INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

PREPARATION AND CHARACTERIZATION OF MICROCRYSTALLINE CELLULOSE FROM RICE STRAW USING CHEMICAL AND ENZYMATIC TECHNIQUES

Harrizul Rivai [1]*, Ramdani Ramdani [2], Regina Andayani [1], Fithriani Armin [1], Akmal Djamaan [1]
[1]Faculty of Pharmacy, Andalas University, Limau Manih Campus, Padang 25163, Indonesia
[2]School of Pharmaceutical Science (STIFARM), Jl. Siteba Kurao Pagang Dalam RT02/RW08, Padang 25147, Indonesia
*Corresponding Author Email: harrizul@phar.unand.ac.id

Article Received on: 30/04/19 Approved for publication: 02/06/19

DOI: 10.7897/2230-8407.1007213

ABSTRACT

The preparation and characterization of microcrystalline cellulose (MCC) from rice straw have been done by using chemical and enzymatic techniques. Alpha cellulose was prepared by a multistage pulping method from rice straw. MCC was prepared by using chemical and enzymatic techniques from alpha cellulose. In this study, the physicochemical properties of microcrystalline cellulose were evaluated and compared to Avicel PH 101. The physicochemical properties include organoleptic, loss on drying, solubility in water, starch test, morphology by scanning electron microscopy (SEM), functional groups by Fourier Transform Infrared (FTIR) Spectroscopy, and analysis by differential scanning calorimetric (DSC). Results showed that MCC obtained by chemical technique was 86 % of alpha cellulose, by enzymatic techniques were 83 % (for 5 % enzyme), 82.67 % for (10 % enzyme) and 84.2 % (for 15 % enzyme), respectively. The SEM image showed the morphology of MCC; the functional groups present in the MCC and DSC analysis of MCC was the same as Avicel PH 101.

Keywords: microcrystalline cellulose, rice straw, chemical, and enzymatic techniques

INTRODUCTION

Rice straw (*Oryza sativa*) is an agricultural waste that is available in relatively more quantities than other agricultural wastes and is found in almost every province in Indonesia. In general, rice straw and other lignocellulose materials are composed of cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are composed of sugar monomers. This cellulose is in the form of twisted fibers and bound by hemicellulose, then protected by solid lignin. Due to the protection of lignin and hemicellulose, cellulose becomes challenging to cut into sugar[1]. Rice straw has been utilized in the production of bioethanol, microcrystalline cellulose, and lactic acid. However, the utilization is still limited; most of them are burned[2, 3]. Contents of sulfur, nitrogen, chlorine lead to the formation of gaseous pollutants such as SO_2, NO_x, N_2O, HCl, and to some extent, dioxins and furans. These pollutants can pose a health hazard[4].

In general, rice straw (*Oryza sativa*) and other lignocellulose sources contain about 32-47% cellulose, 19-27% hemicellulose and 5-24% lignin. Cellulose is a polymer of β-glucose with β-1-4 bonds between glucose units. Cellulose found in wood, cotton, hemp, and other plants. Cellulose is an organic compound found in the cell walls together lignin role in cementing the structure of plants. Wood cellulose generally ranges from 40-50 %, whereas in cotton almost 98 %.[4, 5]

Cellulose consists analytically of three fractions, alpha, beta, and gamma. Alpha cellulose is widely used in the paper industry.[6] Up-scale production has obtained microcrystalline cellulose at the optimal hydrolysis time for 60 minutes using 2.5 N HCl at a temperature of 100 °C from rice straw. Microcrystalline cellulose from rice straw is not significantly different from Vivacel by organoleptic inspection, identification, pH, and dissolved substances in water, solubility, drying shrinkage, apparent specific gravity, starch test, and have as well as the infrared spectrum. In testing the density of compressed, Hausner ratio and Carr's index, there is a noticeable difference between the microcrystalline cellulose from rice straw and Vivacel.[7]

The production of alpha cellulose from rice straw has been done. Alpha cellulose was prepared by maceration of rice straw with ethanol, delignification with 3.5 % sodium hydroxide, extraction of alpha cellulose with 17.5% sodium hydroxide, and bleaching with hydrogen peroxide 20%. In this study, the physicochemical properties of alpha cellulose were evaluated and compared to previous studies. The physicochemical properties include morphology by Scanning Electron Microscopy (SEM), crystallinity by X-ray Diffraction (XRD) analyses, functional groups by Fourier Transform Infrared (FTIR) Spectroscopy, loss on drying, pH and organoleptic. Results showed the SEM image shows the morphology of cellulose fibers, the sample was semi-crystalline with crystallinity index 78 %, the functional groups present in the sample are the same as the functional groups in alpha cellulose, loss on drying 8 is % w/w, pH 7 is and the organoleptic are white powder, odorless and tasteless. The yield of alpha cellulose produced is 30.11 % w/w.[8]

Based on the description above, the researchers want to create and characterize MCC which is hydrolyzed from alpha cellulose using chemicals and microbes producing cellulase enzymes. Cellulose enzyme used comes from filamentous fungi such as *Trichoderma viride* and *Aspergillus niger* which are rich in an endocellulase activity which selectively removes amorphous parts from cellulose, so the potential to hydrolyze cellulose to microcrystalline cellulose.

MATERIAL AND METHODS

Plant Material

Rice straw was collected from a rice field in Padang, West Sumatera, Indonesia on March 2018. Plant material was identified at Andalas University Herbarium, Padang. It was cut into chips and dried.

Chemicals

Ethanol, sodium hydroxide, hydrogen peroxide, and distilled water were purchased from PT Bratachem, Indonesia.

Preparation of alpha cellulose

Preparation of alpha cellulose was conducted as a procedure by Rivai *et al.*[8]

Chemical preparation of microcrystalline cellulose (MCC)

50 g of alpha-cellulose was hydrolyzed with 2.5 N HCl (1.2 L) by boiling for 15 minutes in a beaker glass. Then the hot mixture is poured into cold water and stirred actively using a spatula and left overnight. Microcrystalline cellulose obtained was washed with distilled water to neutral, filtered with a Buchner funnel, then dried in an oven at a temperature of 57-60 °C for 60 minutes and then crushed. MCC obtained is stored at room temperature in the desiccator.[9]

Enzymatic microcrystalline cellulose production

Sterilizing Tools

The tools used are first washed and dried. Glassware that has a mouth covered with cotton wrapped in gauze, then all glassware is wrapped using parchment paper, then sterilized in an autoclave at 121ºC, a pressure of 15 lbs, for 15 minutes. The micropipette tip is arranged in a glass cup, covered with aluminum foil, then sterilized in an autoclave at 121ºC, a pressure of 15 lbs, for 15 minutes. Spatula and needles are sterilized in a flame way over the flame of the spirit lamp for 20 seconds. Aseptic cabinets are cleaned of dust and sterilized by spraying 70% alcohol into all parts of the closet. All works are carried out in aseptic techniques.

Cellulase Enzyme Production

The seeding medium used in this study was Potato Dextrose Agar (PDA). PDA powder is weighed as much as 3.9 grams, dissolved in 100 mL of distilled water, then heated on a heating device, then stirred until it dissolves completely and is bright in color, then covered with a cotton plug wrapped in sterile gauze. Furthermore, this solution is sterilized by autoclaving with a temperature of 121ºC, a pressure of 15 lbs, for 15 minutes.

Trichoderma viride fungi, which have been isolated and purified, are transferred with the help of an ose needle to the PDA media. The work was carried out aseptically on laminar air flow, then incubated at 28 ºC for 7-10 days, then stored at 4 ºC when the spores were formed.[10]

Enzyme production was carried out in 250 ml Erlenmeyer containing mineral salt media (0.05% KH_2PO_4, 0.05% $Ca(NO_3)_2$ and 0.05% $MgSO_4$) and five grams of wheat bran. The ratio of solids and liquids is 1: 3. Erlenmeyer is closed and sterilized at 121 ºC for 20 minutes. After cold, 0.5 ml of the suspension of fungi spores were inoculated into the fermentation medium and incubated for three days.[11]

The enzyme was extracted by adding 25 ml of 0.05 M phosphate buffer to the Erlenmeyer containing the enzyme. The mixture is stirred for 45 minutes at a speed of 150 rpm and filtered with filter paper. The extract was centrifuged for 10 minutes at 10,000 rpm. The supernatant formed is a source of cellulase enzymes.[11]

Hydrolysis of alpha cellulose by enzyme cellulase

Alpha cellulose is dispersed in acetate buffer (0.1 M, pH 5) with a ratio of 1:10. Hydrolysis is carried out using cellulase enzyme produced by fungi with variations in the concentration of enzymes 5, 10 and 15% v / v at 50ºC for variations of time 0.5, 1 and 1.5 hours at a speed of 150 rpm. The mixture was centrifuged at 10,000 rpm for 20 minutes. The precipitate formed is washed with distilled water to remove enzyme residues, then dried in an oven.[12]

Characterization of Microcrystalline Cellulose

Physicochemical of MCC

The organoleptic characteristic, identification, organic impurities, starch and dextrin, solubility, total ash, and water-soluble substances were carried out following BP 2012 specifications.[13]

pH determination

Determination of pH was done by shaking 2 g of the powder material with 100 ml of distilled water for 5 min, and the pH of the supernatant liquid was determined using a pH meter.

Scanning electron microscopy

Scanning electron microscopy was performed using a Joel 6310 (Joel Instrument, Tokyo, Japan) system running at 10 KeV.

Fourier-transform infrared spectra

The surface of each sample was characterized using PerkinElmer Spectrum 1000 Fourier transform infrared (FTIR) Spectrophotometer. Each sample was scanned 64 times at a resolution of 4 cm^{-1} between 4000 and 650 cm^{-1}.

X-ray powder diffractometer studies

Diffraction patterns were obtained using Phillips X-ray diffractometer. The diffraction patterns were recorded using Cu-Kα radiation at 40 kV and 25 Ma. The samples were pressed into pellets (25 mm in diameter) by compression of 0.25 g in a mold under a pressure of 50 MPa. The crystallinity index (CrI) calculated as follows:

$$CrI = [(I_{002} - I_{am})]/I_{002}$$

where I_{002} is the intensity of the peak (at about $2\theta = 22$) and I_{am} is the intensity corresponds to the peak at about $2\theta = 18$

Differential scanning calorimetry

Differential scanning calorimetry (DSC) scans of the powdered samples were recorded using the DSC 204 F1 (Netzsch Geratebau, GmbH, Selb, Germany), a heat-influx DSC equipped with Netzsch Thermokinetic Analysis Software. The thermal traces were obtained by heating from 26°C to 500°C at a heating rate of 10°C under inert nitrogen dynamic atmosphere (70 ml/min) in a closed aluminum pan with lid pierced and an empty pan was used as the reference. The parameters evaluated were: (a) transition temperatures: MCC water loss (T_{wl}), MCC thermal decomposition (T_{MCC}) and (b) heats of fusion or thermal decomposition: MCC thermal decomposition (ΔH_{MCC}).

RESULT AND DISCUSSION

Preparation of alpha cellulose

In the preparation of alpha cellulose from 500-gram rice straw using multistage pulping method produced 210-gram alpha cellulose powder (% yield = 42%). Powdered alpha cellulose from rice straw from this study was yellowish white (Figure 1).

Figure 1: Alpha cellulose from rice straw

Chemical preparation of microcrystalline cellulose

In the manufacture of microcrystalline cellulose (MCC) chemically, 50 grams of alpha cellulose was hydrolyzed using 2.5 N HCl, and 43 grams of microcrystalline cellulose (% yield = 86%) were produced (Figure 2B). Avicel PH 101 (Figure 2A) was used as a comparison of microcrystalline cellulose. The microcrystalline cellulose produced in this study was in the form of a fine powder, white, tasteless and odorless (Figure 2B-E).

Figure 2: Microcrystalline cellulose (A. Avicel pH 101; B. MCC 1 (Chemically); C. MCC 2 (Enzymatic 5%); D. MCC 3 (Enzymatic 10%); E. MCC 4 (Enzymatic 15%))

Enzymatic preparation of microcrystalline cellulose

In enzymatic preparation of microcrystalline cellulose (MCC), 15 grams of alpha cellulose was hydrolyzed using an enzyme with a concentration of 5% resulting in 12.3792 grams of microcrystalline cellulose (% yield = 83%). Microcrystalline cellulose in the results of this study fine powder, white, tasteless and odorless (Figure 2C)

In enzymatic preparation of microcrystalline cellulose (MCC), 15 grams of alpha cellulose was hydrolyzed using an enzyme with a concentration of 10% resulting in microcrystalline cellulose as much as 12.4019 grams (% yield = 82.67%). Microcrystalline cellulose in the results of this study fine powder, white, tasteless and odorless (Figure 2D).

In enzymatic preparation of microcrystalline cellulose (MCC), 15 grams of alpha cellulose was hydrolyzed using an enzyme with a concentration of 15% resulting in microcrystalline cellulose as much as 12.6301 grams (% yield = 84,2 %). Microcrystalline cellulose in the results of this study fine powder, white, tasteless and odorless (Figure 2E)

Characterization of microcrystalline cellulose

The results of the physicochemical properties of microcrystalline cellulose (which are made chemically and enzymatic [5%], [10%] and [15%]) are by the British Pharmacopeia, 2012 as shown in Table 1.

Table 1: Results of characterization of comparable microcrystalline cellulose and those produced from this study

No	Testing	Requirements	Avicel PH101	MCC1	MCC2	MCC3	MCC4
1	Description: • Form • Color • Smell • Taste	 Fine powder White Odorless Tasteless	 Fine powder White Odorless Tasteless	 Fine powder White Odorless Tasteless	 Fine powder White Odorless Tasteless	 Fine powder White Odorless Tasteless	 Fine powder White Odorless Tasteless
2	Identification	The color obtained is blue-violet (BP, 2012)	The color obtained is blue-violet	The color obtained is blue-violet	The color obtained is blue-violet	The color obtained is blue-violet	The color obtained is blue-violet
3	Loss on drying	Loss cannot be more than 6% (BP, 2012)	Loss of 5.287% ± 0.0896%	Loss of 5.35% ± 0.1452%	Loss of 5.35% ± 0.1452%	Loss of 4.97% ± 0.3842%	Loss of 4.33% ± 0.4526%
4	Solubility in water	Solubility must not exceed 0.25% (BP, 2012)	0.102%	0.105%	0.108%	0.106%	0.111%
5	pH test	pH 5-7.5 (BP, 2012)	6.7	6.5	6.3	6.1	6.4
6	Test for starch	Blue is not formed (BP, 2012)	Blue is not formed	Blue is not formed	Blue is not formed	Blue is not formed	Blue is not formed

Description: MCC 1 (chemically made cellulose microcrystalline), MCC 2 (enzymatically made microcrystalline cellulose at a concentration of 5%), MCC 3 (enzymatically made microcrystalline cellulose at a concentration of 10%) and MCC 4 (Microcrystalline cellulose made enzymatically at a concentration of 15%)

Chemically and enzymatically made microcrystalline cellulose after organoleptic testing was found to be the same as Vivacel PH

101® which is white powder, odorless and tasteless. The results meet the requirements of the British Pharmacopoeia 2012. The loss on drying test for both chemical and enzymatic microcrystalline cellulose, the results obtained met the requirements of the British Pharmacopeia 2012, namely: 5.287% ± 0.0896% (MCC 1), 5.35% ± 0.1452% (MCC 2), 4.97% ± 0.3842% (MCC 3) and 4.33% ± 0.4526% (MCC 4). The results of the identification test for microcrystalline cellulose (chemically and enzymatically) using a solution of iodine chloride zinc turned out to be a violet-blue color. Reagents used are specific reagents for microcrystalline cellulose. The result shows that the microcrystalline cellulose powder (chemically and enzymatically) obtained from true microcrystalline cellulose rice straw and there are similarities with Avicel PH 101® with the result of the formation of violet blue. The results obtained to meet the requirements of the British Pharmacopoeia 2012.

The water solubility test for microcrystalline cellulose (chemically or enzymatically) was obtained from the results of initial and final weight differences of no more than 12.5 mg (0.25%) ie 0.102% (Avicel pH 101®), 0.105% (MCC1), 0.108% (MCC 2), 0.106% (MCC 3) and 0.111% (MCC 4). These results indicate that cellulose microcrystalline is made to meet the requirements of the British Pharmacopeia 2012. Then, for the pH test of microcrystalline cellulose (chemically or enzymatically) with a pH range of 5.0-7.5 the results that meet the requirements of the British Pharmacopeia 2012, namely pH 6.7 (Avicel PH 101®), 6.5 (MCC 1), 6.3 (MCC 2), 6.1 (MCC 3) and 6.4 (MCC 4). Pure microcrystalline cellulose does not contain starch in it. The result can be tested by the presence of starch in cellulose by reacting cellulose with iodine. According to the requirements, the color results obtained from the starch test are not blue (British Pharmacopoeia 2012). The results of testing of microcrystalline cellulose (which is made chemically or enzymatically) and Avicel PH 101® meet these requirements.

The results of Fourier Transformation Infra Red (FT-IR) spectroscopic analysis is to identify functional groups in a compound based on the magnitude of the vibrations produced by the atoms in action. The vibrations of the atoms that interact will produce a certain frequency and appear in a certain number of waves in the spectrum. Each absorption band at a particular wavenumber illustrates the existence of a specific functional group. The results of the analysis are in the form of the signal spectrum, the relationship of percentage transmittance to wave numbers. From the functional group analysis with FT-IR, the results showed that microcrystalline cellulose contained OH functional groups at wave numbers 3875.39 cm^{-1} for MCC 3, Avicel pH 101®, MCC 1 and MCC 2. Then there were also spectra which showed bond hydrogen at wave number 3330.61cm^{-1} (for Avicel pH 101®), 3329.78 cm^{-1} (MCC 1), 3340.85 cm^{-1} (MCC 2), 3346.52 cm^{-1} (MCC 3) and 3414.92 cm^{-1} (MCC 4). The absorption band around this area illustrates the stretching vibration of the hydroxyl group. Moreover, there is also an area of absorption bands of around 2874.87-3024.00 cm^{-1} associated with CH stretch vibrations and associated with aliphatic groups in polysaccharides and associated with asymmetric and symmetrical C-H vibrations. The tensile vibration adjacent to the hydrogen atom is depicted by a vibration band of around 1645.04 cm^{-1} (Avicel pH 101®), 1644.72 cm^{-1} (MCC 1), 1648.04 cm^{-1} (MCC 2), 1627.77 cm^{-1} (MCC 3) and 1616.17 cm^{-1} (MCC 4). The absorption band observed around 899.78 cm^{-1} was related to β-1,4 glycosidic bonds while the band around 1051 cm^{-1} was related to ring vibrations and the C-O-C relationship. Absorbances around 1431, 1360, 1325, 1051, 1044, 1030, 1027 and 899 cm^{-1} are the results of pure cellulose.

The use of Scanning Electron Microscopy (SEM) aims to see the surface morphology of one sample microscopically and provide information about the surface texture of the sample. The morphological form of a sample can be seen from three sides, namely: the upper surface, the side surface and the surface of the inner space. Based on particle shape analysis using Scanning Electron Microscopy (SEM) with various magnifications showing the characterization of microcrystalline cellulose (chemically and enzymatically) seen in the form of crystalline solids with rod shape at a magnification of 1000 times. This SEM result shows that microcrystalline made chemically and enzymatically the crystalline form resembles the crystalline form of Avicel pH 101® (Figure 3-7).

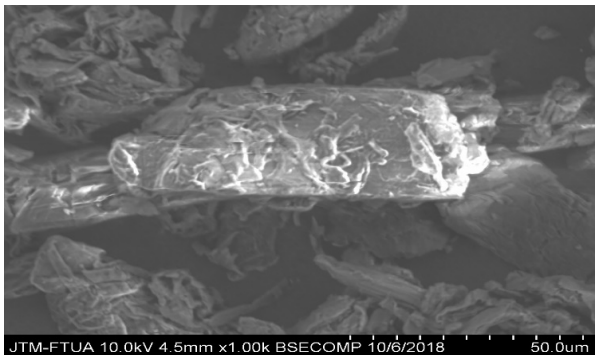

Figure 3: SEM of Avicel PH 101

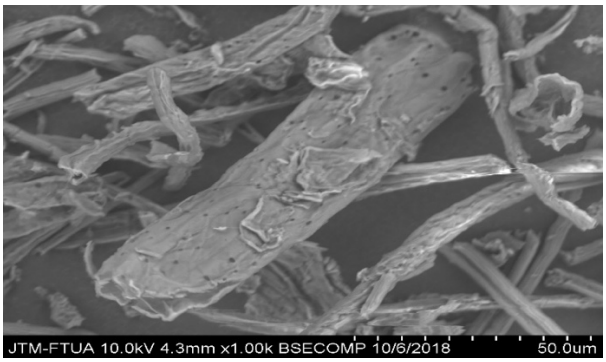

Figure 4: SEM of MCC1

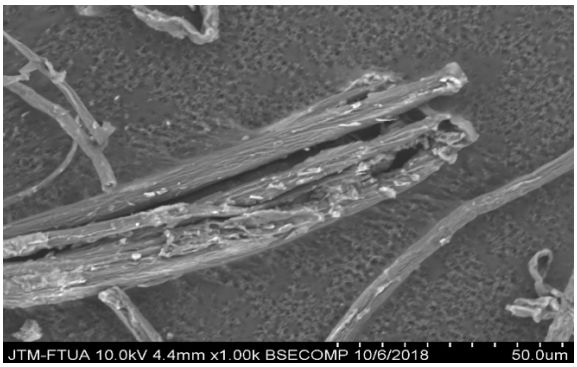

Figure 5: SEM of MCC2

Figure 6: SEM of MCC3

Figure 7: SEM of MCC4

DSC analysis describes the thermo-grams obtained for Avicel PH 101®, MCC 1, MCC 2, MCC 3 and MCC 4. Significant endothermic peaks of around 80 °C were recorded during the first heating scan for all microcrystalline cellulose samples. The result is associated with water loss during heating. The remaining endothermic peaks are obtained according to the peak decomposition. At temperatures below 140 °C water evolution occurs and both samples (MCC 3 and MCC 4) show peak profiles similar to Avicel PH 101®. At higher temperatures, between 300 and 480 ° C, thermal decomposition occurs (Figures 8-11).

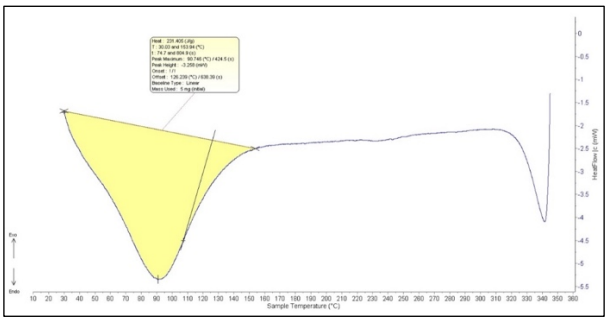

Figure 8: DSC of Avicel PH 101

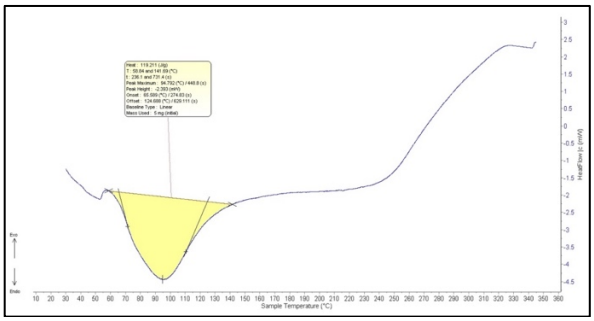

Figure 9: DSC of MCC1

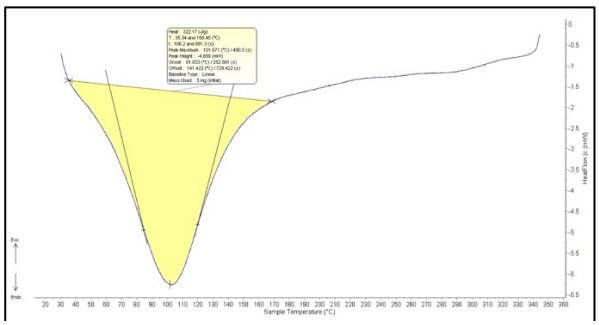

Figure 10: DSC of MCC2

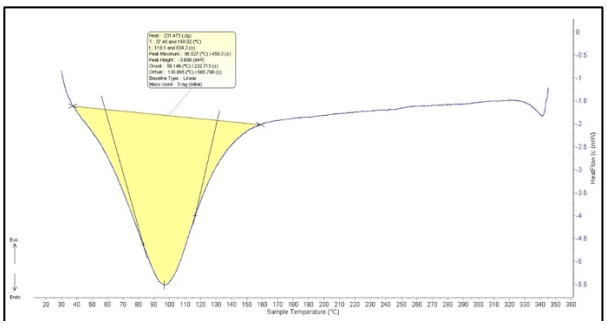

Figure 11: DSC of MCC3

CONCLUSION

Microcrystalline cellulose from rice straw obtained chemically was 86% of alpha cellulose, whereas microcrystalline cellulose from rice straw obtained enzymatically was 83% for enzymes [5%]; 82.67% for enzymes [10%] and 84.2% for enzymes [15%]. Chemical hydrolysis and enzyme concentration did not give a difference in the microcrystalline cellulose produced, seen from the results of the FTIR, SEM and DSC spectra.

ACKNOWLEDEGMENT

The author would like to say special thanks to The Rector of Andalas University for his support this research under Professor Research Grant Project, Andalas University 2018, with Contract Number: 13/UN.16.17/PP.RGB/LPPM/2018.

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Cite this article as:

Harrizul Rivai *et al*. Preparation and characterization of microcrystalline cellulose from rice straw using chemical and enzymatic techniques. Int. Res. J. Pharm. 2019;10(7):27-32 http://dx.doi.org/10.7897/2230-8407.1007213

Source of support: Professor Research Grant Project, Andalas University, Indonesia, Conflict of interest: None Declared