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Nanocellulose production from rice straw derived-cellulose by enzymatic hydrolysis and its effect on lipase activity

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Abstract

Rice straw derived-cellulose was applied as a potential source containing 92.77±0.71% (w/w) of cellulose for nanocellulose production using enzymatic hydrolysis. The nanocellulose content was found to be 28.28±0.38% (w/w), the cellulose residue remaining was 19.93±0.69%, while the efficiency of cellulase hydrolysis was approximately 80%. The rice straw nanocellulose was granular-shaped with a particle size range of 295-396 nm and gave a highly stable suspension due to a high zeta potential value of -45.8±0.4 mV. Furthermore, the effect of the granular nanocellulose on lipase activity was investigated on various substrates. The results showed that the granular nanocellulose significantly reduced lipase activity with a relative lipase activity of 90.51±1.39% and 74.32±0.96% of total activity using *p*-nitrophenyl laurate and olive oil as substrates, respectively. This research has shown that granular nanocellulose can be successfully produced from rice straw-derived cellulose by enzymatic hydrolysis under controlled reaction conditions. Moreover, the granular nanocellulose exhibited attractive physicochemical properties for the composite of nanocellulose with other materials to obtain novel property products, especially for dietary supplements and food ingredients in fat food applications.

Keywords: Enzymatic hydrolysis, Lipase activity, Nanocellulose, Rice straw, Lignocellulosic material

1. Introduction

Cellulose is the most abundant natural and renewable macromolecule in various types of lignocellulosic biomass such as agricultural residues and industrial wastes. The cellulose structure comprises both crystalline and amorphous regions. This structure can be broken down into small particles, generally referred to as nanocellulose, by physical, chemical, enzymatic hydrolysis, and combination processes. By definition, nanocellulose has the geometry of at least one dimension in the nanometer range. For nanocellulose extraction from agricultural residues, a group of enzymes known as cellulase is favorable. Nanocellulose mainly includes cellulose nanocrystals (CNC), cellulose nanofibrils (CNF), and bacterial nanocellulose (BC). The morphology, size, and other characteristics of nanocellulose depend on the cellulose origin, isolation method, and the processing conditions [1-3]. Consequently, a number of nanocellulose forms can be produced using different methods and cellulosic sources. The nanocellulose production process involves various approaches such as acid hydrolysis, mechanical methods, and enzymatic hydrolysis [4]. Numerous agricultural residues such as cotton [1,5], corncob [6], rice straw [7], sugarcane bagasse [8-10], and banana peel [3] have been used as cellulose sources for nanocellulose production.

In Thailand, the production of rice straw as an agricultural residue of the rice crop, is produced annually about 26.2 million tons on average [11]. About 69% of rice straw is burnt in the open field due to the limited time available to prepare the field for the next crop and ease of field maintenance [12]. However, this causes serious environmental problems resulting in pollution. Therefore, proper management for the disposal of rice straw is critical. In terms of its chemical composition, rice straw contains about 34.1-40.5% (w/w) of cellulose [7,13]. Thus, rice straw as a cellulose source for nanocellulose production is attractive economically. In a

previous study, Thakur et al. [14] prepared the rod-shaped CNC from rice straw using a high concentration of sulfuric acid (75%, w/v) for hydrolysis for 5 h of hydrolysis time. As a result, a high cellulose nanocrystal content of 90.28% was obtained. Nasri-Nasrabadi et al. [15] extracted cellulose from rice straw and produced nanocellulose by a chemo-mechanical method via sonication and sulfuric acid hydrolysis. The results revealed that 50% of the cellulose nanofibers had a diameter in the range of 70-90 nm with a length of several microns. However, using a high concentration of sulfuric acid in nanocellulose production has several significant drawbacks; limited thermal stability, a large amount of hazardous waste, and risk of corrosion of the equipment. Thus, enzymatic hydrolysis is one of the potential methods for nanocellulose preparation which is less toxic and uses only mild reaction conditions. Furthermore, the nanocellulose has an easily functionalized structure with high thermal stability and a high aspect ratio for further feasibility utilizing several applications, such as in the food and pharmaceutical industries [16]. Moreover, nanocellulose represents unique physical, chemical, and biological properties. Therefore, this research has been aimed at producing nanocellulose from rice straw-derived cellulose by enzymatic hydrolysis. In addition, the effects of nanocellulose on lipase activity in food and pharmaceutical applications has been investigated, especially in terms of the reduction of lipid digestion. Looking further ahead, nanocellulose might also be a potential candidate for reducing the digestion and absorption of fat for weight management, especially in noncommunicable diseases (NCD) patients.

2. Materials and methods

2.1 Materials

The rice straw cellulose was obtained from the Biorefinery Research Laboratory in the Department of Chemistry, Chiang Mai University (Thailand). The cellulose was extracted from rice straw via alkali pretreatment using 10% sodium hydroxide under autoclaving condition for 20 min, and followed by the bleaching process with 2% sodium chlorite at 75°C for 2 h. The commercial cellulase (endoglucanase, EC 3.2.1.4) (13,000 IU/mL) and lipase (EC 3.1.1.3) (30,000 IU/g) were purchased from iKnowZyme (Thailand). *p*-nitrophenyl laurate (*p*-NP) laurate was supplied by Sigma-Aldrich (Switzerland) while olive oil was selected from a local supermarket in Chiang Mai, Thailand.

2.2 Granular nanocellulose production by enzymatic hydrolysis

The rice straw cellulose (0.13%, w/v) was hydrolyzed by using cellulase (107.06 U/mL) in sodium acetate buffer (0.05 M, pH 5) at 29.5ºC with a shaking rate of 120 rpm for 1 h [17]. The mixture was boiled in a water bath for 10 min and then centrifuged at 10,000 rpm for 15 min. The supernatant was collected for measuring reducing sugar and the precipitate dispersed in deionized water by sonication for 10 min. The suspension was screened through a 200 mesh sieve to remove the cellulose residue and the granular nanocellulose solution was collected for further characterization. The reducing sugar, cellulose residue, and nanocellulose content (%, w/w) were each determined.

2.3 Characterization

2.3.1 Lignocellulosic composition

The lignocellulosic composition of the rice straw cellulose was determined using the detergent fiber analysis method, a modified method based on [17]. The cellulose, hemicellulose, lignin and ash contents were determined.

2.3.2 Scanning electron microscopy (SEM)

The morphology of the rice straw cellulose was observed by scanning electron microscopy (Jeol JSM-6610LV) at an acceleration voltage of 15 kV. All samples were prepared on metallic stubs and then coated with gold under vacuum. In addition, a field-emission scanning electron microscope (FE-SEM) (Jeol JSM 6335 F) was used for characterizing the morphology of the nanocellulose product. A drop of nanocellulose suspension was deposited on a metallic stub and dried at 65ºC for 1 h after which it was coated with gold under vacuum at a 15 kV voltage.

2.3.3 Fourier transform infrared (FTIR) spectroscopic analysis

A FTIR spectrometer (Tensor 2, Bruker) was used to investigate the functional groups of the rice straw cellulose and nanocellulose. All samples were mixed with potassium bromide, compressed into pellet form and then added to the sample holder. Spectra were measured at 4 cm^{-1} resolution over the wavenumber range of 4,000 to 400 cm^{-1}.

2.3.4 X-ray diffraction (XRD) analysis

The crystallinities of the rice straw cellulose and nanocellulose were analyzed by an XRD (Rigaku MiniFlex II). The detection was in the step-scan mode ranging from 5º to 60º at a scan rate at 4º min^{-1}. The crystallinity of each sample was reported as the crystallinity index (*CrI*) calculated from the following formula [18].

$$CrI = (Ic\text{-}Ia)/Ic \times 100$$

where Ic is the peak intensity of the crystal plane at 22.7° and Ia is the peak intensity of the amorphous plane at 18.0°.

2.3.5 Dynamic light scattering (DLS) and zeta potential (ZP) analysis

The particle size distribution and ZP of the nanocellulose suspension (0.01%, w/v) were determined by a Zetasizer (Malvern Nano particle analyser series). Measurements were performed at a reflective index of 1.47 and temperature at 25ºC.

2.4 Effect of granular nanocellulose on lipase activity.

The effects of the granular nanocellulose obtained from rice straw cellulose on lipase activity were investigated using *p*-NP laurate and olive oil as substrates according to Liu and Kong [19] and Stoytcheva et al. [20], respectively. A mixture of 0.02% (w/v) of nanocellulose suspension, olive oil /p-NP laurate, lipase solution (350 U/mL) in a ratio of 3.5:3:1 (v/v/v) was mixed and incubated in an incuba tor shaker at 37ºC with a shaking rate of 120 rpm for 30 min. For olive oil as the substrate, the reaction was stopped by adding 3 mL of 95% ethanol and titrated with 50 mM sodium hydroxide using a thymolphthalein as an indicator. The relative lipase activity (% of total activity) and free fatty acid (FFA) content (μM) were determined. For *p*-NP laurate as the substrate, the reaction was stopped by boiling for 10 min and the absorbance was measured at 400 nm. The relative lipase activity and p-nitrophenol content (mM) were calculated.

2.5 Statistical analysis

The experimental results were carried out in triplicate and their statistical significance was expressed in terms of average mean and standard deviations (SD). The IBM SPSS Statistics of Windows version 25.0 (IBM Corp, Armonk, NY, USA) was used to evaluate the statistical differences using One-way ANOVA at a p-value of less than 0.05 ($P<0.05$).

3. Results and discussion

3.1 Rice straw cellulose

The rice straw cellulose was extracted from rice straw by an alkali pretreatment and bleaching process. Figure 1A shows a photograph of the rice straw cellulose as a white, soft, fluffy material. The morphology of the rice straw cellulose was observed by SEM, as shown in Figure 1B. The results showed that the rice straw cellulose had a smooth fibril-like structure which, after removal of lignin and hemicellulose produced almost pure cellulose fibers. The increased surface area of the rice straw cellulose facilitated its enzyme hydrolysis [21,22]. The rice straw cellulose had a high cellulose content of 92.77±0.71% (w/w) together with slight amounts of hemicellulose (1.41±0.41%), lignin (1.41±0.41%), and ash (0.36±0.04%). Thus, this research has been able to produce a higher content of cellulose than from other sources. In comparison, De Aguiar et al. [10] extracted cellulose from sugarcane bagasse and sugarcane straw in about 81.00% (w/w) yield for use as a raw material for nanocellulose production. Liu et al. [23] prepared cellulose nanofibril from bleached softwood pulp with a cellulose content of 89.84 % (w/w), while Abraham et al. [24] produced cellulose nanofibril from banana, jute and pineapple leaf fibers containing 88.30-97.30% (w/w) cellulose. These studies demonstrate that the

extracted cellulose contents differ according to species, types, and sources of biomass [25,26]. Thus, this research has indicated that the rice straw-derived cellulose is a high potential cellulose source for nanocellulose preparation by enzymatic hydrolysis of cellulase.

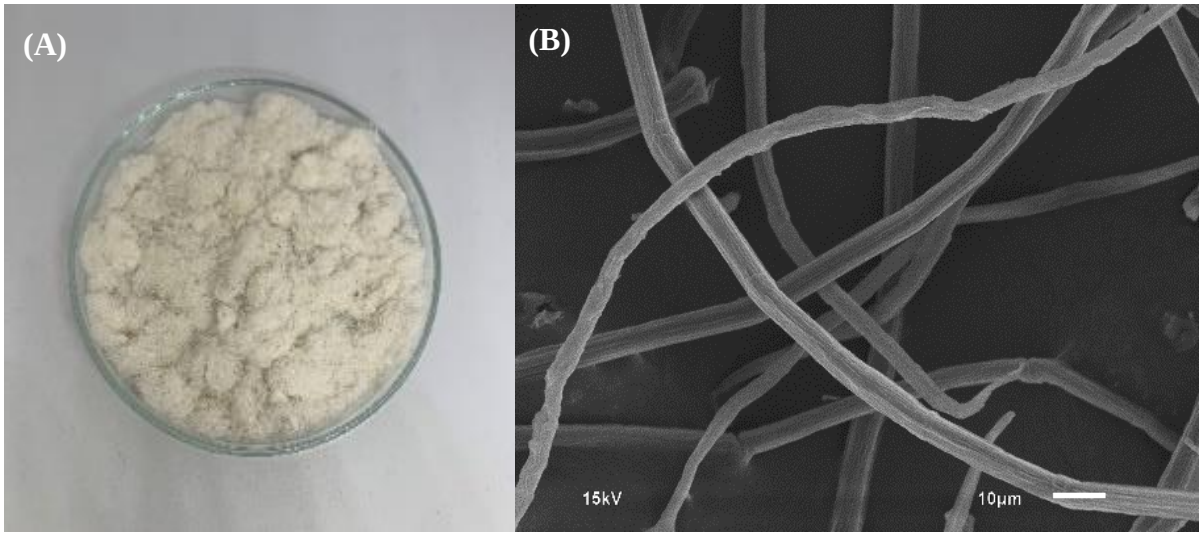

Figure 1 Appearance of (A) the rice straw cellulose and (B) the cellulose fiber from SEM (1000x).

3.2 Granular nanocellulose from rice straw

After enzymatic hydrolysis of the rice straw cellulose using cellulase 107.06 U/mL for 1 h, the nanocellulose suspension exhibited excellent dispersibility leading to a stable aqueous with no aggregation (Figure 2A). The SEM image (Figure 2B) of the obtained nanocellulose demonstrated a granular-shaped particle in accordance with previous studies. Chen et al. [2] produced nanocellulose from cotton pulp fiber by cellulase hydrolysis using 200 U/mL for 5 h. The results indicated that the spherical nanocellulose produced also contained irregular rod-like particles which may have been the intermediate state of the final spherical particles. The results also showed the incompleteness and low efficiency of the enzymatic reaction. Moreover, Chen et al. [2] pretreated cotton pulp by a swelling treatment and obtained ribbon-like nanocellulose by cellulase hydrolysis at a low concentration of cellulase (< 100 U/mL). Granular-shaped particles were obtained at 100 U/mL cellulase, while the nanocellulose was completely granular when using 300 U/mL. de Aguiar et al. [10] hydrolyzed sugarcane bagasse and straw by Cellic CTec3 cellulase hydrolysis for 24-96 h. The nanocellulose obtained exhibited rod-like shapes, while the enzymatic hydrolysis of sugarcane straw produced different morphologies at different reaction times. Consequently, it was concluded that the nanocellulose shape depended on the source of biomass and process methodology.

The contents of reducing sugar, cellulose residue, and granular nanocellulose are compared in Figure 2C. The results show that the granular nanocellulose content was 28.28±0.38%, while the cellulose residue remaining was 19.93±0.69%. Moreover, the reducing sugar liberated in the cellulose hydrolysate was about 51.79±0.46%. The high content of reducing sugar displayed the efficiency of the cellulase hydrolysis, amounting to approximately 80% of the initial rice straw cellulose. However, for nanocellulose production, reducing sugar is an undesirable product. The appearance of reducing sugar affected to decreasing the nanocellulose yield [27,28]. This research demonstrated that a high content of nanocellulose was obtained after 1 h hydrolysis time compared to that obtained from pulp fiber (20.17%), sugarcane bagasse (11.3%) and straw (12%) and wheat microcrystalline cellulose (22.57%) after 5, 96 and 120 h of hydrolysis [1,10,30]. However, the nanocellulose content varied with the extraction process and the duration of an enzymatic hydrolysis reaction. Moreover, the morphology of the nanocellulose can be varied by controlling certain reaction conditions. This present research has indicated the high efficiency of cellulase in rice straw cellulose hydrolysis for the production of granular nanocellulose.

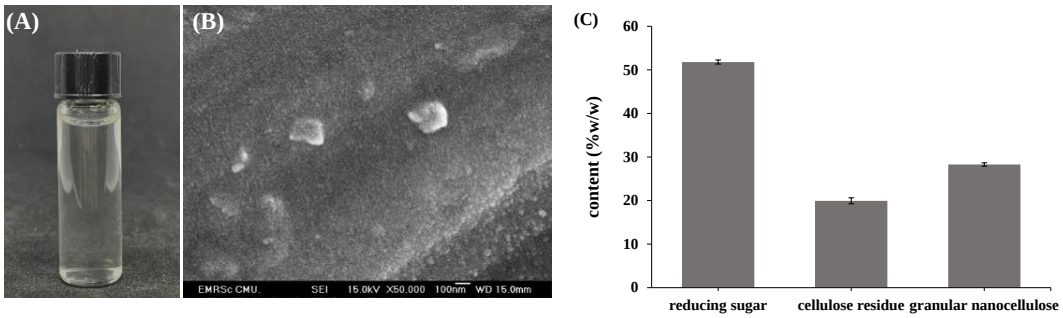

Figure 2 Rice straw nanocellulose (A) nanocellulose suspension (B) SEM image of granular nanocellulose (50,000x) and (C) reducing sugar, cellulose residue, and nanocellulose contents from enzymatic hydrolysis.

3.3 FTIR spectra

The functional groups present in the rice straw cellulose and granular nanocellulose chemical structures are shown in Figure 3, both FTIR spectra exhibiting similar functional groups. The O-H and C-H stretching vibrations appear at around 3400 and 2900 cm^{-1}. The peaks at around 1400 cm^{-1} are due to the asymmetric deformations of the C-H and C-OH groups, while the symmetric deformations of the C-H groups were located at around 1315-1380 cm^{-1}. The cellulose C-O and C-H stretching vibrations show peaks at around 1057 cm^{-1} and 831 cm^{-1}, with the O-H out-of-plane bending located at around 665 cm^{-1}. For the rice straw cellulose structure, the acetyl or ester groups of the hemicellulose or lignin structures and the axial asymmetric deformation of C-O-C, which is normally observed in ester groups, ether and phenol are absent at 1730 cm^{-1} and 1256 cm^{-1}, respectively. Similarly, the vibration of the C=C groups in the aromatic rings of lignin at 1517 cm^{-1} was not observed. These results indicate that the rice straw cellulose is of high purity, emphasizing its practical use as a potential source for nanocellulose production. In addition, the high-intensity peak at 1630 cm^{-1} can be assigned to water absorption in the rice straw nanocellulose. These peaks due to the aliphatic structure of nanocellulose were more clearly resolved than those found in cellulose.

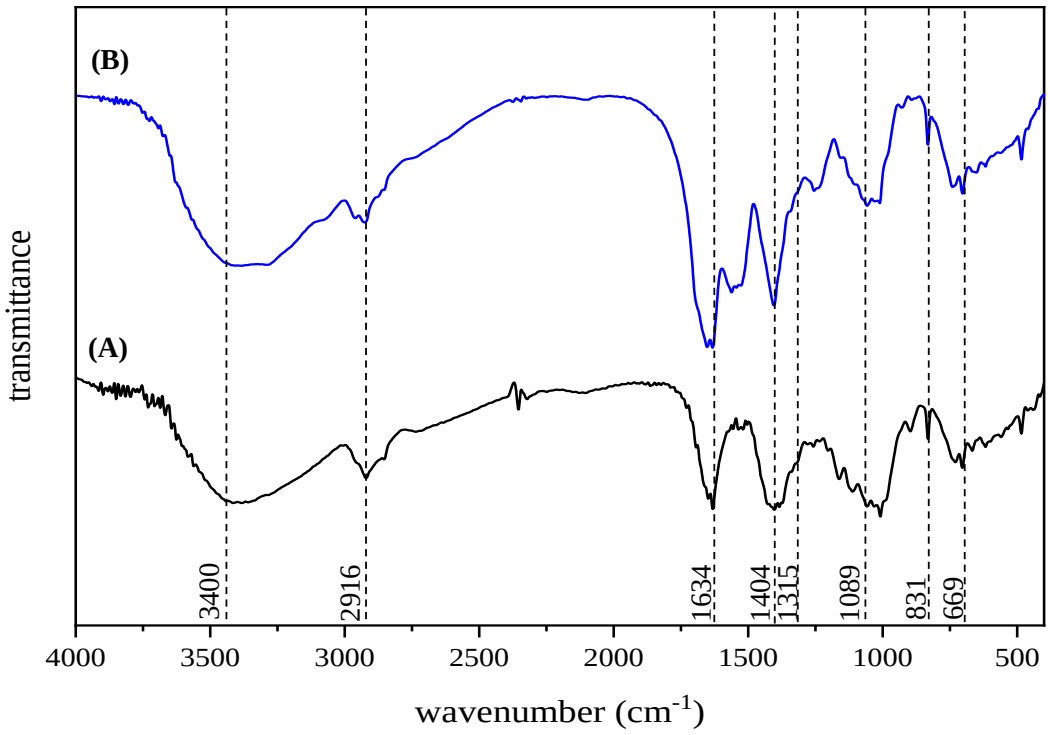

Figure 3 FTIR spectra of (A) rice straw cellulose and (B) granular nanocellulose.

3.4 Crystallinity of granular nanocellulose

The X-ray diffraction patterns of the rice straw cellulose and nanocellulose are shown in Figure 4. The peak intensity of the crystal plane at 22.7° and amorphous at 18.0° of rice straw cellulose revealed cellulose type I characteristics. Cellulose type I or native cellulose has an apparent abundance of crystalline structure in most of the higher plants, with the crystalline regions typically 20-50Å in diameter. Moreover, the crystallinity indices (*CrI*) of rice straw cellulose, granular nanocellulose and cellulose residue are estimated as 46.28, 32.13, and 27.57%, respectively. Generally, native cellulose is composed of both crystalline and amorphous regions. Its crystallinity can vary from 40 to 70% depending on the natural source as well as the extraction procedure [30]. At the same time, the *CrI* values of nanocellulose from enzymatic hydrolysis varied in the range of 45.0-92.3%, with the cellulose nanocrystal [31] depending on the production process.

The results showed that the *CrI* value of the granular nanocellulose was less than the initial rice straw cellulose due to the multicomplex cellulase hydrolyzing the cellulose structure randomly. As a result, a granular or spherical to elliptical shape was obtained, which is referred to as amorphous nanocellulose, one of the nanocellulose categories [30,32]. This is similar to the Zhang et al. [33] study, which reported that nanocellulose from bleach pulp had a *CrI* value of 48.85%, which was less than that of the original pulp of 64.42%. Decreasing the crystallinity of nanocellulose increases the accessibility of the hydroxyl groups in the nanocellulose, which is essential for the effective mixing of nanocellulose with other materials to obtain products with novel properties [34].

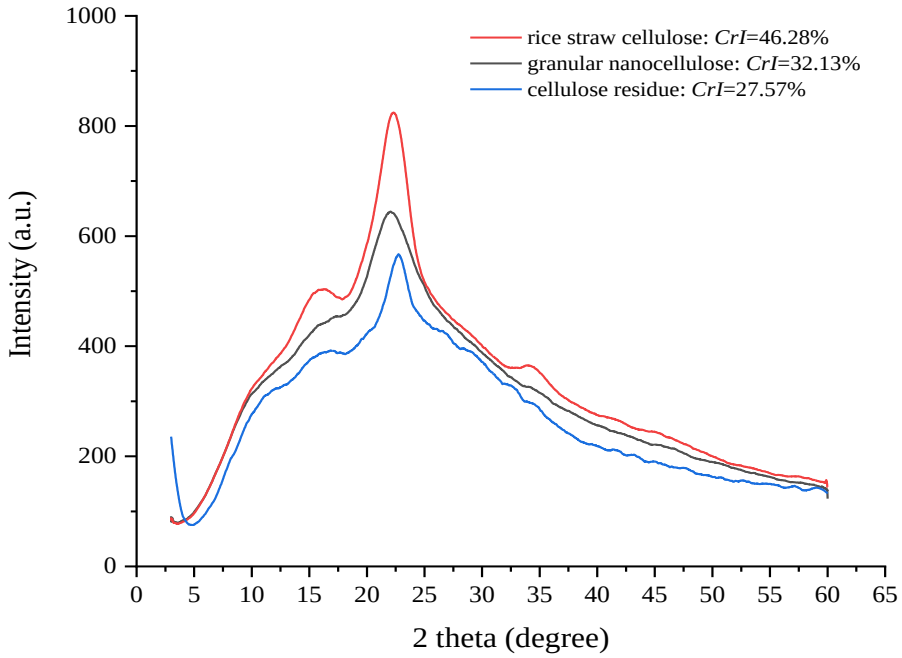

Figure 4 X-ray diffraction patterns and crystallinity indices of rice straw cellulose, granular nanocellulose and cellulose residue.

3.5 Particle size distribution and ZP

The DLS analysis is used to verify the particle size distribution and stability of the granular nanocellulose suspension. Figure 5. shows the particle size distribution in the region of 295-396 nm with 100% size inclusion. Ioelovich [35] prepared spherical-shaped amorphous nanocellulose using cellulolytic enzyme hydrolysis resulting in an average diameter of the nanocellulose in the range of 100-200 nm. According to the work of Zhang et al. [33], spherical-nanocellulose with a particle diameter of 60-570 nm was obtained from cellulose fiber hydrolysis. The high cellulase activity (300 U/mL) after 5 h of pulp fiber hydrolysis resulted in granular nanocellulose with a particle size of about 30 nm [2].

In addition, the granular nanocellulose suspension exhibited high stability with a ZP value of -45.8±0.4 mV. A ZP value of at least 30 mV with a positive or negative charge is suitable for a stable suspension and to prevent agglomeration of the nanocellulose particles. Therefore, this work has demonstrated a high ZP value compared to that of De Aguiar et al. [10] who reported a ZP values of CNC from sugarcane bagasse and straw of -25 mV indicating an unstable colloidal suspension.

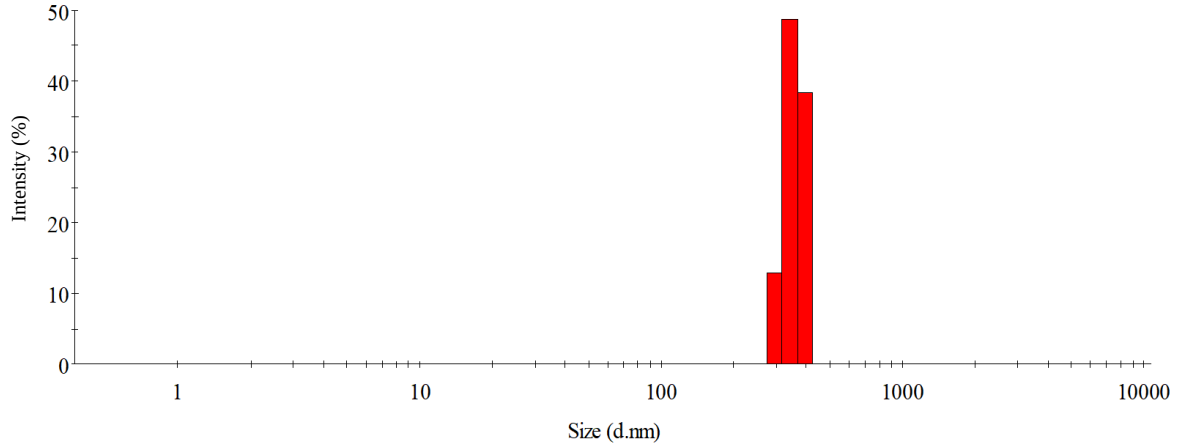

Figure 5 Particle size distribution of the granular nanocellulose suspension produced from rice straw cellulose by enzymatic hydrolysis.

3.6 Effect of granular nanocellulose on lipase activity

The effect of rice straw cellulose and granular nanocellulose on lipase activity was investigated using two different substrates, *p*-NP laurate and olive oil. The *p*-NP laurate refers to a small lipid molecule that releases *p*-NP and FFA [36]. For triacylglycerol hydrolysis, olive oil is used as the substrate and liberates glycerol and FFA products of the hydrolysate, which are detected by alkali titration [37]. Figure 6 shows that 0.02% of granular nanocellulose significantly reduced lipase activity with relative lipase activities of 90.51±1.39 and 74.32±0.96% of total activity when using *p*-NP laurate and olive oil as the substrates, respectively. The *p*-NP, hydrolysate product of *p*-NP laurate decreased from 1.37±0.02 to 1.26±0.05 mM while the FFA decreased from 550±8.66 to 408.75±5.30 µM. These results indicate that the granular nanocellulose has a significant effect on lipase activity similar to cellulose nanofibril [38,39]. The previous study reported that the hydrolysis of FFA from triglycerides in a high-fat food model was reduced by 48.4% when cellulose nanofibril was added at 0.75% (w/w) to the food. Moreover, nano-fibrillated cellulose showed a potential to retard bile acid absorption *in vivo* with a slight effect on cholesterol adsorption capacity [40]. When comparing cellulose's lipase activity, the p-nitrophenol and FFA contents were not significantly different from the control (lipase and substrate mixture). For granular nanocellulose, the effect on lipase activity was greater than cellulose which might be due to an increase its surface area for absorption of the lipase or its substrate (olive oil/*p*-NP laurate), resulting in the granular-amorphous enhanced sorption characteristics [41]. However, the potential metabolic effects of granular nanocellulose need to be investigated further and performed in the context of its potential applications with food or supplements.

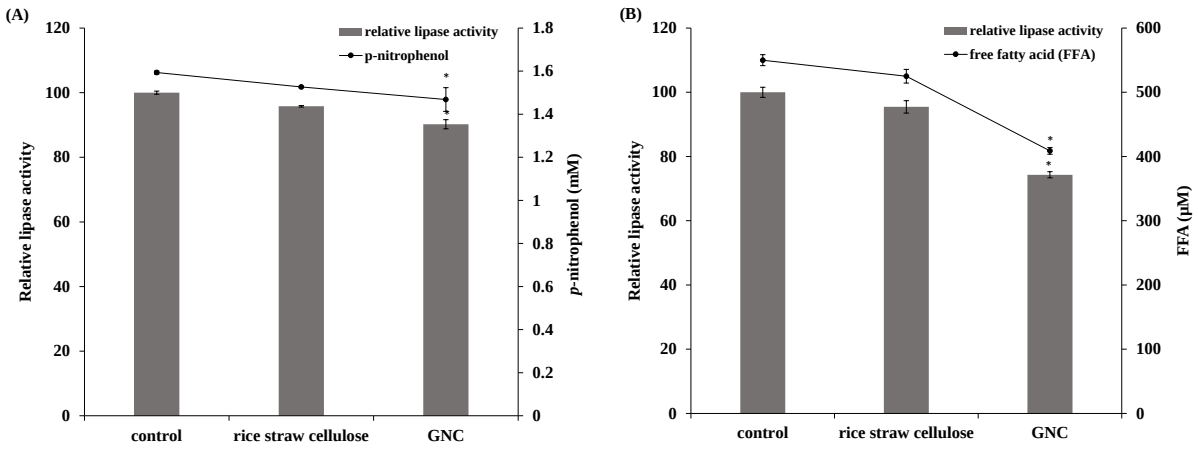

Figure 6. Effects of rice straw cellulose and granular nanocellulose (GNC) on lipase activity using (A) p-NP laurate and (B) olive oil as substrates

4. Conclusions

This work has demonstrated the effectiveness of enzymatic hydrolysis on rice straw-derived cellulose containing a high cellulose content of 92.77±0.71% (w/w) under an optimal set of conditions. The nanocellulose content obtained was about 28.28±0.38%. The characteristics of the nanocellulose were as a granular-shaped material with a homogenous size distribution, good stability suspension and reduced crystallinity which enhanced the availability and accessibility of the functional groups in the nanocellulose structure. Furthermore, the rice straw granular nanocellulose showed potential for the reduction of lipase activity. Therefore, this research has indicated that granular nanocellulose from rice straw has potential for applications in food and supplements for weight reduction and in the treatment of obesity. Moreover, the enzymatic route for nanocellulose production revealed a more environmentally friendly and sustainable alternative process for the utilization of value-added agricultural residues.

5. Acknowledgements

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6. Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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