

Biobased Polystyrene Foam-like Material from Crosslinked Cassava Starch and Nanocellulose from Sugarcane Bagasse

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This research aimed to study the effect of lignin, natural rubber latex (NRL), nanocellulose, and talc on production of biobased foam using cassava starch as matrix. Comparison study on lignin extraction from sugarcane bagasse (SCB) for different types of base (KOH and NaOH), concentration (10 %w/w and 40 %w/w), and temperatures (60 °C for 3 h and 120 °C for 1 h) was performed. The most suitable isolation condition giving the highest yield of lignin and lowest hemicellulose contamination was 40 %KOH at 120 °C for 1 h. A mechanical method was superior to a chemical method for cellulose size reduction owing to more appropriate size distribution and uniformity of nanocellulose. The most favorable proportion of foam contained 20% nanocellulose, 3% talc, 0.1% NRL, 38.5% water, and 76.9% crosslinked cassava starch. These conditions resulted in favorable flexural strength, modulus, and percentage of elongation, analogous to polystyrene foam. An appropriate amount of added lignin increased the elasticity of biofoam.

Keywords: Biobased foam; Nanocellulose; Lignin additive; Natural rubber latex; Thermal compression molding; Water drop test; Hydrophobic surface

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INTRODUCTION

Biobased foam from agro-industrial residues has gained much more attention to replace petroleum-based foam from polystyrene, as the latter requires more than 100 years to be degraded. Cassava starch, natural rubber latex (NRL), and sugarcane bagasse (SCB), which are abundant and can be easily found in Thailand, are potential renewable sources for biobased foam production. Starch can be used as a matrix of foam; however it exhibits higher water absorption in comparison to petroleum-based polymers. The crosslinking reaction between starch molecules and crosslinking agents was proposed to improve the water resistance of starch. Glyoxal was used as one of the crosslinking agents for starch, and it was reported to improve the internal structure of bio-foam and to yield a more homogeneous foam (Uslu and Polat 2012). The crosslinking reaction additionally resulted in increased strength, decreased density, and reduced water absorption of biofoam made of starch. Cellulose fibers isolated from lignocellulosic biomass were investigated to be used as reinforcement units. To improve the flexural strength of biofoam, cellulose nanofibers were added into the starch matrix; such preparation additionally enhanced the modulus of the materials (Floros *et al.* 2012; Narkchamnan and Sakdaronnarong 2013). In addition, poly(vinyl alcohol) matrix reinforced with nanocellulose resulted in increased tensile modulus and tensile strength of the composite (Floros *et al.* 2012). Interestingly, from a

previous study in the authors' group, a lignin by-product from cellulose isolation significantly improved the water resistance of starch composite, as indicated by an increase of contact angle on the surface of foam by water drop test analysis (Narkchamnan and Sakdaronnarong 2013). Attempts have been made to overcome the brittleness and improve the porosity of bio-based starch composites. NRL was found to increase the flexibility of rigid foam (Teixeira *et al.* 2011). Higher NRL content in biofoam decreased the modulus and stress to breakage but increased strain to breakage of biofoam (Shey *et al.* 2006). Additionally, the effect of nucleating agents such as talc on improving regular porosity was studied. Composite material produced from wood fiber, talc, and poly(hydroxybutyrate-co-valerate) by extrusion had good pore distribution with increased talc content (Singh *et al.* 2010).

To the best of our knowledge, the present work was the first time that a crosslinking technique using glutaraldehyde and thermal compression molding technique has been applied to produce bio-based polystyrene foam-like material. An experimental design by the Box-Behnken method was performed in order to screen the crucial factors and determine the most favorable condition to produce biofoam having similar physical and mechanical properties to polystyrene foam. A study of the influence of nanocellulose, talc, and NRL on biobased foam formation was carried out. Effects of lignin addition on the properties of biobased foam were also tested.

EXPERIMENTAL

Materials

Cellulose fibers and lignin were isolated from SCB provided by Kornburi Sugar factory, Nakornratchasima, Thailand. Cassava starch was purchased from Pla Mungkorn Co., Ltd., Thailand. NRL (50% solids) was supplied from Srijaroen Latex, Co., Ltd. in Nakornsritthammarat province, Thailand. Black liquor from soda pulping process was contributed from Environment Pulp and Paper, Co., Ltd., Nakornsawan, Thailand. Accellerase® enzyme (Genencor, USA) was supplied from Siam Victory Chemicals, Co., Ltd. Glutaraldehyde, talc (Prod. No. 18654, Sigma-Aldrich with the particle size between 10 to 20 μm), dioxane, standard lignin (alkali lignin, low sulfonate), and microcrystalline cellulose and 3,5-dinitrosalicylic acid (DNS) were from Sigma-Aldrich. Other chemicals *e.g.* sulfuric acid, hydrochloric acid, acetic acid, sodium hydroxide, potassium hydroxide, and sodium acetate, were purchased from Ajax.

Lignin Extraction and Nanocellulose from Sugarcane Bagasse (SCB)

SCB was milled by centrifugal mill, sieved to 20-40 mesh, and then dried at 50 °C until constant weight before use. For lignin extraction, 30 g SCB was soaked in 300 mL of alkaline solution by varying types and concentrations of base (10% NaOH, 10% KOH, 40% NaOH and 40% KOH) as well as temperature and time at 120 °C for 1 h and 60 °C for 3 h. Subsequently, solid and liquid parts were separated. To remove the solid lignin from the liquid part, lignin was precipitated by adding concentrated sulfuric acid until pH 1, and then the whole solution was centrifuged at 5000 rpm for 5 min. The supernatant solution was rich in hemicelluloses. Wet lignin slurry was dried at 50 °C under vacuum condition until constant weight and ground to a fine powder. Solid lignin was analyzed for hemicellulose contamination and by spectroscopic analysis.

Solid residue from the lignin extraction, containing mainly cellulose, was washed with distilled water, and then the pH of pulp was adjusted to the range 4 to 5 by acetic acid. For the bleaching step, the cellulose pulp was soaked in 11% w/w hydrogen peroxide and stirred using magnetic stirrer for 24 h. Bleached fibers were then soaked in 10% w/w hydrochloric acid in an ultrasonicator at 60 °C for 2 h prior to size reduction step. Two size reduction methods used in this work were by 1) mechanical and 2) chemical means. For the mechanical size reduction method, bleached fibers were adjusted to neutral pH by adding distilled water (1: 60 of pulp dry weight: water ratio) then high shear homogenization (Heidolph, DIAx 900, Germany) was performed at 20,000 rpm for 15 min. For chemical size reduction method, bleached fibers were soaked in 6 M sulfuric acid at 45 °C for 30 min then washed with distilled water until neutral pH. Never-dried nanocellulose was determined for the moisture content and used for biofoam reinforcement. Vacuum-dried nanocellulose at 50 °C was used for analyses.

Biobased Foam Production

100 g native cassava starch was added into 0.4% glutaraldehyde solution and stirred using a magnetic stirrer for 24 h at room temperature. Crosslinked starch was washed three times using distilled water and then dried at 50 °C before grinding to a fine powder. The effects of cellulose fibers, talc, NRL, and water proportions were studied for biobased foam formation. 18 g dry weight of mixture was filled into a stainless steel mold having dimensions of 6.25 cm × 7.5 cm × 0.2 mm. Thermal compression molding was performed at 200 °C for 3 min. Response surface methodology using Box-Behnken method was performed to investigate the proper foam formulation to produce PS-like material. Experimental data and quadratic models of all responses were statistically analyzed by ANOVA (Design Expert version 9, www.statease.com). The suitable condition that gave the foam's mechanical and physical properties analogous to polystyrene foam was selected for the study of influence of lignin isolated from sugarcane bagasse on foam's properties.

Analytical Methods

The yield of lignin and cellulose was determined from the dry weight of isolated material based on the initial dry weight of SCB. Hemicellulose contamination was determined by hemicellulase hydrolysis in 15 mL of hydrolysate containing 2 mL of black liquor, 10 mL of sodium acetate buffer (pH 4.8), and 3 mL of Accellerase® enzyme. The hydrolysis took place at 50 °C for 48 h. Reducing sugar was measured by DNS assay (Miller 1959). Hemicellulose contamination onto precipitated lignin expressed as reducing sugar based on dry weight of lignin as shown in Eq. 1.

$$\text{Hemicellulose contamination (mg sugar mg lignin}^{-1}\text{)} = \frac{\text{Reducing sugar (mg mL}^{-1}\text{)} \times 15 \text{ mL hydrolysate}}{\text{lignin (g dry weight)}} \quad (1)$$

UV-Vis spectrophotometry (T60U, PG instrument, China) of isolated lignin solubilized in dioxane-water solution (lignin: dioxane ratio of 1: 9) was measured between 200 and 600 nm wavelength. Functional groups of lignin were analyzed by FTIR spectroscopy (Spectrum 2000, Perkin ELMER, USA (at 4000 to 400 cm⁻¹ wave number at 4 cm⁻¹ resolution and 128 numbers of scan for triplicates) when lignin pellet was prepared at a lignin:KBr ratio of 1:99 by weight. An X-ray diffractometer (XRD) (Bruker, D8-Discover, Germany) was used for crystallinity analysis of nanocellulose. Crystallinity

index (CrI) was calculated from the intensity of the 200 peak (I_{200} , $2\theta = 22.6^\circ$) and the minimum intensity between the peaks at 200 and 110 (I_{am} , $2\theta = 18.7^\circ$) by using the empirical equation, $CrI = (I_{200}-I_{am})/I_{200} \times 100$ (Segal *et al.* 1959). Transmission electron microscope (TEM) analysis (JEM-1230, JEOL, Japan (for mechanical and chemical size reduction nanocellulose was conducted.

Mechanical properties measurements of biobased foam, namely flexural strength, modulus, and elongation, were carried out with a Universal Testing Machine (Instron 4467, USA) using a three-point bending method at 0.5 kN loading cell, 4 mm min⁻¹ crosshead speed, and 40 mm span. Foam density was calculated from the weight of dried biofoam (50 °C under vacuum for 24 h) divided by the volume of foam having a dimension of 7.5 cm × 7.5 cm × 2 mm. Light transmission digital microscopy was performed for analysis of cell distribution of biofoam at $\times 5$ magnification. The morphology of the cellulose fibers was examined using a scanning electron microscope (SEM) (Hitachi S4800, Japan). The specimens were coated with a thin platinum layer for 15 min in a vacuum in order to improve the conductivity and prevent electron charging. Images were taken at 5kV. Water resistance analysis was determined by drop shape instrument (FTA1000, Brand, Canada). A dynamic change of contact angle between biofoam surface and water drop was calculated between 1 sec and 60 sec by image analyzer equipped with water drop test apparatus.

RESULTS AND DISCUSSION

Lignin Extraction from Sugarcane Bagasse

Sugarcane bagasse was extracted by alkaline solution for lignin, and the results showed that when the temperature and base solution were varied, the yield of lignin was different, as shown in Fig. 1(A). Results of the analysis showed similar levels of lignin yields (2.6% w/v) from all extraction conditions from 10% NaOH, 40% NaOH, and 40% KOH at 120 °C for 1 h, as well as 40% NaOH at 60 °C for 3 h. The highest yield of lignin at 6.3% w/v was isolated from black liquor from the pulp and paper factory. Higher lignin yield from the factory was obtained compared with lignin yield isolated from SCB because higher temperature and pressure were applied in the soda pulping process (14% NaOH at 170 °C) (Jiménez *et al.* 1999). It was reported that an increase of lignocellulose treatment severity, *e.g.* temperature and catalyst concentration, enhanced lignin yield (Fernández-Bolaños *et al.* 1999). However, hemicelluloses contamination within lignin extract seems to be enhanced when increasing alkaline concentration and temperature.

Hemicellulose has amorphous structure, and the presence of hemicellulose has been found to increase the moisture absorption of biofilm (Bahcegul *et al.* 2012). To select a suitable condition for lignin extraction, not only high lignin yield but also the lowest hemicellulose contamination were considered. As shown in Fig. 1(B), lignin extracted by 40% KOH at 120 °C for 1 h had less hemicellulose contamination (133.4 mg sugar/g lignin) than that extracted by 40% NaOH at 120 °C for 1 h (182.6 mg sugar g lignin⁻¹). The highest hemicellulose contamination was obtained from lignin in black liquor from pulp and paper factory (205.4 mg sugar g lignin⁻¹). Therefore, 40% KOH at 120 °C for 1 h was the most suitable condition for lignin extraction because of higher yield of lignin and least hemicellulose contamination. The result was in accordance with the study of hemicellulose contamination in extracted lignin by Bahcegul and colleagues (2012), who evaluated the effect of alkaline pretreatment temperature on a multi-product basis for the co-production

of glucose and hemicellulose based films from lignocellulosic biomass. They reported that 9.8% hemicellulose recovery from 10% KOH was obtained from lignin extraction at 25 °C and 18.2% hemicellulose recovery was obtained from lignin extraction at 90 °C. Thus, higher temperature for lignin extraction provided higher hemicellulose released from lignocellulosic material.

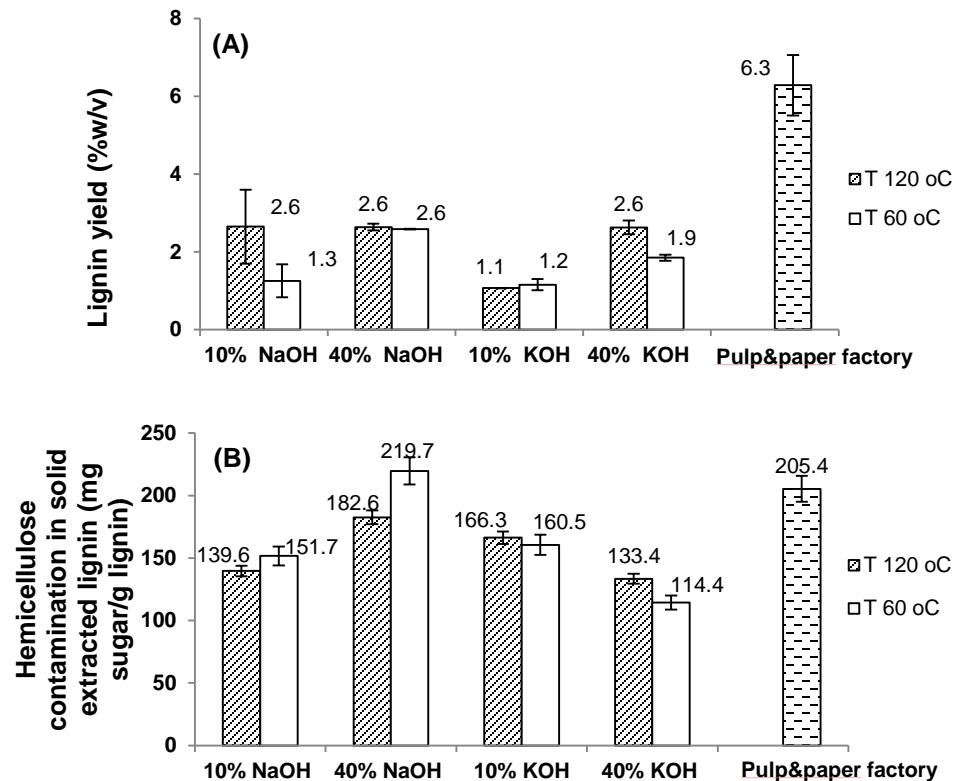


Fig. 1. (A) Yield of isolated lignin at different temperatures, base types, and concentrations, and (B) hemicellulose contamination in solid extracted lignin

As illustrated in Fig. 2(A), analysis of lignin by UV spectrophotometer showed a typical peak at a wavelength of 280 nm, which is attributable to non-conjugated phenolic units, and a peak at wavelength of 315 nm can be assigned to conjugated phenolic units (Maziero *et al.* 2012). Figure 2(A) shows that lignin extracted using 10% KOH at 120 °C for 1 h gave the maximum absorbance at a wavelength of 280 nm and 315 nm. The second highest spectrum at 280 and 350 nm was from lignin extracted with 40% KOH at 120 °C for 1 h. Moreover, the absorbance ratio of A₂₈₀/A₃₁₅ was higher for lignin extracted from KOH compared to NaOH extracted lignin. This result indicated that lignin extraction with KOH led to higher yield of unconjugated lignin than conjugated lignin; thus KOH extraction yielding small molecules of lignin was more suitable for use as an additive in biocomposite formation, in accordance with the results from a previous work (Sun *et al.* 2004). As illustrated in Fig. 2(B), FTIR spectroscopy of isolated lignin typically shows a peak at 1500 cm⁻¹ (I_{1500}) assigned to aromatic lignin. Peak intensity at 1734 cm⁻¹ (I_{1734}) is assigned to hemicellulose, peaks at 1373 and 1161 cm⁻¹ (I_{1373} and I_{1161}) are polysaccharides, and peak intensity at 900 cm⁻¹ (I_{900}) is attributed to cellulose (Krongtaew *et al.* 2012). The FTIR spectrum indicated that the more purified lignin gave a lower percentage of transmittance or higher absorbance at the peak near 1500 cm⁻¹ (Zeng *et al.* 2012). The FTIR spectrum of lignin from pulp and paper factory was similar to the FTIR spectrum of

extracted lignin from 40% KOH (120 °C, 1 h) as well as that of standard lignin. Nevertheless, the intensity near polysaccharide absorption was different among all extracted lignin specimens; thus the ratio of lignin to carbohydrate transmittance intensity was more appropriately considered to indicate hemicellulose contamination in lignin molecules, as demonstrated in Table 1.

Table 1. Relative Intensity Ratios of FTIR Spectroscopy of Hemicellulase Enzyme Treated and Untreated Lignin

Enzymatic treatment	Sample	Relative intensity ratio			
		I_{1510}/I_{1734}	I_{1510}/I_{1373}	I_{1510}/I_{1161}	I_{1510}/I_{898}
Untreated	40% NaOH	0.95	1.07	1.31	0.95
	40% KOH	0.94	1.13	1.36	1.02
	Pulp & paper factory	0.96	1.21	1.54	1.06
Treated	40% NaOH	0.85	1.01	1.12	0.90
	40% KOH	0.89	1.10	1.28	0.91
	Pulp & paper factory	0.87	1.09	1.26	0.95
-	Standard lignin	0.87	1.02	1.08	0.87

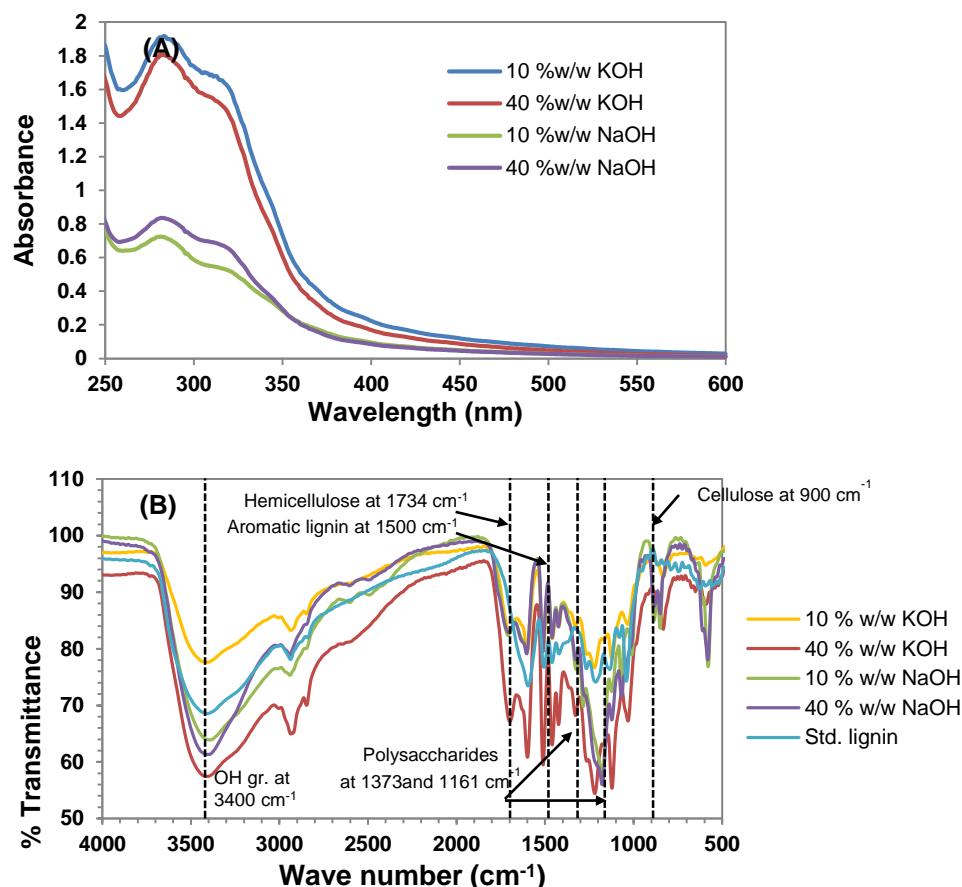


Fig. 2. (A) Lignin extracted at 120 °C analyzed by UV-vis spectrophotometer, (B) Lignin extracted at 120 °C after treated by hemicellulase enzyme analyzed by FTIR spectroscopy

In transmittance mode, less intensity of lignin-to-carbohydrate ratio represents higher lignin content compared with carbohydrate content, thus representing more purified lignin. From the results, the intensity ratio of extracted lignin after hemicellulase enzyme treatment was less than untreated lignin, showing that enzymatic treated lignin was more purified than untreated lignin. The average intensity of extracted lignin from 40% KOH at 120 °C for 1 h had the same value as lignin from pulp and paper factory. Due to similar FTIR spectra and relative intensity ratios of lignin-to-carbohydrate components, both extracted lignin from SCB (40% KOH at 120 °C for 1 h) and from the pulp and paper factory were able to be used as an additive for biofoam production.

Cellulose Isolation and Size Reduction

Delignified SCB from 40% KOH at 120 °C for 1 h and subsequent acid hydrolysis/H₂O₂ bleaching yielded 44.8% cellulose. A lower yield of cellulose (37.9% yield of cellulose) was achieved from 40% NaOH at 120 °C for 1 h and subsequent acid hydrolysis/H₂O₂ bleaching. The results were in good agreement with those of Sun and coworkers, who reported that lignin yield from KOH extraction was higher than that from NaOH extraction (Sun *et al.* 2004). It was suggested that KOH was preferential for hemicellulose removal from sugarcane bagasse, and more than 90% of the original hemicelluloses were released. This significant solubility of hemicelluloses was probably due to the hemicelluloses being present to a greater degree on outer fiber surfaces, from where they could dissolve easily in the alkaline solution. By contrast, cellulose is more located in the inner parts of the fibres and therefore is not easily dissolved. In the present study, the findings suggested that the most appropriate extraction condition yielding highest lignin and cellulose yields was at 40% KOH at 120 °C for 1 h.

After the bleaching step, isolated cellulose from SCB was subjected to size reduction. Nanocellulose was added in biofoam to increase its mechanical properties. TEM analysis illustrated morphology and size distribution of nanocellulose fibers for mechanical (Fig. 3(A) and 3(B)) and chemical methods (Fig. 3(C) and 3(D)).

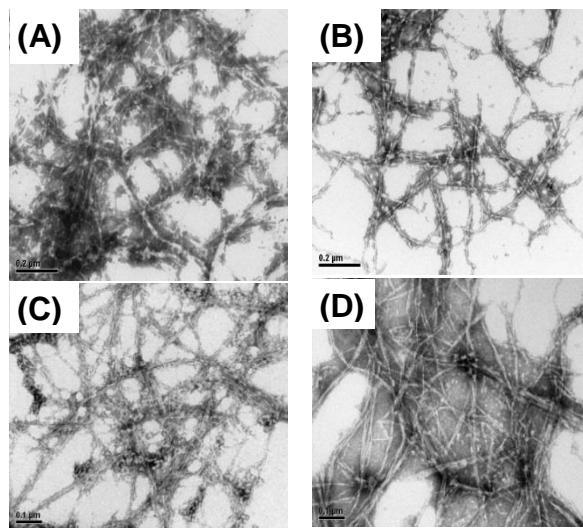


Fig. 3. TEM images for reduce size of cellulose by mechanical method ($\times 80,000$ magnification) (A) cellulose from 40% NaOH at 120 °C extraction for 1 h, and (B) cellulose from 40% KOH at 120 °C extraction for 1 h. For reducing size of cellulose by chemical method ($\times 100,000$ magnification), (C) cellulose from 40% NaOH at 120 °C extraction for 1 h, and (D) cellulose from 40% KOH at 120 °C extraction for 1 h

Both size reduction methods decreased the diameter of cellulose fibers from micro-scale (data not shown) to nano-scale with the same average diameter ranging from 10 to 20 nm. The average length of the nanocellulose ranged between 200 and 800 nm, which is the same length (100 to 500 nm) as cellulose whiskers isolated from sugarcane bagasse in previous work (Teixeira *et al.* 2011). In the present study, mechanical method was selected to produce nanocellulose due to superior shape distribution, simplicity of handling, and less amount of effluent compared with the chemical method.

An XRD diffractogram of isolated nanocellulose indicating crystallinity of cellulose apparently showed a mixture of polymorphs of cellulose (typical peaks near $2\theta = 22.6^\circ$) (Sun *et al.* 2004) and amorphous moieties near $2\theta = 16.8^\circ$, as depicted in Fig. 4. Nanocellulose isolated from 40 %KOH at 120 °C for 1 h using the chemical method for size reduction exhibited a higher typical peak intensity of cellulose ($2\theta = 22.6^\circ$) than that using the mechanical method, as shown in Fig. 4(A) and 4(B). Higher crystallinity of cellulose was obtained from the chemical size reduction method (69.3 % CrI) relative to the mechanical size reduction method (66.5 % CrI). Accordingly, analysis of TEM and XRD results for nanocellulose from different reducing size methods showed that mechanical method was more suitable than chemical method to use as reinforcement units in biofoam.

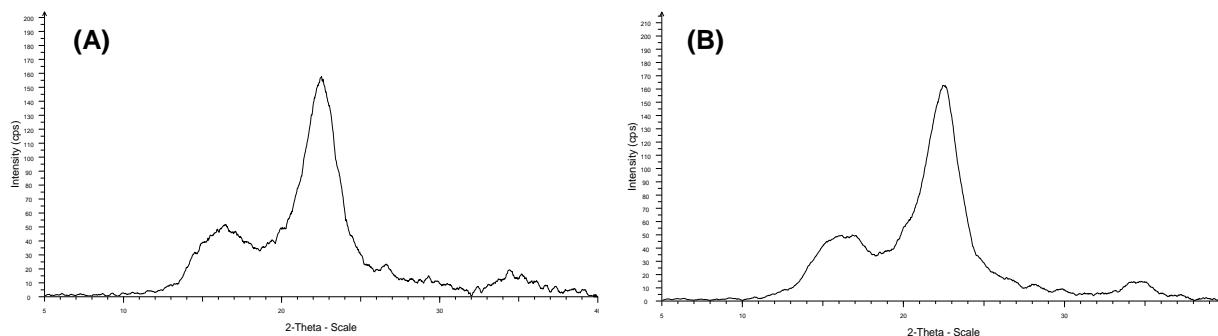


Fig. 4. X-ray diffractogram of 40 %KOH which reduce size of cellulose by (A) mechanical method, and (B) chemical method

Production of Biobased Foam

It has been widely known that biobased foam from starch has disadvantages in terms of high stiffness, high brittleness, low elasticity, and high water absorption. Many researchers have investigated this issue and found that addition of petroleum-based plastics *e.g.* polypropylene (Bagheri 2009), polystyrene (Pushpadass *et al.* 2010), polyvinyl-chloride (Viña *et al.* 2007), as well as plasticizers, *e.g.* glycerol and sorbital (Das *et al.* 2011), is able to overcome the aforementioned problems. In the present work, addition of NRL into biobased foam made of starch matrix was hypothetically applicable, as it improved the water resistance as well as elasticity of composite, although it could diminish the biodegradability of the composite (Boutheoud *et al.* 2011).

Effect of NRL on biofoam properties

As shown in Table 2, the ingredients for production of biobased starch foam included nanocellulose, talc, NRL, and distilled water. The dry weight of the mixture was calculated based on cassava starch (dry weight). The responses of varying biofoam compositions were demonstrated as mechanical and physical properties of biofoam consisting of flexural strength, elongation, modulus, and density. From the results, NRL gave increasing elongation and flexural strength of biobased foam, as demonstrated in sample no. 20 and sample no. 23. Addition of 10% NRL increased the percentage of elongation up to 5.93%, compared with 3.1% elongation when adding 0.1% NRL. An increase of 91.3% elongation was achieved when adding 10% NRL into biofoam containing 15% nanocellulose, 5% talc, 38.5% distilled water, and 70% crosslinked cassava starch (dry weight). The solids in natural rubber latex are comprised mainly of *cis*-1,4-polyisoprene (Shey *et al.* 2006). When this latex cures or dries during thermal compression molding, an elastic and hydrophobic material is produced. However, before the latex is cured, it is in an aqueous emulsion, which allows the rubber to be evenly distributed throughout the batter in the production of thermal compressed starch foams. Table 2 also shows that the flexural strength was slightly decreased, and a 43.5% decrease of modulus was obtained when adding 10% NRL compared with 0.1% NRL. The results agreed with those of Shey and coworkers (2006), who reported that addition of NRL increased strain by approximately 60% compared with biofoam without rubber latex. Therefore, enhancement of NRL content increased elasticity of biofoam caused by polyisoprene in NRL. In other words, at low content of NRL (sample no. 20) the higher cassava starch loading was used compared with the sample no. 23, and thus more crystalline regions were introduced compared to the control biofoam. This occurs as a consequence of the retrogradation process of starch, which realigns the amylose and amylopectin chains (Mohamed *et al.* 2008). It has been reported that as the crystalline region is increased, the NRL composite becomes vulnerable to the formation of cracks and failure upon stress application. At higher starch loading, the formation of bigger sizes and numbers of crystal regions contribute to a composite's failure during mechanical testing. The formation of starch crystalline regions also decreased NRL composite's ability to elongate further (Afiq and Azura 2013). Nevertheless, from the ANOVA of flexural strength and elongation in Table 3, it was found that talc content also gave significant effect on these two responses. An increase of NRL content gave significant effect on flexural strength and elongation enhancement ($P<0.05$). However, an increase of talc content as well as interaction between talc and NRL contents additionally augmented flexural strength and elongation. Therefore, at 3% talc content (sample no. 2 and no. 5) which had low content of talc compared with sample no. 20 and 23 (5% talc), an increase of NRL content seemed to give insignificant effect on flexural strength and elongation. An increase of NRL proportion in the biofoam ingredients made the density of biofoam increase, which was in accordance with a previous work (Shey *et al.* 2006). This could possibly be improved by addition of talc, a foam nucleating agent.

Effect of nanocellulose on biofoam properties

Addition of nanocellulose into biofoam significantly enhanced flexural strength and modulus because cellulose has a parallel chain orientation and crystalline structure; however the percentage of elongation was decreased when nanocellulose was added, as demonstrated in samples no. 29 and sample no. 6 (Table 2). An increase of nanocellulose content from 10% to 20% substantially enhanced flexural strength and modulus of

biobased foam from 1.4 and 74.4 MPa to 14.4 and 533.5 MPa, respectively. These results corresponded to 956.6% and 616.9% increases of flexural strength and modulus, respectively. This can be explained by strong interaction in the interface region between nanocellulose, crosslinked starch, and NRL. It was reported that the increase of crosslink density of rubber restricts the mobility of rubber chains in the presence of CNs (Cao *et al.* 2013). The results agreed with those of Floros and colleagues (2012), who studied the addition of cellulose into a polyurethane (PU) matrix (Floros *et al.* 2012). An increase of nanocellulose fiber added to PU caused increase of flexural strength and modulus while the %elongation was reduced. However, in the present study when nanocellulose was increased from 10% to 20%, percentage of elongation was insignificantly decreased from 2.8% to 2.6%, respectively. The results suggested that inappropriate nanocellulose distribution throughout biofoam ingredients can cause the failure during the elongation test, and therefore the orientation of nanocellulose is required to be arranged in parallel to improve the biocomposite's mechanical properties, as designed. It is important to note that the mechanical disruption of bacterial cellulose (BC) and the development of an reconstituted BC biofilm produce better fibre association and orientation of the fibrils, which can promote higher thermal stability (de Souza *et al.* 2013). Nanocellulose provided a higher stiffness and induces brittleness of the material. Therefore, the dispersion of the filler is expected to be lower, and long entangled nanocellulose and its higher hydrophobic character are required, resulting in a higher level of adhesion with the NRL and starch matrix. Moreover, the biofoam density from the present study was slightly increased from 0.61 to 0.82 g cm⁻³ when nanocellulose content in batter was increased from 10% to 20%. This was in good agreement with the work of Glenn and coworkers (2001), who found that addition of softwood pulp fiber to the formulation of the baked starch foams improved flexural properties and lowered the density (Glenn *et al.* 2001). The foam density could be presumably reduced by adding nucleating or blowing agents. Nevertheless, from ANOVA of flexural strength and modulus (Table 3), it was not the only effect of addition of nanocellulose content that enhanced the flexural strength and modulus. In addition, interaction between nanocellulose and NRL contents as well as nanocellulose and talc contents gave considerable influence on flexural strength and modulus. Solely the influence of an increase of NRL content significantly enhanced flexural strength and modulus, however. The interaction effect of NRL and nanocellulose showed that an increase of NRL content reduced flexural strength and modulus of biofoam. Thus, if comparing the pair of sample no. 5 and 28, which contained high NRL content at 10%, the flexural strength and modulus of biofoam were decreased, although the nanocellulose content was increased.

Effect of talc on biofoam properties

To improve the cellular size distribution of biofoam, several studies have been conducted of the effects of adding salts *e.g.* NaCl and CaCl₂ (Zhou *et al.* 2006), nucleation agent *e.g.* talc (Lee *et al.* 2009; Singh *et al.* 2010; Zhang and Sun 2007), as well as blowing agent *e.g.* CaCO₃ (Glenn *et al.* 2001) into the foam's batter. Solid, insoluble particles (*e.g.* talc particles) are often dispersed in the polymer matrix to provide heterogeneous nucleation sites and control the number density and/or size distribution of bubbles in the final foam (McClurg 2004). In the present work, talc was selected as an additive for the biofoam formulation, as it is simple way to improve cellular size distribution of biofoam. As demonstrated in Table 2, adding talc into biofoam gave increasing flexural strength but decreasing percentage of elongation, as shown for sample no. 22 and sample no. 9. An

increase of talc content from 1% to 5% considerably increased flexural strength from 2.1 MPa up to 5.5 MPa, which was a 158% increase of flexural strength. Similarly, the modulus of biofoam was increased from 278.2 MPa to 323.3 MPa when talc content was increased from 1% to 5%. In accordance, the results showed that increasing talc content from 1% to 5% caused an insignificant decrease of percentage of elongation from 2.0% to 1.7%. The results agreed with those of Singh and coworkers (2010), who studied on improvement of biocomposite properties containing cellulose fiber, talc, and polyhydroxybutyrate-co-valerate (PHBV). This previous work reported that 20% talc and 20% cellulose fiber resulted in a doubling of the elongation and increased flexural strength up to 22%. Thus, the interaction effect of talc and nanocellulose significantly influenced elasticity and strength of biofoam. With the presence of 5 wt% of untreated talc particles, the onset time of cell nucleation was earlier (data not shown), and the foam density increased significantly as shown in sample no. 33 compared to sample no. 9 in Table 2. Nonetheless, additional theoretical studies on the effect of viscosity and elasticity of the polymer–nucleating agent mixture on mechanical properties of biofoam are needed.

Surface appearance and light transmission images of biobased foam from sample no. 25 are shown in Figs. 5(A) and 5(B), respectively. Light transmission imaging of polystyrene (PS) foam is additionally illustrated in Fig. 5(C). It was observed that cell distribution of PS foam was more uniform and homogeneous in the whole specimen analyzed compared with those observed in biobased foam. However, the mechanical properties of the biofoam sample having flexural strength of 0.4 MPa, percentage of elongation of 3.1%, modulus of 43.5 MPa, and density of 0.44 g cm^{-3} were similar to those of polystyrene foam having flexural strength of 0.5 MPa, elongation of 3.2%, modulus of 21.2 MPa, and density of 0.03 g cm^{-3} (Table 2). As illustrated in Fig. 5, denser foam made of cassava starch/NRL/nanocellulose/talc was obtained (Fig. 5(A) and 5(B)) and looser foam made of polystyrene (PS) was shown in Fig. 5(C). In this context, denser foams tend to have thicker cell walls or higher solid fraction and hence are able to resist deformation better than lower density foams with thinner cell walls or lower solid fraction. Addition of nucleation agent (*e.g.* talc powder) in the present study increased foam densities; thus the significant increases of the compressive strength, elastic modulus, and elastic deformation energy were obtained. Hence, nucleation agents are suitable to refine the foam cell structures to produce more rigid foams having high load-bearing capacity and yet able to deform at higher stress levels to absorb impact energy and hence suitable for protecting heavy goods (Zhou *et al.* 2006). Consequently, biofoam made of cassava starch/NRL/nanocellulose/talc having thicker cellular wall (Fig. 5(B)) provided higher resistance of impact energy and thus it was more suitable for packaging applications relative to polystyrene (PS) foam having thinner cellular wall (Fig. 5(C)). Figures 5D to 5G additionally show SEM images of biofoam surface and cross-sectional area on biofoam sample no. 20 (15% nanocellulose, 5% talc, 0.1% NRL, 38.5% distilled water) and sample no. 7 (15% nanocellulose, 1% talc, 0.1 % NRL, and 38.5% distilled water). It was found that higher content of talc (5% talc) in the biofoam formulation gave foam with lower density (0.1 g cm^{-3}) but smoother surface (Fig. 5(D)) and better cellular size distribution (Fig. 5(E)). On the other hand, denser foam with 0.25 g cm^{-3} density was obtained from sample no. 7 (1% talc), and a rougher surface and poor cellular size distribution were obtained, as shown in Fig. 5(F) and 5(G), respectively.

Addition of water significantly decreased flexural strength, as demonstrated in sample no. 3 and sample no. 4 (Table 2). An increase of water content from 33% to 44% in the biobased foam caused decreased flexural strength from 4.6 MPa to 0.6 MPa.

Likewise, the modulus of biofoam significantly decreased from 256.2 MPa to 38.0 MPa. In contrast, the percentage of elongation increased from 2.7 to 3.7 % when water content in biofoam was enhanced from 33% to 44%. The great importance lies in the starch composition (amylose and amylopectin ratio) during thermal processing. Influence of water on the T_g showed that the very branched amylopectin had a slightly lower glass transition temperature than the amylose and starchy material containing water that is generally in the glassy state, and therefore brittle under natural conditions (Mitrus and Moscicki 2014). Nevertheless, an optimal water content in biofoam ingredients in the present study substantially depended on the temperature of starch processing, as well as quantity and type of added plasticizers, nanocellulose fiber and crosslinked starch. A suitable water content in the ingredients of biofoam was required to improve the biofoam's mechanical and water resistance properties for production of packaging or other short-lived materials derived from agro-polymers (Narkchamnan and Sakdaronnarong 2013).

ANOVA for Response Surface Quadratic Models

ANOVA results for Response Surface Reduced Quadratic models of each factor namely flexural strength, elongation, modulus, and density are listed in Table 3. According to the accuracy of the Box Behnken design and degrees of freedom of the quadratic models, 24 runs and 5 center points were performed. It was found that the term NRL in the model gave significant effect on namely flexural strength, elongation, modulus, and density ($P<0.05$). For flexural strength, the model F-value of 2.48 implies the model is significant. There is only a 4.78% chance that an F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case C or NRL content is a significant model term ($P<0.05$). Values greater than 0.1000 indicate the model terms are not significant. In case of elongation model, the model F-value of 2.43 implies the model is significant. There is only a 4.85% chance that an F-value this large could occur due to noise. In this case B (Talc content) and C^2 (NRL^2) are significant model terms ($P<0.05$). In terms of modulus, the model F-value of 3.07 implies the model is significant. There is only a 1.92% chance that an F-value this large could occur due to noise. In this case B (Talc), BC (Talc*NRL), and B^2 ($Talc^2$) are significant model terms ($P<0.05$). For the biofoam density, C (NRL) is only significant model term. The model F-value of 2.71 implies the model is significant. There is only a 3.62% chance that an F-value this large could occur due to noise. An increase of NRL content in biofoam ingredient enhanced the foam density. Similarly, an increase of NRL content tended to slightly increase the flexural strength. Meanwhile, elongation and density of material depended significantly on interaction effect of NRL and talc contents as shown in the quadratic models. From the 3D surface plot of talc and NRL contents (data not shown), an increase of NRL up to 10% and Talc content up to 5% achieved the maximum elongation and density of material. From statistical analysis, standard deviation of flexural strength, elongation and modulus and density of foam from the quadratic models were 3.12 MPa, 0.95%, 181.11 MPa, and 0.2 g/cm³, respectively with corresponding R-square of 0.50, 0.57, 0.70, and 0.73, respectively. Therefore the regression models to predict all responses were relatively robust.

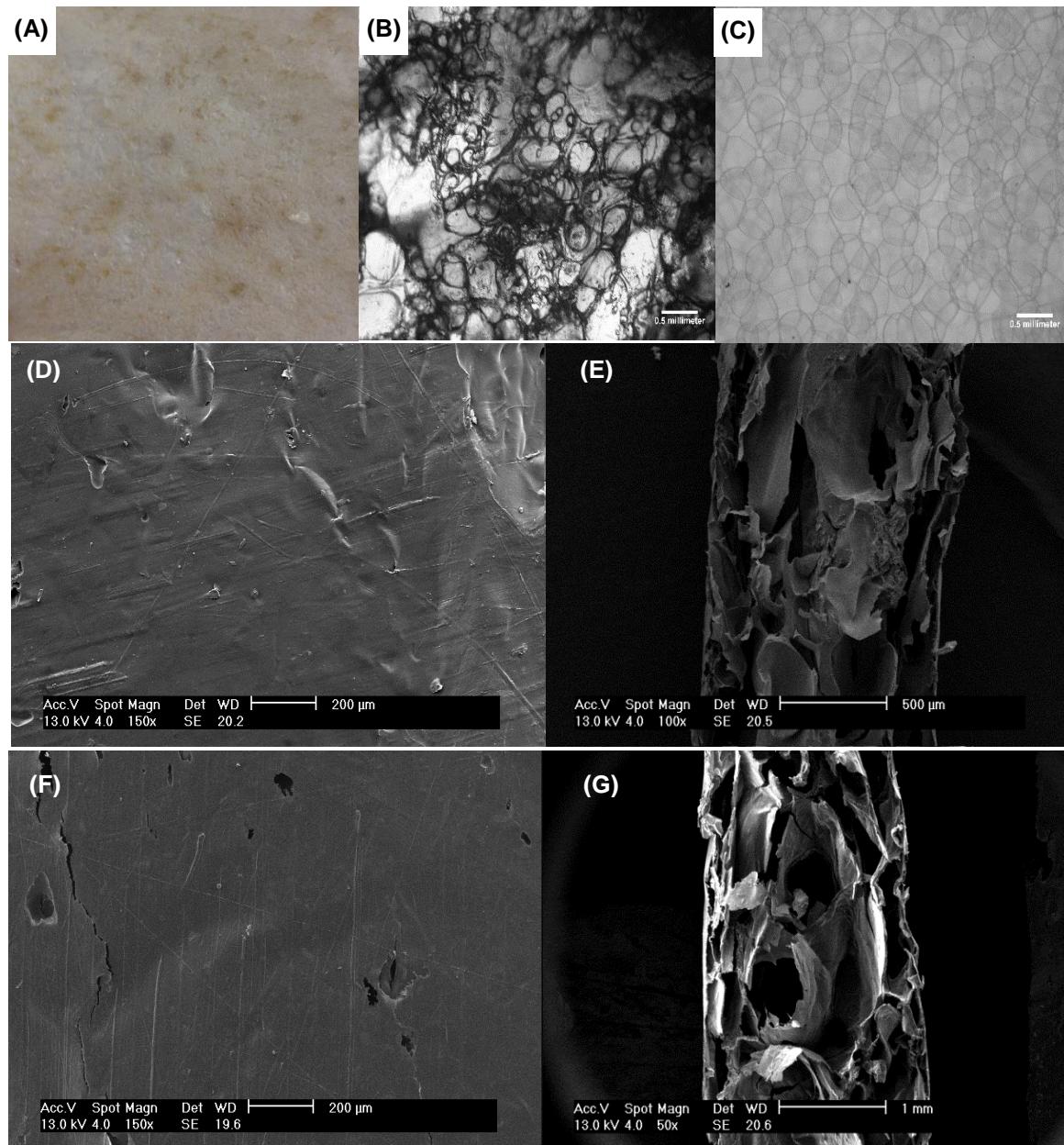


Fig. 5. Surface of biobased foam had mechanical properties similar to polystyrene foam (A) surface of biobased foam sample no. 25 from digital camera, (B) surface of biobased foam sample no. 25 from digital microscope ($\times 5$ magnification), and (C) surface of polystyrene foam from digital microscope ($\times 5$ magnification), and scanning electron microscopic images of (D) biofoam surface (sample no. 20, $\times 150$ magnifications), (E) cross-sectional area (sample no. 20, $\times 50$ magnifications), (F) biofoam surface (sample no. 7, $\times 150$ magnifications), and (G) cross-sectional area (sample no. 7, $\times 50$ magnifications)

Table 2. Mechanical and Physical Properties of Biobased Foam from the Experimental Design

Sample no.	Factors ^a					Responses			
	Nanocellulose (%)	Talc (%)	NRL (%)	Distilled water (%)	Lignin (%)	Flexural strength (MPa)	Elongation (%)	Modulus (MPa)	Density (g cm ⁻³)
1	10	1	5.05	38.5	-	3.5±0.8	1.3±0.3	398.4±76.6	0.60
2	10	3	0.10	38.5	-	2.4±0.5	3.2±1.0	96.2±27.0	0.20
3	10	3	5.05	33.0	-	4.6±1.1	2.7±0.7	256.2±93.5	0.63
4	10	3	5.05	44.0	-	0.6±0.1	3.7±1.8	38.0±5.0	0.50
5	10	3	10.00	38.5	-	8.6±3.2	2.0±0.6	415.7±175.5	0.73
6	10	5	5.05	38.5	-	1.4±0.6	2.8±1.0	74.4±6.4	0.61
7	15	1	0.10	38.5	-	2.5±1.00	1.9±0.5	178.2±13.7	0.25
8	15	1	5.05	33.0	-	5.8±1.9	1.4±0.7	465.0±20.9	0.49
9	15	1	5.05	44.0	-	2.1±1.2	2.0±0.7	278.2±65.5	0.40
10	15	1	10.00	38.5	-	14.9±1.2	1.4±0.1	993.7±130.1	1.00
11	15	3	0.10	33.0	-	0.9±0.2	1.6±0.5	68.5±23.9	0.38
12	15	3	0.10	44.0	-	0.2±0.1	4.9±1.4	6.0±1.9	0.40
13 ^b	15	3	5.05	38.5	-	5.7±3.8	3.0±1.4	146.2±24.6	0.70
14 ^b	15	3	5.05	38.5	-	1.7±1.0	2.1±1.3	149.0±10.0	0.53
15 ^b	15	3	5.05	38.5	-	2.8±0.4	1.3±0.2	259.5±92.3	0.80
16 ^b	15	3	5.05	38.5	-	3.9±0.5	1.2±0.5	457.5±20.8	0.45
17 ^b	15	3	5.05	38.5	-	2.2±2.6	1.3±0.4	70.5±70.3	0.38
18	15	3	10.00	33.0	-	4.6±0.3	2.0±0.1	268.2±81.6	0.87
19	15	3	10.00	44.0	-	2.8±4.1	4.9±0.7	17.6±1.4	1.34
20	15	5	0.10	38.5	-	1.0±0.8	3.1±1.0	54.9±6.8	0.10
21	15	5	5.05	33.0	-	2.4±1.1	2.2±0.5	148.5±69.5	0.54
22	15	5	5.05	44.0	-	5.5±1.4	1.7±0.1	323.3±74.1	0.66
23	15	5	10.00	38.5	-	1.0±0.0	5.9±1.5	38.2±19.0	1.10
24	20	1	5.05	38.5	-	8.9±1.1	1.5±0.8	921.8±71.7	0.63
25	20	3	0.10	38.5	-	0.4±0.3	3.1±1.8	43.5±6.3	0.44
26	20	3	5.05	33.0	-	1.5±0.7	1.7±0.6	117.1±48.8	0.78
27	20	3	5.05	44.0	-	2.2±1.4	1.4±0.5	291.6±30.2	0.30
28	20	3	10.00	38.5	-	3.1±2.5	2.6±1.2	37.3±3.2	0.72
29	20	5	5.05	38.5	-	14.4±4.4	2.6±0.7	533.5±178.4	0.82
L1	20	3	0.10	38.5	0.5	0.3±0.0	3.7±0.7	18.2±2.2	n.d.
L2	20	3	0.10	38.5	2.5	0.3±0.0	1.2±0.5	35.4±3.1	n.d.
Polystyrene foam						0.5±0.1	3.2±1.1	21.2±0.2	0.03

^a Calculated based on total solid dry weight and crosslinked cassava starch was added to 100% solid dry weight^b Center points of the design

n.d. = not determined

Water resistance of biofoam

The contact angle of the water drop test was used as indicator to demonstrate the water resistance of the biofoam surface. An increase of contact angle corresponds to a more highly hydrophobic character of the biofoam surface (Narkchamnan and Sakdaronnarong 2013). Extensive studies showed that starch could be used as ideal sustainable biobased resource for biofoam production. The only drawback is its association with hydrophilicity (Kumar and Siddaramaiah 2007). As illustrated in Fig. 6, the result showed that addition of NRL (10 %) into biofoam ingredient significantly increased water resistance of biofoam, as the contact angle was highest at 76.06 degrees (Fig. 6(C)). The result was in good agreement with several previous work studying the influence of NRL introduced into starch foam to anchor fillers (Shey *et al.* 2006; Afiq and Azura 2013; Konar *et al.* 2013). Rubber latex is widely known to reduce the water sensitivity of starch-filled biocomposite (Buchanan *et al.* 1975). In a combination of addition of cellulose, water resistance of biocomposite was considerably improved due to the particular presence of residual lignin, extractive substances and fatty acids at the surface of cellulose limits, comparatively, the hydrophilic character of the filler (Bendahou *et al.* 2010). Reduction of talc content from 5% to 1% slightly increased the contact angle from 59.45 to 55.72 degrees (Fig. 6(B) and 6(D)). High water content in biofoam additionally reduced contact angle of water drop, in other words high water content caused the biofoam to be more hydrophilic (Fig. 6(A)). Addition of lignin into biofoam ingredient significantly enhanced surface hydrophobicity of biofoam. As shown in Fig. 6(E), biofoam containing 20% nanocellulose, 3% talc, 0.1% NRL, 38.5% distilled water without addition of lignin gave the water drop test contact angle at 76.28 degrees. An increase of lignin content from 0% to 0.5% and 2.5% enhanced the water drop contact angle to 76.53 and 80.07 degrees, respectively. Approximately 5% enhancement of contact angle was achieved from biofoam containing 2.5% lignin. However, addition of lignin at levels of more than 2.5% required additional reaction, *e.g.* hydrolysis to produce polyols from lignin (Mahmood *et al.* 2013), in order to increase the dissolution and compatibility of lignin molecules in biofoam ingredient. Thus, further systematic study to increase lignin content in biofoam containing starch, talc, NRL, and nanocellulose is necessary.

In addition to the cross-linking reaction of cassava starch, it has been revealed that the use of the less polar milled wood lignin or phenolic lignin could increase the water resistance of biocomposites in combination with laccase enzyme and a mediator, which is an examples of a so-called laccase mediator system (Huttermann *et al.* 1980; Fackler *et al.* 2008; Narkchamnan and Sakdaronnarong 2013). Therefore, in the present work the influence of phenolic lignin on mechanical properties of biofoam from cassava starch/NRL/nanocellulose/talc was investigated.

As demonstrated in Table 2, proportion of biofoam sample no. 25 having similar mechanical properties as PS foam was selected to test the effect of lignin on its properties. Isolated lignin from sugarcane bagasse was added into the biofoam formulation at 0% (sample no. 25), 0.5% (L1), and 2.5% (L2), while other ingredients were 20% nanocellulose, 3% talc, 0.1% NRL, and 38.5% distilled water.

It was found that an optimal lignin content added into biofoam needed to be determined. As shown in Table 2, addition of 0.5% lignin into biofoam containing 20% nanocellulose, 3% talc, 0.1% NRL, and 38.5% distilled water gave the most favorable effects on flexural strength, percentage of elongation, and modulus of biofoam, thus mimicking PS foam.

Table 3. ANOVA for Response Surface Reduced Quadratic Models of each Response

Response 1 Flexural strength						
Source	Sum of Squares	df	Mean Square	F Value	p-value (Prob > F)	
Model	192.50	8	24.06	2.48	0.0478	significant
<i>A-Nanocellulose</i>	7.36	1	7.36	0.76	0.3944	
<i>B-Talc</i>	12.00	1	12.00	1.23	0.2796	
<i>C-NRL</i>	63.48	1	63.48	6.53	0.0188	
<i>AB</i>	14.44	1	14.44	1.49	0.2370	
<i>AC</i>	3.06	1	3.06	0.32	0.5808	
<i>BC</i>	38.44	1	38.44	3.96	0.0606	
<i>BD</i>	11.56	1	11.56	1.19	0.2884	
<i>B²</i>	42.16	1	42.16	4.34	0.0503	
Residual	194.35	20	9.72			
Response 2 Elongation						
Source	Sum of Squares	df	Mean Square	F Value	p-value (Prob > F)	
Model	23.03	10	2.30	2.43	0.0485	significant
<i>A-Nanocellulose</i>	0.65	1	0.65	0.69	0.4172	
<i>B-Talc</i>	6.45	1	6.45	6.81	0.0177	
<i>C-NRL</i>	0.083	1	0.083	0.088	0.7702	
<i>D-water</i>	4.08	1	4.08	4.31	0.0525	
<i>AC</i>	0.12	1	0.12	0.13	0.7233	
<i>AD</i>	0.42	1	0.42	0.45	0.5127	
<i>BC</i>	2.72	1	2.72	2.87	0.1073	
<i>CD</i>	0.040	1	0.040	0.042	0.8395	
<i>C²</i>	8.30	1	8.30	8.76	0.0084	
<i>D²</i>	0.61	1	0.61	0.64	0.4338	
Residual	17.05	18	0.95			
Response 3 Modulus						
Source	Sum of Squares	df	Mean Square	F Value	p-value (Prob > F)	
Model	1.210E+006	12	1.008E+005	3.07	0.0192	significant
<i>A-Nanocellulose</i>	36951.90	1	36951.90	1.13	0.3043	
<i>B-Talc</i>	3.545E+005	1	3.545E+005	10.81	0.0046	
<i>C-NRL</i>	1.459E+005	1	1.459E+005	4.45	0.0510	
<i>D-water</i>	11334.45	1	11334.45	0.35	0.5648	
<i>AB</i>	1033.62	1	1033.62	0.032	0.8613	
<i>AC</i>	26520.12	1	26520.12	0.81	0.3819	
<i>AD</i>	38553.32	1	38553.32	1.18	0.2944	
<i>BC</i>	1.731E+005	1	1.731E+005	5.28	0.0354	
<i>BD</i>	32688.64	1	32688.64	1.00	0.3330	
<i>B²</i>	2.231E+005	1	2.231E+005	6.80	0.0190	
<i>C²</i>	56491.81	1	56491.81	1.72	0.2079	
<i>D²</i>	47594.87	1	47594.87	1.45	0.2459	
Residual	5.248E+005	16	32800.89			

Response 4 Density						
Source	Sum of Squares	df	Mean Square	F Value	p-value (Prob > F)	
Model	1.52	14	0.11	2.71	0.0362	significant
A-Nanocellulose	0.015	1	0.015	0.37	0.5540	
B-Talc	0.018	1	0.018	0.44	0.5174	
C-NRL	1.33	1	1.33	33.18	< 0.0001	
D-water	6.750E-004	1	6.750E-004	0.017	0.8985	
AB	8.100E-003	1	8.100E-003	0.20	0.6595	
AC	0.016	1	0.016	0.39	0.5419	
AD	0.031	1	0.031	0.77	0.3962	
BC	0.016	1	0.016	0.39	0.5419	
BD	0.011	1	0.011	0.28	0.6077	
CD	0.051	1	0.051	1.27	0.2794	
A^2	2.530E-003	1	2.530E-003	0.063	0.8050	
B^2	6.815E-004	1	6.815E-004	0.017	0.8980	
C^2	0.017	1	0.017	0.43	0.5225	
D^2	2.998E-003	1	2.998E-003	0.075	0.7882	
Residual	0.56	14	0.040			

Slightly decreased flexural strength and increased elongation was achieved when 0.5% lignin was added. Thus, lignin improved the elasticity of biofoam. The results agreed with those of Sahoo and colleagues, who reported that addition lignin into polybutylene succinate composite enhanced the tensile, flexural, and impact strength simultaneously (Sahoo *et al.* 2011). Important functional groups, chemical units, and inter-unit linkages present in lignin are phenolic AOH, aliphatic hydroxyl, carbonyl, alkyl aryl ether, biphenyl, diaryl ether, phenylpropane, guaiacyl, and syringyl, *etc*. Therefore, there were reports on considerable compatibility of lignin in starch matrix, natural fibers, and particularly polystyrene. However, too much lignin added into biofoam (2.5% lignin) caused problems related to reduction of elongation and led to stiffer foam that was unsatisfactory for packaging applications.

The increase in the tensile strength at higher lignin content indicates a reinforcing effect of lignin in crosslinked starch/NRL matrix that may be attributed to the similarity in the solubility parameter of lignin and crosslinked starch/NRL matrix, crosslinking ability, and the adhesive nature of lignin. The enhancement in the properties of biofoam indicates an interaction, possibly polar–polar interaction between lignin and crosslinked starch matrix via a hydrogen bond formation that could be possible between the hydroxyl group of the crosslinked starch matrix and the hydroxyl group of lignin.

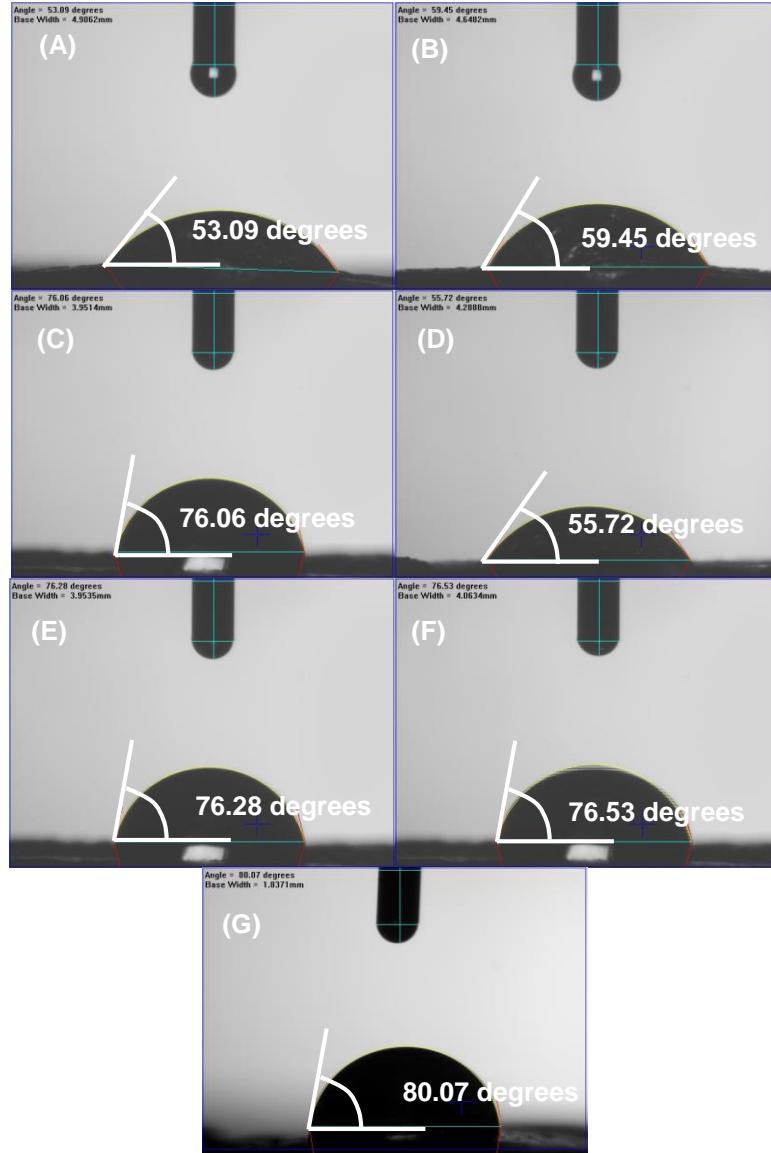


Fig. 6. Water drop test at 60 sec of biobased foam made of (A) 15% nanocellulose, 3% talc, 0.1% NRL, 44% distilled water (sample no. 12), (B) 15% nanocellulose, 5% talc, 0.1% NRL, 38.5% distilled water (sample no. 20), (C) 15% nanocellulose, 3% talc, 10 % NRL, 44% distilled water (sample no. 19), (D) 15% nanocellulose, 1% talc, 0.1% NRL, 38.5% distilled water (sample no. 7), (E) 20% nanocellulose, 3% talc, 0.1% NRL, 38.5% distilled water, (sample no. 25), (F) 20% nanocellulose, 3% talc, 0.1% NRL, 38.5% distilled water, 0.5% lignin (sample no. L1), and (G) 20% nanocellulose, 3% talc, 0.1% NRL, 38.5% distilled water, 2.5% lignin (sample no. L2).

CONCLUSIONS

1. Lignin extraction from SCB in 40 % KOH solution at 120 °C for 1 h gave relatively high lignin yield with the least hemicellulose contamination. The isolated lignin structure was similar to that of lignin from black liquor from the pulp and paper industry.
2. Analysis of cellulose yield, XRD diffractometry, ATR-FTIR spectroscopy, and TEM imaging indicated that 40 % KOH at 120 °C for 1 h was the most suitable condition for cellulose extraction. To obtain nanocellulose, size reduction by the mechanical method was selected according to superior fibril distribution and homogeneous shape.
3. Biocomposite foam produced from 20% nanocellulose fiber, 3% talc, 0.1% natural rubber latex (NRL), 38.5% water, and 76.9% crosslinked cassava starch by weight had equal mechanical properties as polystyrene foam. An increase of NRL resulted in increased percent elongation of the biocomposite.
4. Addition of an optimal amount of lignin slightly decreased the flexural strength and improved elasticity of biofoam, mimicking polystyrene foam's mechanical properties, which is beneficial for packaging application.

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Article submitted: August 5, 2014; Peer review completed: October 19, 2014; Revised version received and accepted: November 11, 2014; Published: November 20, 2014.