Sains Malaysiana 43(5)(2014): 767–773

Effect of Different Drying Methods on the Morphology, Crystallinity, Swelling Ability and Tensile Properties of *Nata De Coco*

(Kesan Kaedah Pengeringan Berbeza pada Morfologi, Penghabluran, Kebolehan untuk Mengembang dan Kekuatan Tegangan *Nata De Coco*)

NORHAYATI PA'E, NUR IDAYU ABD HAMID, NOZIEANA KHAIRUDDIN, KHAIRUL AZLY ZAHAN, KOK FOOK SENG, BAZLUL MOBIN SIDDIQUE & IDA IDAYU MUHAMAD*

ABSTRACT

Nata de coco *or bacterial cellulose produced by* Acetobacter xylinum *is a unique type of biocellulose. It contains more than 90% of water. Dried* nata *was preferred compared to wet form since it is more convenient and portable with stable properties. Therefore, drying process is necessary in order to produce dried* nata de coco. *Drying method is a key factor that influenced the properties of dried* nata de coco *produced. The aim of this study was to investigate the effect of different drying methods on morphology, crystallinity, swelling ability and tensile strength of dried* nata de coco. Nata de coco *samples were dried using three physical drying methods such as oven, tray dryer or freeze dryer until it achieved 3-5% moisture content. Obviously, the three drying techniques produced web-like structured* nata de coco *and quite similar crystallinity which was in range between 87 and 89%. Freeze dried sample showed the largest swelling capacity and tensile strength which was found to be 148 MPa. Different drying method gave different properties of* nata de coco. *Therefore, the present work proposed the most suitable drying method can be utilized based on the properties of end product needed.*

Keywords: Acetobacter xylinum*; bacterial cellulose; drying process;* nata de coco

ABSTRAK

Nata de coco *atau selulosa bakteria yang dihasilkan oleh* Acetobacter xylinum *merupakan bioselulosa yang unik. Ia mengandungi lebih daripada 90% air. Oleh itu, proses pengeringan sangat diperlukan untuk menghasilkan* nata de coco *kering. Kaedah pengeringan adalah faktor utama yang mempengaruhi sifat* nata de coco *yang telah dikeringkan. Kajian ini dijalankan untuk mengkaji kesan kaedah pengeringan yang berbeza pada morfologi, penghabluran, kebolehan untuk mengembang dan kekuatan tegangan* nata de coco *yang telah dikeringkan. Sampel* nata de coco *dikeringkan menggunakan tiga kaedah pengeringan fizikal iaitu ketuhar, pengering dulang atau pengering beku sehingga ia mencapai kurang daripada 5% kandungan lembapan. Jelas sekali, tiga teknik pengeringan tersebut menghasilkan* nata de coco *dengan struktur jaringan yang tersusun dan nilai penghabluran yang hampir sama iaitu dalam lingkungan 87-89%. Sampel daripada pengering beku menunjukkan kapasiti penyerapan air terbesar dan dengan kekuatan tegangan 148 MPa. Kaedah pengeringan yang berbeza memberikan sifat yang berbeza kepada* nata de coco. *Oleh itu, hasil kajian ini dapat mencadangkan kaedah pengeringan yang paling sesuai digunakan berdasarkan sifat produk akhir yang dikehendaki.*

Kata kunci: Acetobacter xylinum; nata de coco*; proses pengeringan; selulosa bakteria*

INTRODUCTION

Nata or bacterial cellulose is a white gelatinous cellulose which is produced from fermentation of *Acetobacter aceti* ssp. *xylinum* using fruit juices as a medium (Sheu et al. 2000). Cellulose formed by fermentation using coconut water were called *nata de coco*. *Nata* is one of the famous foods served as dessert in Asian countries including Philippine, Indonesia, Japan and Taiwan (Okiyama et al. 1992). *Nata* has high fiber content, low calorific value and high water content which exceeds 90%. Its nutritional value and palatable taste makes *nata* a dessert that is suitable for all ages.

Previous research reported that film produced by *Acetobacter aceti* subspecies *xylinum* contained water and cellulose as the main component (Yeoh et al. 1985) Therefore, drying methods are crucial to structure, for performance and application of bacterial cellulose (Zhang et al. 2011). The same makes drying as a definite processing step used in order to produce various types of products from *nata*. Besides, previous researches by Wei et al. (2011) reported that the dried *nata* was preferred compared with wet form since it is more convenient and portable for practical application. The dried *nata* which possess stable properties is suitable for powder processing. The cellulose powder from *nata* met the specification of pure cellulose as proven by Fourier transform infra-red (FTIR) (Nadia et al. 2012). In food application, *nata* can be used in diverse ways such as low-calorie additive, thickener,

stabilizer and texture modifier (Chawla et al. 2009). Recent works also reported the use of bacterial cellulose as active packaging (Iuliana et al. 2012), carrier support for probiotic (Jagannath et al. 2010) and for enzyme immobilization (Sheng & Ying 2008). Gardner (1982) explained drying is a removal of water or other volatile liquid from another liquid or gas, or the removal of water from a suspension or solution of a solid. Drying process is mainly to enhance the stability of the final products by reducing the chemical reactivity (Bashaiwoldu et al. 2004). The objective of this study was to investigate the influence of different drying methods on the *nata de coco* properties.

Three different drying techniques which were commonly used for drying *nata de coco* were investigated. They were oven drying (OD), tray drying (TD) and freeze drying (FD). Heat was involved in both OD and TD. However, in TD, the heat was circulated by moving air or fan which happens to aid the drying process. Using FD, the substance to be dried is usually frozen by exposure to very cold air. In FD, the water is removed as a vapor by sublimation from the frozen material in a vacuum chamber. Drying method is crucial to structure, performance and application of *nata de coco* films (Zhang et al. 2011). A series of analysis were performed in order to compare the properties of the product. This includes morphology test, swelling measurement, crystallinity and tensile strength of the *nata de coco*.

Materials and Methods

Production of *Nata de Coco*

Nata de coco was produced by slightly modified the method used by Pa'e et al. (2007). About 200 mL of coconut water was filtered in a 1 L shake flask and further added with 200 mL of distilled water (dilution of 1:1). About 6 g yeast extract, 4 g sucrose, 0.8 g bactopepton, 0.6 g KH_2PO_4 and 0.1 g $MgSO_4$ were added into the medium. The pH of the medium was adjusted to 5.0 by using NaOH before autoclaved at 121°C for 15 min.

The medium was cooled to room temperature then 40 mL of inoculums were added in sterile condition. The solution was mixed apparently by shaking the shake flask slowly. The medium was left for 14 days for fermentation at room temperature (23 ± 2°C). The *nata de coco* produced was harvested followed by washing with acetic acid and rinsing with ample of water until the pH of the rinsing water became neutral.

Drying Procedure

Sample Preparation

A plate of cleaned *nata de coco* sample was squeezed to reduce its water content. The sample was wrapped with tissue paper before the squeezing process to protect its surface. The sample was then cut into the size of 15 cm length × 7 cm width before weighed using weighing scale model AA-200 from Denver Instrument Company.

Oven Drying

The sample was put in Petri dish (padded with aluminum foils) and dried in oven (Memmert) at 80°C. The sample was weighted using weighing scale until its moisture content achieved below 5% (Geankoplis 2003). The experiment was repeated using two other samples.

Tray Drying

The sample was put in a Petri dish (padded with aluminum foils) and dried in tray dryer (Ringwood Haunpshire, England) at 80°C with 2.5 m/s air velocity. The samples were weighted until the moisture content reached below 5% (Geankoplis 2003). The experiment was repeated twice.

Freeze Drying

The sample was put in a Petri dish (padded with aluminum foils) and dried in a freeze dryer (Heto FD 4.0, Denmark) at -50°C and -0.03 hPA. Sample was weighted using weighing scale until its moisture content achieved below 5% (Geankoplis 2003). This experiment was also repeated twice.

Scanning Electron Microscopy

The morphology of dried *nata de coco* was observed using scanning electron microscope (SEM) model Q659, Oxford Instrument England to comprehend the differences between the products produced by different drying methods. The samples sized 1 × 1 cm were coated with gold using sputter coater 240V.AC, Polaron Division prior to study under SEM with 10000× magnification.

X-ray Diffraction

An X-ray diffractometer (XRD) model D5000 Siemens was used. The angle 2θ was set between 10^0 and 45^0 (Mihranyan et al. 2004). The degree of crystallinity percentage was calculated as:

$$\text{Degree of crystallinity } (\%) = \frac{I_{002} - Iam}{I_{002}} \times 100,$$

where I_{002} is the overall intensity of the peak at 2θ and *Iam* is the intensity of the baseline at 2θ (Segal et al. 1959).

Swelling Measurement

The swelling percentage was determined by modification of the method used in other research (Maneerung et al. 2008). The samples were cut into a size of 2 cm length × 1 cm width × 1.2±0.5 cm thickness. The prepared oven dried samples (OD), tray dried samples (TD) and freeze dried samples (FD) were weighted using Electronic balance model AA-200. Then the samples were soaked in distilled water for 72 h. The percentage of swelling was calculated as follows:

$$\text{Swelling } (\%) = \frac{ws - wi}{wi} \times 100,$$

where *Wi* is the initial weight of dried sample and *Ws* is the weight of sample after 72 h soaking in water.

TENSILE STRENGTH

The tensile test was done using LRX, LLOYD Instruments, United Kingdom with maximum load of 2.5 kN. This test was conducted using ASTM D 882 for thin film less than 1.0 mm in the condition of room temperature (23°C ± 2°C).

The test specimens were prepared using Hollow Die Punch 6935 with dumble type five. The specimen was attached to the extension indicator and the speed of testing was set to 10 mm/min. The load-extension curve was recorded to obtain the tensile strength, elongation at break and Young's modulus.

RESULTS AND DISCUSSION

PHYSICAL APPEARANCES

The physical appearances of dried samples of *nata de coco* from OD, TD and FD were demonstrated in Table 1. From the observation, physical appearances of the sample were different for each drying method. However, the colours for all the films were not significantly different which is in agreement with previous research done with chitosan film (Thakhiew et al. 2010). At the end of drying process, all the films from three different drying methods exhibited brownish colour especially for OD and TD. This is most probably caused by the heat applied during the drying processes. Besides that, the surface of the samples was no longer smooth. The surface of TD samples shrunk more than that of OD samples indicating TD to have the highest shrinking rate during the removal of water content in drying process. This might be due to the heat was being substituted by moving air as drying aid. The moving air forced the water in the sample to be evaporated rapidly compared with oven and freeze dryer. On the other hand, smooth surface was observed on FD samples.

MORPHOLOGY STRUCTURE OF DRIED *NATA DE COCO*

Figure 1 shows the texture of OD, TD and FD obtained by SEM at 10000 magnifications. Obviously, the surfaces were found to have composed of numerous intertwined strings which produced an aggregated web-like structure. This result is in agreement with the previous study by Nadia et al. (2012) that reported of fine fibrils which overlapping each other as a layer of cellulose ribbons that were randomly oriented for oven dried *nata*. These structures were similar to high crystalline cellulose structure (Cladiphora) reported in previous work (Mihranyan et al. 2004). The surface analysis by SEM showed that the surface texture for OD,

TABLE 1. Physical appearance for dried *nata de coco*

Method	Colour	Surface	Shrinkage
Oven dried	Brown	Wrinkled	Shrink
Tray dried	Light brown	Wrinkled	Highly shrink
Freeze dried	Light brown	Smooth	Not shrink

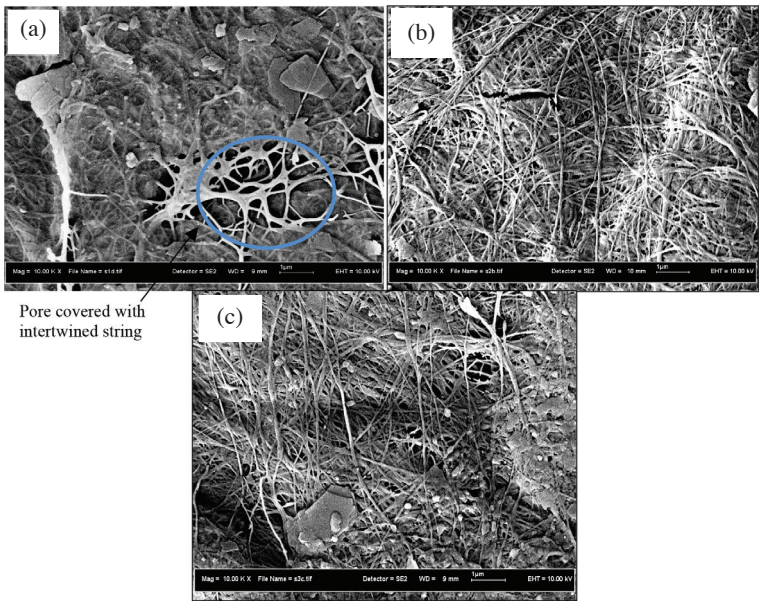

FIGURE 1. SEM image for (a) oven drying, (b) tray drying and (c) freeze drying

TD and FD were slightly different. OD structure composed of few pores covered by rough string-like structures. However, for TD, the structure was more compact and no pores were observed on the surface. As for FD, few pores were observed covered with string which is quite similar with the OD samples. Nevertheless, the strings that covered the pores were thin and fine compared to that in OD.

ANALYSIS OF CRYSTALLINITY

The X-ray diffractograms of *nata de coco* samples from different drying methods were shown in Figure 2. From the result, the peak can be observed at 14.4°, 16.9° and 22.5° which were in agreement with earlier study (Philasaphong & Jatupaiboon 2008). These peaks were attributed to the bacterial cellulose produced in static fermentation (Keshk & Sameshima 2006). From the diffractogram, it shows that *nata de coco* contain cellulose (green line), ammonium sulphate/mascagnite (red line) and sucrose octaacetate (pink line). Cellulose and sucrose octaacetate are produced from the fermentation while ammonium sulphate is a chemical residue that was used during fermentation. Table 2 listed the degree of crystallinity of all dried samples obtained from the X-ray diffractograms. The degrees of crystallinity were in the range of 87-90%. This finding is in agreement with the previous work by Czaja et al. (2004) which reported 89% of crystallinity. In other words, all dried *nata de coco* showed similar crystallinity. However, FD has slightly higher crystallinity compared with other drying methods, followed by OD and TD.

SWELLING ABILITY

Swelling ability is the ability of water to penetrate into the structure (Castellano et al. 1997). Swelling and crystallinity have close relation in demonstrating the hydrophilicity or hydrophobicity of the material. Amorphous region is more hydrophilic than the crystalline region (Segal et al. 1959). Theoretically, water easily enters the amorphous region of cellulose in *nata de coco* to break the Van der Waals force between cellulose molecules and then form cellulose-water hydrogen bonds (Norihiro & Gehrke 1997). However, the crystalline region of cellulose does not allow water to enter its region as it caused lower swelling ability for the material (Jie et al. 2005). The theory was proved by preceding study (Mihranyan et al. 2004) who found that the lower the crystallinity of cellulose powder, the higher was the moisture sorption.

Swelling abilities of the samples were predicted from the calculated degree of crystallinity. The higher the degree of crystallinity, the lower was the swelling ability of the samples. However, Figure 3 shows that this fact did not influence FD sample. FD showed the highest amount of swelling which were 490%. As for TD and OD samples, the

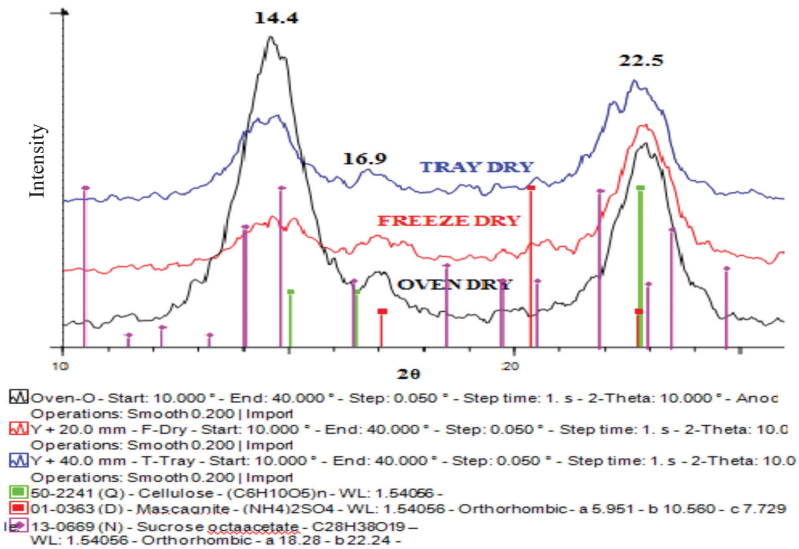

FIGURE 2. X-ray diffractograms of dried *nata de coco* for different drying methods

TABLE 2. Degree of crystallinity of *nata de coco* from different drying methods

Drying method	Degree of crystallinity
Oven dried	87.80%
Tray dried	87.01%
Freeze dried	88.90%

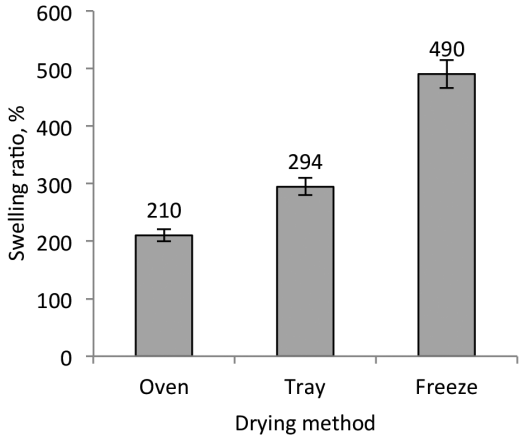

FIGURE 3. The swelling percentage of *nata de coco* for different drying method

results showed lower swelling ability which was 294% for TD and 210% for OD. This is in agreement with work done by Clasen et al. (2006) which reported higher swelling ratio for FD sample compared with OD sample. A prominent factor is the structural rigidity afforded by the samples during drying time. This prevented the remaining porous structure to collapse after drying. When water was added later, the rehydrated product retains much of its original structure (Geankoplis 2013).

The swelling of OD and TD samples were lower than FD sample. This might be due to the heat that involved in the OD and TD contributed to the lower structure rigidity that collapsed the porous structure. As a consequence, the samples could not retain more water. Nevertheless, the swelling for OD sample is greater than TD sample resulting from the internal pores generated on the surface of OD sample as shown in SEM. As a fibrous material, OD samples can hold water by physically entrapping it in fine capillaries and internal pores comparing to tray dried where no internal pores could be seen on the surface.

TENSILE PROPERTIES

Figure 4 shows the effect of different drying techniques on the tensile strength, Young's modulus and elongation at break of dried *nata de coco*. The tensile properties of these samples were related to the drying method. From the results, FD sample has the greatest tensile strength and Young's Modulus which was 148.01 and 2037.4 MPa, respectively. It was followed by TD with tensile strength of 122.46 MPa and Young's Modulus of 280.35 MPa. The OD shows the lowest tensile strength and Young's Modulus which was 91.96 and 189.7 MPa, respectively. This was proven in earlier work by Bashaiwoldu et al. (2004) who found freeze-dried pellet made from microcrystalline cellulose, water and ethanol gave the highest tensile strength. This might be due to low drying temperature used

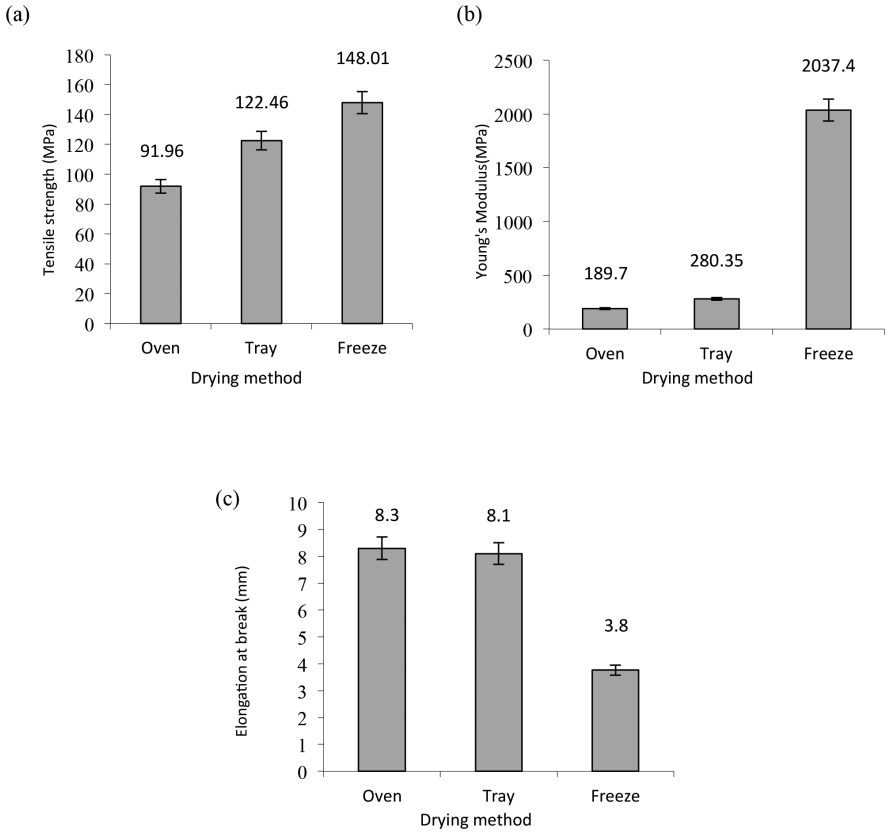

FIGURE 4. The effect of different drying techniques on the (a) tensile strength, (b) Young's modulus and (c) elongation at break of dried *nata de coco*

in FD that helped to reserve the strength of the structure. Low tensile strength for OD can be linked to the structure that can be seen by SEM. A few pores that covered with intertwined string at OD surface increased the surface area where larger surface area may lead to lower strength (P=F/A). The elongation at break of the samples shows inverted pattern of tensile strength and Young's Modulus where the elongation decrease with the increase of tensile strength and Young's Modulus. This is in agreement with Phisalaphong and Jatupaiboon (2008) which reported the percentage of elongation was opposites of tensile strength and Young's Modulus. The OD samples gave the highest elongation at break which was 8.3 mm followed by TD (8.1 mm) and FD (3.8 mm).

CONCLUSION

This study showed that different drying techniques produced dried *nata de coco* with different morphology, crystallinity, swelling and tensile strength. Dried *nata de coco* produced by freeze drying showed the highest crystallinity (88.90%), swelling ability (490%) and tensile strength (148.01 MPa) compared with oven and tray drying. This was contributed by the structure rigidity which was achieved by the frozen substance when sublimation occurred. This process subsequently helped to preserve the porous structure and strength. The electron microscopy showed that oven-dried *nata de coco* had pores covered by intertwined string on the surface. The presence of pores may entrap water and weakens the structure that lead to low tensile strength for the sample.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Science, Technology and Innovation (MOSTI) Malaysia and Research Management Centre of Universiti Teknologi Malaysia (UTM) for the financial support of this project.

REFERENCES

Bashaiwoldu, A.B., Podczeck, F. & Newton, J.M. 2004. A study on the effect of drying techniques on the mechanical properties of pellets and compacted pellets. *European Journal of Pharmaceutical Sciences* 21(2-3): 119-129.

Castellano, I., Gurruchaga, M. & Goni, I. 1997. The influence of drying on the physical properties of some graft copolymers for drug delivery systems. *Journal of Carbohydrate Polymers* 34(1-2): 83-89.

Chawla, P.R., Bajaj, I.B., Survase, S.A. & Singhal, R.S. 2009. Microbial cellulose: Fermentative production and applications. *Food Technol. Biotechnol.* 47(2): 107-124.

Clasen, C., Sultanova, B., Wilhelms, T., Heisig, P. & Kulicke, W.M. 2006. Effects of different drying processes on the material properties of bacterial cellulose membranes. *Macromol. Symp.* 244: 48-58.

Czaja, W., Romanovicz', D. & Brown, R.M. Jr. 2004. Structural investigations of microbial cellulose produced in stationary and agitated culture. *Cellulose* 11: 403-411.

Gardner, A.W. 1982. *Industrial Drying*. Texas: Gulf Publishing Company.

Geankoplis, C.J. 2003. *Transport Processes and Separation Processes Principles (Includes Unit Operations)*. USA: Prentice Hall.

Iuliana, M.J., Anicuta, S.G. & Marta, S. 2012. Controlled release of sorbic acid from bacterial cellulose based mono and multilayer antimicrobial films. *LWT - Food Science and Technology* 47: 400-406.

Jagannath, A., Raju, P.S. & Bawa, A.S. 2010. Comparative evaluation of bacterial cellulose (*Nata*) as a cryoprotectant and carrier support during the freeze drying of probiotic lactic acid bacteria. *LWT - Food Science and Technology* 43: 1197-1203.

Jie, X., Cao, Y., Qin, J.J., Liu, J. & Yuan, Q. 2005. Influence of drying method on morphology and properties of asymmetric cellulose hollow fibre membrane. *Journal of Membrane Science* 246(2): 157-165.

Keshk, S. & Sameshima, K. 2006. Influence of lignosulfonate on crystal structure and productivity of bacterial cellulose in a static culture. *Enzyme and Microbial Technology* 40: 4-8.

Maneerung, T., Tokura, S. & Rujiravanit, R. 2008. Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. *Carbohydrate Polymers* 72: 43-51.

Mihranyan, A., Llagostera, A.P., Karmhag, R., Stromme, M. & Ek, R. 2004. Moisture sorption by cellulose powders of varying crystallinity. *International Journal of Pharmaceutical* 269(2): 433-442.

Nadia, H., Mohd Cairul Iqbal, M.A. & Ishak, A. 2012. Physicochemical properties and characterization of *nata de coco* from local food industries as a source of cellulose. *Sains Malaysiana* 41(2): 205-211.

Norihiro, K. & Gehrke, S.H. 2004. Microporous, fast response cellulose ether hydrogel prepared by freeze-drying. *Journal of Colloid and Surfaces* 38(3-4): 191-196.

Okiyama, A., Shirae, H., Kano, H. & Yamanaka, S. 1992. Bacterial cellulose: Two-stage fermentation process for cellulose production by *Acetobacter aceti*. *Food Hydrocolloids* 6: 471-477.

Pa'e, N., Hui, C.C. & Muhamad, I.I. 2007. Shaken culture fermentation for production of microbial cellulose using pineapple waste. *Proceeding of International Conference on Waste to Wealth*. 26-28 November. Kuala Lumpur. Malaysia Nuclear Agency (MINT).

Phisalaphong, M. & Jatupaiboon, N. 2008. Biosynthesis and characterization of bacteria cellulose-chitosan film. *Carbohydrate Polymers* 74: 482-488.

Segal, L., Creely, J.J., Martin, Jr. A.E. & Conrad, C.M. 1959. An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. *Textile Research Journal* 29(10): 786-794.

Sheng, C.W. & Ying, K.L. 2008. Application of bacterial cellulose pellets in enzyme immobilization. *Journal of Molecular Catalyst B: Enzymatic* 54: 103-108.

Sheu, F., Wang, C.L. & Shyu, Y.T. 2000. Fermentation of Monascus purpureus on bacterial cellulose-nata and the color stability of Monascus-nata complex. *Journal of Food Science (Food Microbiology and Safety)* 65(2): 342-345.

Thakhiew, W., Devahastin, S. & Soponronnarit, S. 2010. Effects of drying methods and plasticizer concentration on some physical and mechanical properties of edible chitosan films. *Journal of Food Engineering* 99: 216-224.

Wei, B., Yanga, G. & Hong, F. 2011. Preparation and evaluation of a kind of bacterial cellulose dry films with antibacterial properties. *Carbohydrate Polymers* 84: 533-538.

Yeoh, Q.L., Lee, G.L. & Fatimah, H. 1985. Teknologi pengeluaran *Nata*. *Jurnal Teknologi Makanan* 4(1): 36-39.

Zhang, C., Wang, L., Zhao, J. & Zhu, P. 2011. Effect of drying methods on structure and mechanical properties of bacterial cellulose films. *Advanced Materials Research* 239-242: 2667-2670.

Department of Bioprocess Engineering
Faculty of Chemical Engineering
Universiti Teknologi Malaysia
81310 Skudai, Johor
Malaysia

*Corresponding author; email: idayu@cheme.utm.my

Received: 11 April 2013
Accepted: 3 September 2013