ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Characteristics of *Nata de coco* of Three Types Coconut Fermentation Media.

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ABSTRACT

Generally, *nata de coco* made from coconut water as a fermentation media instead of coconut milk and skim. Actually coconut milk and skim were also potential as medium of fermentation. They contain components required by the bacteria *Acetobacter xylinum* for its growth. This study, evaluated properties of *nata de coco* from three different types of fermentation media; coconut water, coconut milk and coconut skim. FTIR analysis showed that the peaks spectra of *nata de coco* from coconut water, milk and skim corresponding to cellulose. However, cellulose of *nata de coco* from the three different types of fermentation media had different shape. Thermal analysis showed that there was no significant change in weight loss percentage of *nata de coco* from the three types of media when heated from 50°C to 800 ° C after one hour. SEM analysis showed that the resulting fibrous particles of *nata de coco* had not uniform in size and shapes. XRD analysis showed that diffraction gram of *nata de coco* from coconut skim and milk had greater crystalline index than coconut water.
Keywords: coconut water; coconut milk; coconut skim; *nata de coco*

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INTRODUCTION

Nata is a fermented product made of high sugar solution using *Acetobacter xylinum*. Enzyme from these bacteria converted sugar in fermentation media into a million of cellulose fibril which will become white or transparent and compact. Cellulose produced by *Acetobacter xylinum* is named bacterial cellulose (BC) or microbial cellulose [1,2].

BC as natural cellulose resources only consisted of glucose as monomer with unique characteristics [3] such as a high purity, a high water holding capacity, a high polymerization degree, high mechanical strength and high crystallinity [4,5,6,7,8,9]. Further, it was explained in [10] that BC is high purity, free from hemicellulose, lignin, and non-cellulose contents. Based on these characteristics, BC is potential to be applied in electronics such as electronic paper [11], audio membrane [12]; wound dressing for medical [13], skin substitution [14] and vascular prosthetic device [15,16]. BC is also applied on industry such as food industry, paper and textile industry, electrical applications, and membrane industries [17].

Generally, coconut water becomes basic material or fermentation medium for BC production or *nata de coco* using *Acetobacter xylinum*. Coconut water consisted of good nutritions for *Acetobacter xylinum* such as soluble sugar, protein, salt, few of oil and vitamin [18]. Sugar is a major fraction of dissolved solids in coconut water [19, 20]. The fermentation process to produce BC by the bacterium *Acetobacter xylinum* using coconut water medium takes about 7-8 days [17].

Basically, the fermentation medium to produce BC is a material that can provide good nutrition for growth and development of bacteria *Acetobacter xylinum*. Coconut milk and coconut skim are alternative media that can be used to produce BC. It was acknowledged by [21] that the aqueous extract of endosperm of coconut could produce coconut milk as a natural emulsion (oil in water). Coconut milk contains approximately 54% moisture, 35% fat, and 11% of non-fat solids [22, 23].

Coconut skim is a residual of virgin coconut oil (VCO) production process. It normally disposed of which could contribute to environmental pollution. In [24], it was found that the VCO used to produce health and cosmetic products with predicted growth rate of production up to 0.86%. Coconut skim contains protein, vitamins and minerals could be a potential source to serve as a medium for growth of *Acetobacter xylium* bacteria in production of *nata de coco* [25]. Characteristics of *nata de coco* using coconut water as a fermentation medium have been reported by previous researchers as [26, 27]. However, there was no information about the specific characteristics of *nata de coco* using coconut milk and coconut skim as fermentation media. Hence, this study aimed to obtain the properties of *nata de coco* from three different types of fermentation media, namely coconut water, coconut milk and coconut skim.

MATERIALS AND METHOD

Materials

Materials used in this study were coconut water, coconut milk and skim obtained from a local market in Padang area, Indonesia. Sugar, urea and glacial acetic acid (Merk), *Acetobacter xylinum* was purchased from PT. Cocomas *Nata de coco* Factory. Further, oven (Memmert, Germany), analytical balance (ABJ/NM/ABS-N), a spectrophotometer (Perkin Elmer), X-Ray Diffraction (X'Pert PRO PAN analytical), Thermogravimetric analyzer (Leco TGA 701), SEM-XRD (Hitachi S-3400 N), glass equipment were also used in this research.

Methods

Nata de coco Production

Coconut water, coconut milk and skim was bought from local market in Padang. In order to increase acidity and produce higher yield, raw material for nata particularly coconut water was kept in storage for 3 days. However, fresh coconut milk and skim were directly used for nata production. Fresh coconut milk was diluted with distilled water until obtaining similar fat content to coconut water.

Further, three liters of each materials were filtered to separate impurities then 66 g of sugar and 15 g of urea were added to be boiled for 15 minutes. Next, about 25 ml of glacial acetic acid were added to adjust pH to pH 3-4. Nata sheet was harvested after 25 days. Nata sheet was washed repeatedly and cleaned until its color clear using distilled water and also to remove acid and sour odor. Thus, *nata de coco* named Water Coconut *Nata de coco* (WCN), Coconut Milk *Nata de coco* (CMN), and Skim Milk *Nata de coco* (SMN) regarding its main material. Next, *nata de coco* was cut into 2 cm x 2 cm size then distilled water was added into *nata de coco* with in a ratio of 1:2. *Nata de coco* was washed, screened and dried on glass plate at temperature 75ºC using oven (Memmert, Germany). Dried nata was grinded using a disk mill to obtain bacterial cellulose powder.

Fourier Transform Infrared (FTIR) Spectroscopy

Infrared spectra of cellulose bacterial were recorded using spectrophotometer (Perkin Elmer) at room temperature. Powdered forms of the samples were prepared and analysed over the range of 600-4000 cm^{-1}.

Thermogravimetric Analysis (TGA)

Thermogravimetric analysis was performed using Leco 701 version 1.4x, part number 200-287 US. Ten milligrams (10 mg) of bacterial cellulose powder prepared was placed in a sample cup. The analysis was performed at 10˚C/min from 50˚C to 800 ˚C.

Scanning Electron Microscopy (SEM)

Nata de coco powder was mounted on plate to a thickness of 25-30 nm. Samples were observed under a Hitachi S-3400 N scanning electron microscopy with 1000 magnification.

X-ray diffraction (XRD)

XRD patters of samples were performed in an X'Pert PRO MDP X-ray diffract meter (PAN analytical, Eindhoven, The Netherlands) using a copper (Cu) tube (λ=1,54 Å) at 40 Kv and 30 mA. Samples were examined with a scanning angle of 2θ from 10˚ to 30˚ at rate of 1˚/ min. The crystalline index (CrI) was calculated as a function of maximum intensity of the diffraction peak from the crystalline region (I_{200}), at angle of 2θ – 22.5˚, and minimum intensity from the amorphous region (I_{am}), at angle of 2θ – 18˚, as describe by [28] (Eq.1).

$$CrI\ (\%) = (I_{200}-I_{am})/\ I_{200} \quad (1)$$

RESULTS AND DISCUSSION

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of WCN, CMN and SMN are shown in Fig. 1. The spectra from all of the powder displayed similar characteristic peak wherein frequency of 4000 cm^{-1} – 1300 cm^{-1} shows the main peak, while below 1300 cm^{-1} shows the fingerprint area. The analysis of BC produced from WCN showed peaks at 3320.03 cm^{-1}, 2910.95 cm^{-1}, 1450.53 cm^{-1}, 1341.83 cm^{-1}, 1043.47 cm^{-1} and 821.27 cm^{-1}. The analysis of BC produced from CMN showed peaks at 3339.27 cm^{-1}, 2897.79 cm^{-1}, 1410.15 cm^{-1}, 1327.01 cm^{-1}, 1008.69 cm^{-1} and 858.76 cm^{-1}. The analysis of BC produced from SMN showed peaks at 3340.09 cm^{-1}, 2899.79 cm^{-1}, 1413.45 cm^{-1}, 1326.21 cm^{-1}, 1168.55 cm^{-1} and 863.35 cm^{-1}.

Thus, the spectra of WCN, CMN and SMN shows the typical vibrations of cellulose such as the wave number 3362 cm^{-1} (stretching of the O-H bonds), 1429 cm^{-1} (asymmetric angular deformation of C-H bonds), 1371 cm^{-1} (symmetric angular deformation of CH bonds), 1163 cm^{-1} (asymmetrical stretching of C-O-C glycoside bonds), 1110 cm^{-1} and 1059 cm^{-1} (stretching of C- OH and C- C- OH bonds in the secondary and primary alcohols, respectively), and 897 cm^{-1} (angular deformation of CH bonds) [29].

Instead of the peaks of the spectra of WCN, CMN and SMN corresponding to cellulose, cellulose from WCN, CMN and SMN have different shape. The shape of cellulose WCN spectra are similar to study done by [26], where the cellulose powder from *nata* met the specification of pure cellulose.

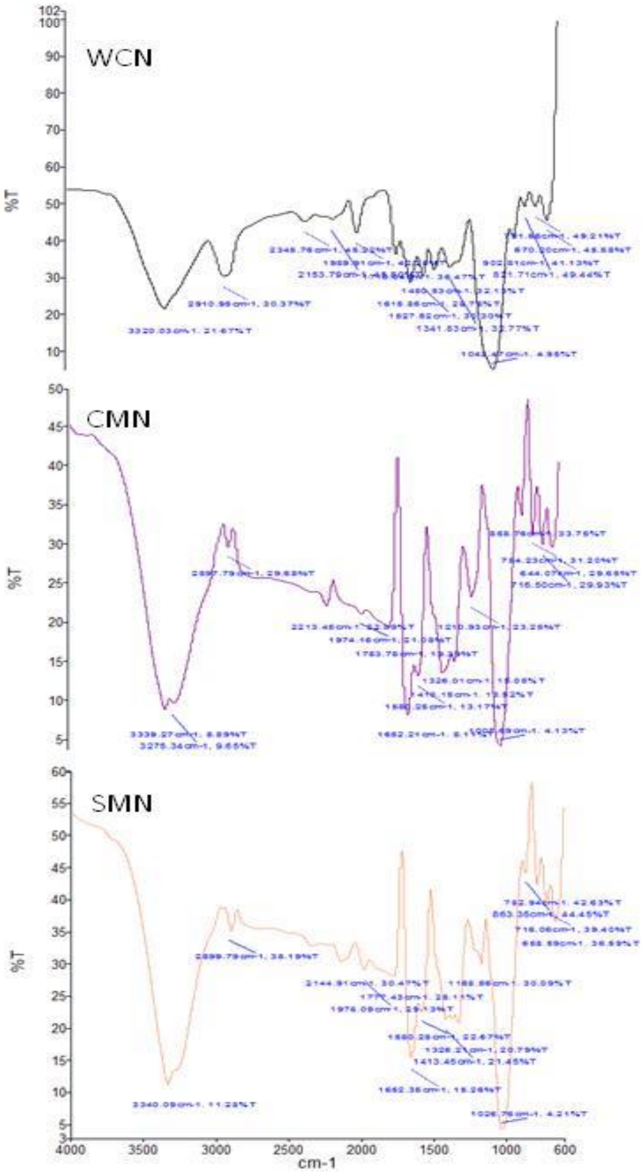


Fig.1 FTIR spectra of WCN, CMN and SMN

Thermogravimetric Analysis (TGA)

As explained by [30], TGA test is related to measure of sample weight during increasing temperature condition under of programed heating. TGA was set up to 800°C as the highest temperature and remained for several minutes until sample weight balance. As the results, percentage of weight loss against temperature was shown as TGA curves as shown in Fig 2. It was obtained that there was no significant change on weight loss percentage of WCN, CMN and SMN at 800°C after one hour. For cellulose as the standard, the weight loss at 800°C was almost 99% while WCN only obtained 96% of weight loss. The weight loss for other CMN and CMN were about 98%.

TG curves of the three fermentation media showing mass-loss profile that is similar to the results of research [31], where there were three mass-loss events. The first one occurred at temperature range 50-150 ^{0}C, was caused by evaporation of residual water present in the material. The second one occurred at 250-400 ^{0}C, was indicated by a set of cellulose reactions degradation, such as dehydration, decomposition and depolymerization of the glycoside units. The third one occurred at 450-600 ^{0}C, was corresponded to oxidation and breakdown of carbonaceous residues which produced gaseous with low molecular weight.

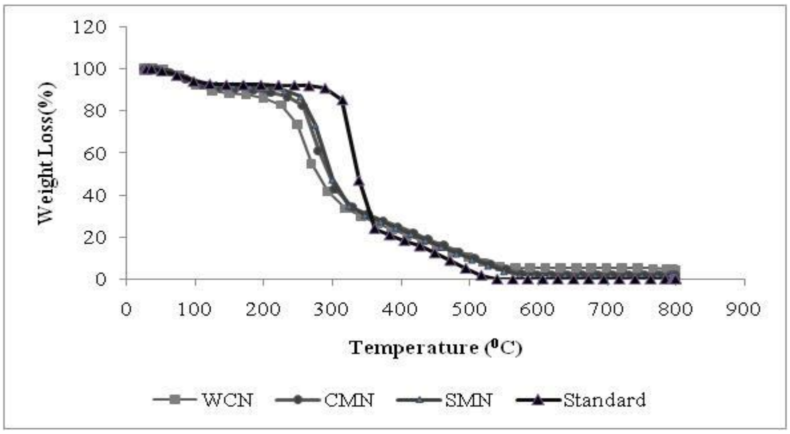
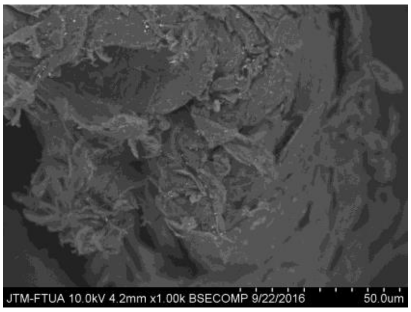


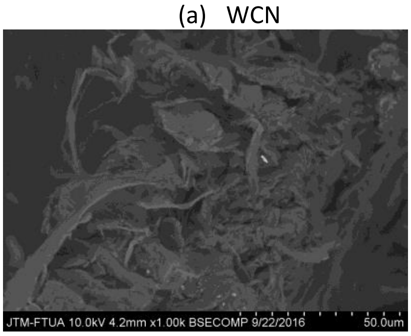
Fig.2 WCN, CMN and SMN and Standard Curves

Scanning Electron Microscopy Analysis

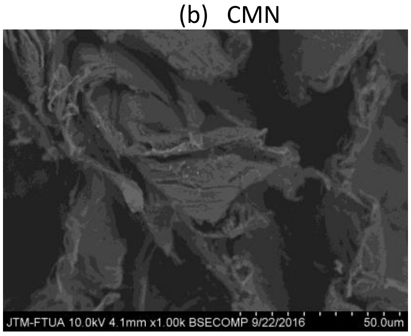
SEM analysis showed that the resulting fibrous particles had not uniform in size and shapes. The range of bacterial cellulose particle size were 0.45 to 1.19; 0.47 to 1.23 and from 0.92 to 1.20 µm respectively for WCN, CMN and SMN (Fig. 3). These particle sizes were larger than reported by [26] is 50-60 nm.



(a) WCN



(b) CMN



(c) SMN

Fig.3 Surface Structure of *Nata de coco*

X-Ray Diffraction (XRD)

XRD diffractogram of bacterial cellulose produced from the fermentation of different media such as WCN, CMN and SMN shown in Fig. 4. X-ray diffraction pattern of the bacterial cellulose of WCN showed three strongest peaks at an angle 2θ: 22.57˚; 34.43˚; 16.85˚ the crystalline index of 77.95%. At the bacterial cellulose of SMN showed three strongest peaks at an angle 2θ: 22. 60˚; 14.51˚; 16.62˚ the crystalline index of 86.43%. While in the bacterial cellulose of CMN showed three strongest peaks at an angle 2θ: 22, 66˚; 22,26˚; 14,48˚ the crystalline index of 87.99%. Therefore, SMN and CMN had greater crystalline index than WCN. It was found by [27] that a crystalline index *nata de coco* from coconut water produced ranges between 87.80% -88.90%.

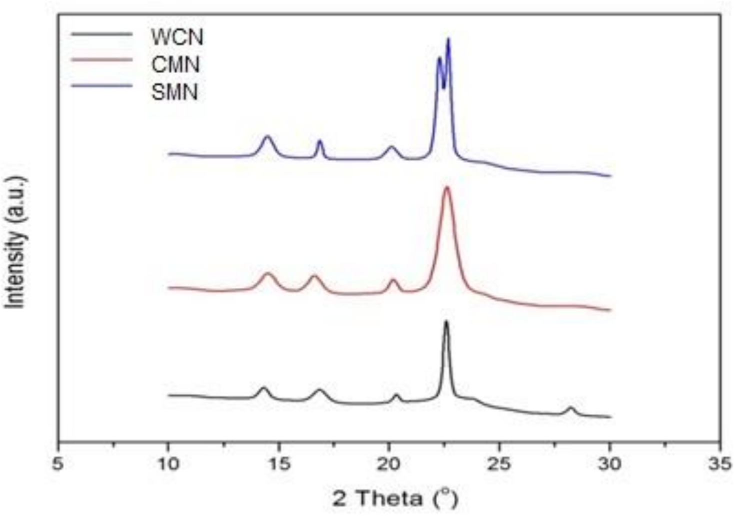


Fig. 4 X-ray diffraction pattern of bacterial cellulose of the three sources of raw materials (-) WCN (-) CMN (-) SMN

CONCLUSION

This study was conducted to evaluate properties of *nata de coco* from three different types of fermentation media; coconut water, coconut milk and coconut skim. The results showed that properties of *nata de coco* from coconut water were different with properties from coconut milk and skim in the form of spectra and crystalline index. Meanwhile, there was no difference in the properties of the three media fermentation based on the thermal analysis and SEM.

ACKNOWLEDGEMENT

This work was supported by the Andalas University under Grant MoA: Sciencefund No. 96/UN.16/HKRGB/LPPM/2016.

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