

Physicochemical Properties and Characterization of *Nata de Coco* from Local Food Industries as a Source of Cellulose

(Sifat Fizikokimia dan Pencirian *Nata de Coco* daripada Industri Makanan Tempatan Sebagai Sumber Selulosa)

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ABSTRACT

Nata de coco, a dessert originally from the Philippines is produced by fermentation of coconut water with a culture of Acetobacter xylinum, a gram negative bacterium. Acetobacter xylinum metabolizes glucose in coconut juice and converts it into bacterial cellulose that has unique properties including high purity, crystallinity and mechanical strength. Because the main component of nata de coco is bacterial cellulose, nata de coco was purified, extracted and characterized to determine whether pure cellulose could be isolated from it. The FTIR spectra of bacterial cellulose from nata de coco showed distinguish peaks of 3440 cm⁻¹, 2926 cm⁻¹, 1300 cm⁻¹, 1440 cm⁻¹, 1163 cm⁻¹ and 1040 cm⁻¹, which correspond to O-H stretching, C-H stretching, C-H bending, CH₂ bending, C-O-C stretching and C-O stretching, respectively, and represent the fingerprints of pure cellulose component. Moreover, the FTIR curve showed a pattern similar to other bacterial cellulose spectra reported by report. Thermal analysis showed a DTG peak at 342°C, which falls in the range of cellulose degradation peaks (330°C - 370°C). On the other hand, the TGA curve showed 1 step of degradation, and this finding confirmed the purity of nata de coco. Bacterial cellulose powder produced from nata de coco was found to be soluble only in cupriethylenediamine, a well known solvent for cellulose; thus, it was confirmed that nata de coco is a good source of bacterial cellulose. The purity of bacterial cellulose produced from nata de coco renders it suitable for research that uses pure cellulose.

Keywords: *Acetobacter xylinum; bacterial cellulose; FTIR; nata de coco*

ABSTRAK

Nata de coco merupakan hidangan pencuci mulut tempatan yang berasal dari Filipina. Ia dihasilkan melalui proses fermentasi air kelapa bersama kultur bakteria Acetobacter xylinum yang merupakan bakteria Gram negatif. Acetobacter xylinum memetabolismekan glukosa dalam air kelapa kepada selulosa bakteria yang mempunyai ciri-ciri unik seperti ketulen yang tinggi, kehabluran dan kekuatan mekanikal yang tinggi. Memandangkan kandungan utama nata de coco adalah selulosa bakteria, ia dituliskan, diekstrak dan seterusnya dilakukan pencirian untuk memastikan kandungan selulosanya. Hasil analisis FTIR nata de coco menunjukkan kehadiran puncak-puncak pada 3440 cm⁻¹, 2926 cm⁻¹, 1300 cm⁻¹, 1440 cm⁻¹, 1163 cm⁻¹ dan 1040 cm⁻¹ yang masing-masing merujuk kepada regangan O-H, regangan C-H, bengkokan C-H, bengkokan CH₂, regangan C-O-C dan regangan C-O yang merupakan cap jari bagi sebatian selulosa tulen. Selain itu corak lengkukan spektra FTIR nata de coco juga menepati corak lengkukan spektra selulosa bakteria yang telah dilaporkan oleh penyelidik terdahulu. Kajian termal pula mendapati puncak pada graf DTG adalah 342°C, menepati julat suhu penguraian termal selulosa (330°C - 370°C) sebagaimana yang dilaporkan sebelum ini. Graf TGA pula menunjukkan nata de coco hanya mempunyai satu langkah penguraian dan membuktikan ianya terdiri daripada satu sebatian tulen. Serbuk nata de coco yang dihasilkan juga didapati hanya larut dalam kuprum (II) etilenadiamina, iaitu pelarut bagi selulosa seterusnya membuktikan bahawa nata de coco adalah sumber selulosa bakteria yang baik. Ketulen selulosa bakteria yang dihasilkan menjadikan ia bahan yang sesuai di dalam penyelidikan yang menggunakan selulosa tulen.

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

INTRODUCTION

Nata de coco is an indigenous dessert of the Philippines and is served as gelatinous squares of 1 cm × 1 cm. In Malaysia, the dessert is prepared by culturing a type of bacteria called *Acetobacter xylinum* through fermentation with coconut

water. After a period of time, a layer of gelatinous sheet forms on the surface of the fermented coconut water. The sheets are allowed to grow to a thickness of 1 cm and then cut into cubes. As a dessert, washed cubes are usually served with flavoured syrup, jelly or other fruit cocktails.

Presently, *nata de coco* is manufactured at an industrial scale not only in Malaysia but also in Indonesia and some are exported to countries like Japan.

The major component of *nata de coco* was shown to be cellulose and not dextran, as was assumed in the past (Iguchi et al. 2000). During the production of *nata de coco*, *Acetobacter xylinum* metabolizes glucose in the coconut water that act as carbon source and converts it into extracellular cellulose as metabolites (Cannon & Anderson 1991). *Acetobacter xylinum* is an acetic acid bacterium, which is known for its ability to oxidize different types of alcohol and sugars to acetic acid. Moreover, the acetic acid bacteria are gram negative and strictly aerobic. The recent classification of acetic acid bacteria included the following genera: *Acetobacter*, *Acidomonas*, *Asaia*, *Gluconacetobacter*, *Gluconobacter*, *Kozakia*, *Swaminathania* and *Saccharibacter* (Trcek 2005). Some genera, such as *Acetobacter*, can eventually oxidize acetic acid to carbon dioxide and water via the activity of the enzymes of the Krebs cycle. Other genera, such as *Gluconobacter*, do not oxidize acetic acid because they lack a complete set of these enzymes. *Acetobacter*, which are now called *Gluconacetobacter*, are well known to produce cellulose. This cellulose exhibits unique features including high purity, crystallinity, uniformity and high mechanical strength (Brown 2004; Jonas & Farah 1998).

Bacterial cellulose synthesized by *Acetobacter xylinum* is the most promising biopolymer and is used in a number of applications as high quality audio membrane (Nishi et al. 1990), electronic paper (Shah & Brown 2005), hydrogel (Halib et al. 2009; Halib et al. 2010) and medical materials such as wound dressing (Czaja et al. 2006), skin substitute (Fontana et al. 1990) and vascular prosthetic device (Bäckdahl et al. 2006; Charpentier et al. 2006).

The bacterial cellulose synthesized in static culture with natural media like coconut water was originally used for the manufacturing of the indigenous dessert, *nata de coco*. Hence, in this study, we characterized *nata de coco* from local food industries as a possible source of pure bacterial cellulose by performing solubility test and pH analysis, Fourier transform infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA) and scanning electron microscopy (SEM) on the cellulose yielded after purification. The bacterial cellulose produced could be used for further celluloses study.

MATERIALS AND METHODS

Nata de coco was purchased as food-grade from the local food industry in the form of cubes ($1 \times 1 \times 1 \text{ cm}^3$). The sodium hydroxide, methanol, acetone and cupriethylenediamine hydroxide (Merck, Germany) were supplied as analytical grade chemicals.

PREPARATION OF BACTERIAL CELLULOSE POWDER

The *nata de coco* was washed and soaked in distilled water until the pH was neutral (pH 5-7) which could require

1-2 weeks. Thereafter, *nata de coco* was blended in a wet blender, poured into trays and dried up in the convectional oven (Memmert, Model UNE 700, Germany) at 60°C for 1 week. The cellulose sheets were then blended with a dry blender (National MX 798S, Malaysia) before they were subjected to grinding (Pulverisette14, Frisch, Germany) to produce a fluffy celluloses powder.

pH DETERMINATION

The bacterial cellulose powder from *nata de coco* was mixed with distilled water and allowed to stand with occasional stirring for 1 h. Thereafter, the liquid supernatant was collected and the pH of the liquid was tested using pH meter (Mettler Toledo MP220, Switzerland).

SOLUBILITY TEST

Two hundred fifty milligrams (250 mg) of bacterial cellulose powder was separately mixed with sodium hydroxide, methanol, acetone and cupriethylenediamine (Cuen). The solubility of the fibre in each solvent was then observed.

FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY OF BACTERIAL CELLULOSE

Infrared spectra of the cellulose were recorded using FTIR Spectra 2000 (Perkin Elmer) at room temperature. Powdered forms of the samples were prepared and analyzed over the range of 500-4000 cm^{-1} by using a Diamond ATR.

THERMOGRAVIMETRIC ANALYSIS (TGA) OF BACTERIAL CELLULOSE

Thermogravimetric analysis was performed using Perkin Elmer, STA6000. Ten milligrams (10.0 mg) of the bacterial cellulose powder prepared was placed in a sample pan. The analysis was performed at 10°C/min from 50°C to 900°C under nitrogen flow. The differential thermogravimetric (DTG) curve of bacterial cellulose was derived using Pyris 1 software (Perkin Elmer).

DETERMINATION OF THE BACTERIAL CELLULOSE PARTICLE SIZE

The particle size of bacterial cellulose powder was determined with Mastersizer 2000 (Malvern, UK). Bacterial cellulose powder has poor flowability because of its tendency to absorb moisture and exhibits particles agglomeration. For this reason, the powder was prepared as dispersion by using a wet method. Readings were recorded in triplicates.

SEM ANALYSIS OF BACTERIAL CELLULOSE

SEM analysis was performed for both the bacterial cellulose dried sheets and powder. The dried sheet was prepared by mixing cellulose powder with distilled water to produce 1% dispersion (w/v). The mixture was poured into a Petri

dish and dried in convectional oven at 60°C for 3 days. The dried sheet was then cut (1 × 1 cm²) and placed on a sample grid. The sample was sputtered coated with gold and examined at 50000× magnifications. The same procedure was carried out for the powdered samples, but the imaging was done at 500× magnification.

RESULTS AND DISCUSSION

pH TEST

The pH of the supernatant liquid from the bacterial cellulose and distilled water mixture was determined to be in the range of pH5-6. According to British Pharmacopoeia, pure cellulose should have pH of supernatant liquid around pH5 – pH7.5. The pH of the cellulose powder prepared in this study was within this specification.

SOLUBILITY TEST

Physical observation showed that bacterial cellulose powder did not dissolved in solvents used namely sodium hydroxide, methanol and acetone. However the powder completely dissolved in cupriethylenediamine (Cuen) the best well known solvent for cellulose. The Cuen system belongs to the class of metal/ion solvent where the composition is 0.5M copper (II) with copper:ethylenediamine molar ratio of 1:2 (Silva 1996).

The solution is highly alkaline since it was prepared directly from copper (II) hydroxide. Cellulose forms a coordinated complex with metal ion where glycol group of a 1,4 anhydroglucose unit of a cellulose molecule chelates to occupy two of the coordination sites of the copper (II)

ion displacing a mole of ethylenediamine (Johnson 1985; Reeves 1951). Reeves (1951) had studied the complex formation between cellulose glycol and copper (II) by examined a series of partly methylated celluloses with different glycol content. It had been evident that, there were direct correspondence between bound copper and the number of glycol groups as measured by optical rotation.

Ethylenediamine is a good swelling agent for cellulose. By disrupting intermolecular hydrogen bond, swelling promotes molecular chain separation allowing thorough penetration of the solvent. This process had enhanced the complex formation with cellulose glycol groups. The complex is sufficiently stable to prevent aggregation of chains and formation of precipitation. Therefore cellulose was being dissolved (Johnson 1985). Hence the results supported that cellulose is the primary component of the bacterial cellulose powder.

FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

The FTIR spectra of bacterial cellulose prepared from *nata de coco* are shown in Figure 1. The FTIR spectra of bacterial cellulose (b) was compared with that of pure cellulose powder (micro granular cellulose powder from SIGMA) of high purity grade reagents (Ramírez-Flores et al. 2009) and with that of bacterial cellulose (a) previously reported by Surma-Ślusarska et al. (2008).

For the pure cellulose spectrum, distinguish peaks of 3350 cm⁻¹ and shouldering around 3400 cm⁻¹ to 3500 cm⁻¹ indicates O-H stretching, 2800 cm⁻¹ to 2900 cm⁻¹ indicates C-H stretching, 1160 cm⁻¹ indicates C-O-C stretching and 1035 cm⁻¹ to 1060 cm⁻¹ indicates C-O stretching. Other

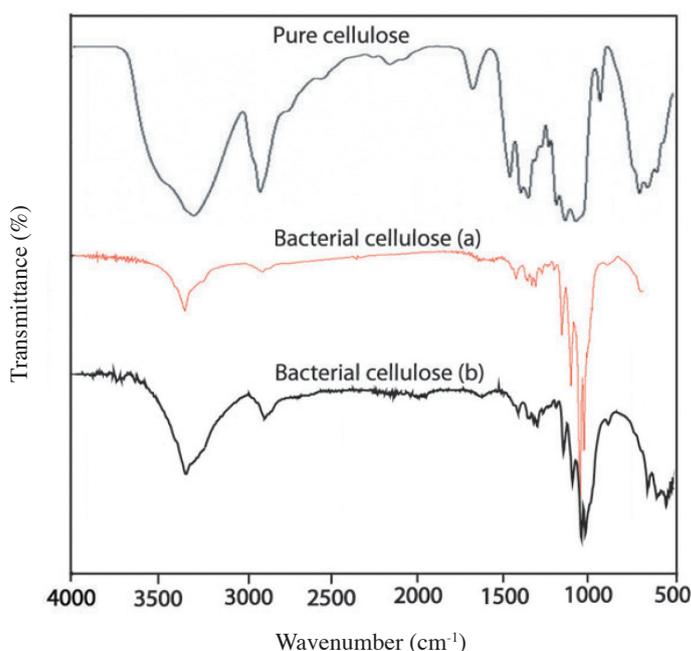


FIGURE 1. FTIR spectra of pure cellulose (micro granular cellulose) from Sigma (Ramírez-Flores et al. 2009), bacterial cellulose (a) from Surma-Ślusarska et al. (2008) and bacterial cellulose (b) from *nata de coco*

fingerprint regions for cellulose are peaks around 1300cm^{-1} indicating C-H bending and around 1400cm^{-1} indicating CH_2 bending (Marchessault & Sundararajan 1983). The analysis of bacterial cellulose produced from *nata de coco* showed peaks at 3440cm^{-1} , 2926cm^{-1} , 1300cm^{-1} , 1440cm^{-1} , 1163cm^{-1} and 1040cm^{-1} thus confirming the purity of the cellulose produced.

Although fingerprint peaks can confirm the structure such as that of cellulose, the curve of peaks may vary, depending on the origin of cellulose. Some examples are provided in Figure 2. All the peaks correspond to celluloses; however, the cellulose from palm kernel (Bono et al. 2009), barley straw (Sun et al. 2005) and *Rhizobium* species (Parthiban et al. 2011) have different shapes. Therefore, the shape of the curve is a signature of the origin of the cellulose. In addition, the spectra of bacterial cellulose from *Acetobacter xylinum* showed its own signature curve and this shape of curve was consistent and reproducible. A comparison of the FTIR spectra was made between bacterial cellulose spectra (a) obtained by researchers from Poland (Surma-Ślusarska et al. 2008) and (b) the bacterial cellulose (*nata de coco*) that had been produced from our local food supplier (Figure 1). These results indicate that our bacterial cellulose is confirmed as pure cellulose synthesised from the bacteria species of *Acetobacter xylinum*.

THERMOGRAVIMETRY ANALYSIS (TGA)

The TGA and DTG curves of bacterial cellulose from *nata de coco* were compared with those of pure cellulose powder

(Aldrich, $20\ \mu\text{m}$) and Whatman filter paper from previous study by Soares et al. 1995 (Figure 3). The pure cellulose powder and Whatman paper have showed maximum rates of weight loss around $330\text{--}335^\circ\text{C}$, whereas the bacterial cellulose showed maximum rate of weight loss at 343°C . However, some researchers have reported the maximum rate of weight loss to occur at 370°C for bacterial cellulose (Surma-Ślusarska et al. 2008), 333°C for cellulose from barley straw (Sun et al 2005) and 350°C for Kraft paper (Soares et al. 1995).

A number of factors may influence the thermogravimetric measurements and one of them is samples preparation. Samples size, morphology and homogeneity may affect heat transfer within the samples and thus influence the diffusion rate of reaction and the course of reaction (Bottom 2008). In this case, the size of the powder cellulose was $20\ \mu\text{m}$, whereas the size of the bacterial cellulose from *nata de coco* was estimated to be $70\text{--}80\ \mu\text{m}$. The powder cellulose was in granular form hence more homogenous than the bacterial cellulose, which has an irregular fibrous form. Therefore, as observed in the DTG curve, the peak of maximum weight loss for pure cellulose was shifted almost 10°C lower than that of bacterial cellulose and this reflects the size and morphology differences between the samples. Although this value may vary, but as reported in literature, the DTG peak prove that bacterial cellulose from *nata de coco* is pure cellulose which have maximum weight loss at the range of $330\text{--}370^\circ\text{C}$ (Surma-Ślusarska et al. 2008).

The TGA curve of bacterial cellulose showed a typical decomposition curve of pure compound where only one step of decomposition was observed (Bottom 2008). Thus

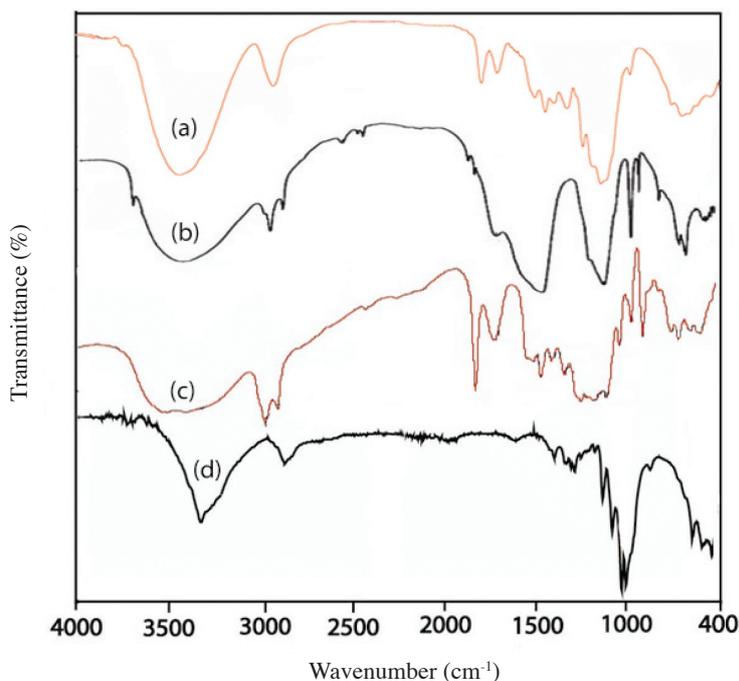


FIGURE 2. FTIR spectra of (a) barley straw cellulose (Sun et al. 2005), (b) bacterial cellulose from *Rhizobium* species (Parthiban et al. 2011), (c) palm kernel cellulose (Bono et al. 2009) and (d) from *nata de coco*

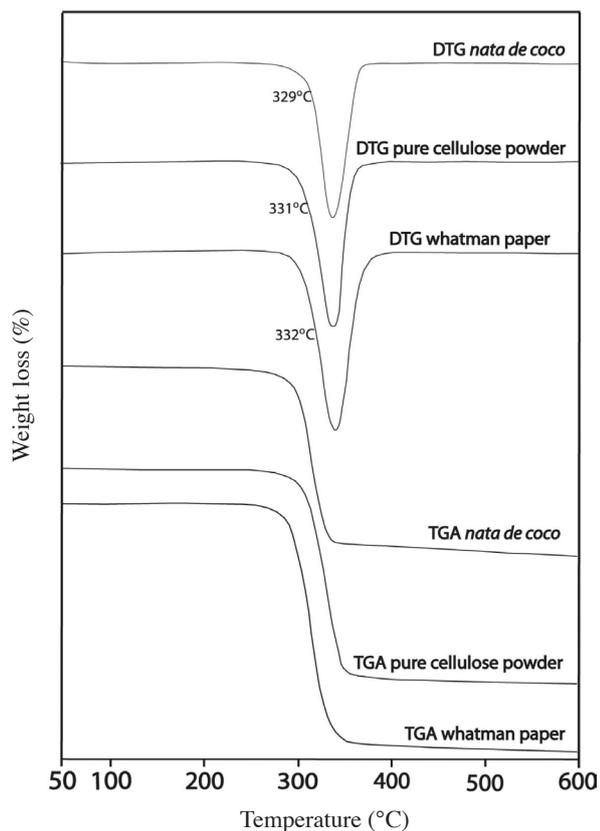


FIGURE 3. TGA and DTG curve of bacterial cellulose from *nata de coco*, pure cellulose powder (Aldrich, 20 μm) and Whatman paper (Soares et al. 1995)

confirming the purity of the polymer extracted from *nata de coco*. Since the analysis was performed under nitrogen atmosphere, no oxidation occurred. In this inert condition, sequence of reactions occurs as cellulose being heated. The first reaction is dehydration of cellulose, an endothermic process known as dehydrocellulose (Kilzer & Broido 1965). After that, depolymerization reaction took place and yielded levoglucosan (1,6-anhydro- β -D-glucopyranose) as an essential intermediate. Dehydrocellulose that was produced in the earlier reaction was then going through decomposition and later produce gaseous products (CO , CO_2) and residual char (Arseneau 1971).



FIGURE 4. *Nata de coco* purchased as food grade

PARTICLE SIZE

Bacterial cellulose powder was found to have particles with size (surface diameter) of $78.2 \pm 0.28 \mu\text{m}$. As mentioned before, bacterial cellulose extracted from *nata de coco* (Figure 4) was dried into sheets. These sheets were then cut and sieved. The cellulose fibres extruded through the 100 μm sieve to form irregular fibrous, fluffy powder as seen in Figure 5. Standard powder cellulose from Sigma, microcrystalline cellulose or cotton linters have granular structure and thus the particle size can be directly determined using SEM. However, the fibrous structure of bacterial cellulose powder has irregular morphology and thus the particle size need to be determined via a different approach. Moreover because the powder shows poor flowability, the test was performed using wet method in which the cellulose was dispersed in distilled water. Bacterial cellulose has many hydroxyl groups, and these groups interact with water to form hydrogen bond; thus, the bacterial cellulose has a tendency to swell. Hence, for accurately determining the particle size, the value acquired by this method should consider the swelling power of the cellulose fibres.

In a recent study, the swelling power of cellulose was found to be in the range of 17%-36% (Stana-Kleinschek et al. 2001). In this case, if the particle size is 78 μm as determined by wet method, the actual size of the particle is at least 17% smaller.

SEM ANALYSIS

SEM analysis of bacterial cellulose was performed on cellulose powder under 500 \times magnification and bacterial cellulose dried sheets under 50000 \times magnification. Under this observation, the particles of the cellulose powder were fibrous with irregular size and shape (Figure 6). Observation of bacterial cellulose dried sheet under 50000 \times magnification (Figure 7) showed the fine cellulose ribbons, sometimes called fibrils. The size of the ribbons was in the range of 50-60 nm, in accordance with the value in the literature (Ben-Hayyim & Ohad 1965; Czaja et al. 2006; Hult et al. 2003; Yamanaka et al. 1989). These ribbons were also appeared as crossed, superimposed layers of cellulose ribbons that were randomly oriented (Ben-Hayyim & Ohad 1965).



FIGURE 5. Bacterial cellulose powder from *nata de coco*

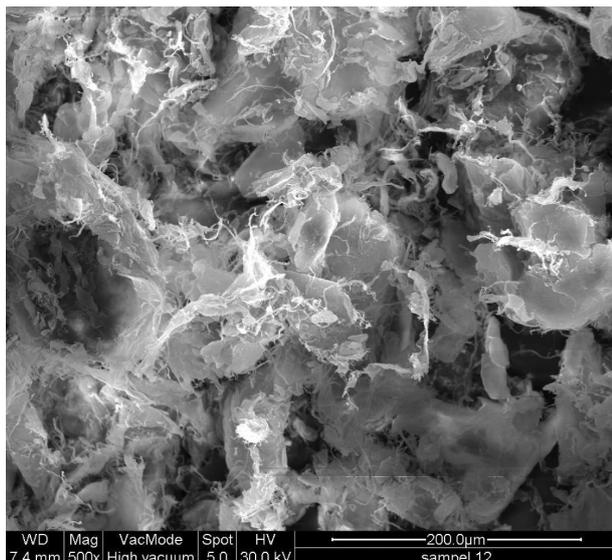


FIGURE 6. SEM of bacterial cellulose powder

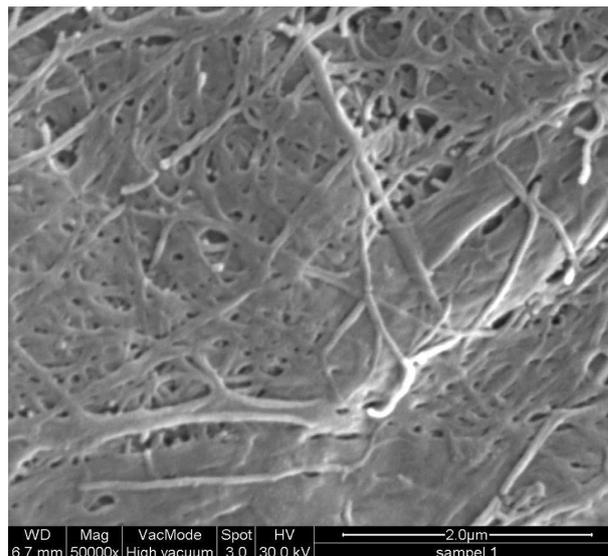


FIGURE 7. SEM of bacterial cellulose dried sheet

CONCLUSION

The cellulose extracted from *nata de coco* met the specifications of pure cellulose, as proven by FTIR, thermal properties and solubility studies. The FTIR spectra of bacterial cellulose from *nata de coco* showed distinguished peaks at 3440 cm^{-1} , 2926 cm^{-1} , 1300 cm^{-1} , 1440 cm^{-1} , 1163 cm^{-1} and 1040 cm^{-1} which correspond to O-H stretching, C-H stretching, C-H bending, CH_2 bending, C-O-C stretching and C-O stretching, respectively. While the DTG showed maximum weight loss at 342°C , this value was within the range of observed cellulose degradation peaks reported in the literature. The solubility of *nata de coco* in cupriethylenediamine solvent indirectly confirms the purity of the cellulose.

Although *nata de coco* was purchased locally as a food-grade material, purification showed that the material was a reliable source of bacterial cellulose and could be used for research activities.

ACKNOWLEDGEMENT

The authors thank Universiti Kebangsaan Malaysia (UKM), the Ministry of Agriculture (MoA) and the Malaysian Nuclear Agency for their support. This project was funded by MoA: Sciencefund 05-01-02-SF1023.

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Received: 13 April 2011
Accepted: 1 August 2011