiling.

Based on chemical and organoleptic characteristics it is known that biocellulose from sago liquid waste can be used as a fiber-rich dessert because its characteristics meet Indonesian national standards (SNI) No. 01-4317-1996. The Food and Drug Administration (FDA) categorizes a food product as a source of fiber if it contains 2 grams of dietary fiber per 100 grams. Biocellulose from sago liquid waste meet the standards as a fiber source food with levels of 2.74-4.53%.

4. Conclusion

Based on the results of this study it can be concluded that biocellulose produced from sago liquid waste with different ammonium sulfate concentrations has different characteristics. Biocellulose products from sago liquid waste with the best characteristics were produced at ammonium sulfate concentrations of 1.5%, with a thickness of 19.55 mm, a yield of 53.01%, fiber content of 3.63% and the level of preference of panelists for texture, taste, and aroma within the limits Organoleptically accepted by panelists. Biocellulose from sago liquid waste meets Indonesian national standards (SNI) to be used as fiber-rich foods.

Acknowledgment

The author would like to thank the Directorate General of Research and Development Strengthening, Ministry of Research, Technology, and Universities who have provided research funding assistance in the National Strategic Research Grant (PSN) project in 2018.

References

[1] Chawla, P.R., Bajaj, I.B., Survase, S.A. and Singhal, R.S. 2009. Microbial cellulose: Fermentative production and applications. Food. Technol. Biotechnol., 47: 107-124.

[2] Esa, F., Tasirin, S.M. and Rahman, N.A. 2014. Overview of Bacterial Cellulose Production and Application. Agriculture and Agricultural Science Procedia, 2 : 113 – 119.

[3] Fifendy, M., Putri, D.H. dan Maria, S.S. 2011. Pengaruh Penambahan Tauge sebagai Sumber Nitrogen terhadap Mutu Nata de Kakao, Jurnal Saintek, 3 (2) : 165-170.

[4] Goh, W.N., Rosma A., Kaur, B., Fazilah, A., Karim A.A. & Rajeev Bhat. 2012. Fermentation of black tea broth (Kombucha): I. Effects of sucrose concentration and fermentation time on the yield of microbial cellulose. International Food Research Journal, 19 (1): 109-117.

[5] Hamad, A. dan Kristiono. 2013. Pengaruh Penambahan Sumber Nitrogen terhadap Hasil Fermentasi Nata de coco. Momentum 9 (1) : 62-65.
[6] Iskandar, Zaki, M., Mulyati, S., Fathanah, U., Sari, I. dan Juchairawati, 2010, Pembuatan Film Selulosa dari Nata de Pina, Jurnal Rekayasa Kimia dan Lingkungan, 7(3):105-111

[7] Keshk, S.M.A.S. 2014. Bacterial Cellulose Production and its Industrial Applications. J. Bioproces Biotechniq. 4 (2) : 1-10.

[8] Mamlouk, D. and Gullo, M. 2013. Acetic Acid Bacteria: Physiology and Carbon Sources Oxidation, Indian J Microbiol. 53(4):377–384.

[9] Melliawati, R. 2015. Bahan baku alternatif Pembuatan Bioselulosa. Biotrends 6 (2) : 1-3.

[10] Patria, A., Muzaifa, M. & Zurrahmah. 2013. Pengaruh Penambahan Gula dan Amonium Sulfat terhadap Kualitas Nata de Soya. Jurnal Teknologi dan Industri Pertanian Indonesia, 5 (3) : 1-5.

[11] Putriana, I. dan Aminah, S., 2013, Mutu Fisik, Kadar Serat dan Sifat Organoleptik Nata de Cassava Berdasarkan Lama Fermentasi, Jurnal Pangan dan Gizi, 4 (7):101-159.

[12] Purwaningsih, S., Salamah, E. dan Setiani, A. 2007. Pengaruh Pemberian Sukroza dan Amonium Sulfat terhadap Mutu Nata Gracillaria sp. Buletin Teknologi Hasil Pertanian 10 (2) : 35-47.

[13] Sudarmadji, S., Haryono, B. dan Suhardi, 1997, Prosedur Analisa untuk Bahan Makanan dan Pertanian, Liberty, Yogyakarta.

[14] Sulistyo, Arif, D.R. dan Nur, A., 2007, Pembuatan Nata dari Limbah Cair Tahu dengan Menggunakan Molasses sebagai Sumber Karbon Acetobacter xylinum, Jurnal Ekuilibrium, 6 (1):1-5.

[15] Sulistiwati, I. dan Suhartiningsih. 2016. Pengaruh Jumlah Gula dan Natrium Metabisulfit terhadap Sifat Organoleptik Nata de Bogem Mangrove (Sonneratia caseolaris), e-journal Boga 5 (1) : 192-200.

[16] Yamada, Y. and Yukphan, P. (2008). Genera and species in acetic acid bacteria. International Journal of Food Microbiology, 125 (1): 15–24.

[17] Yanti, N.A., Ahmad, S.W., Ambardini, S., Muhiddin, N.H. and. Sulaiman, L.O.I. 2017. Screening of Acetic Acid Bacteria from Pineapple Waste for Bacterial Cellulose Production using Sago Liquid Waste, Biosaintifika, 9 (3) : 387-393.

[18] Yunianta, 2010. Limbah Cair Industri Kakao sebagai Bahan Pembuat Nata. Jurnal Teknik Industri, 1 (1) : 31–34.