Carbohydrate Polymers 96 (2013) 109–117

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Thermo-molded biocomposite from cassava starch, natural fibers and lignin associated by laccase-mediator system

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ARTICLE INFO

Article history:
Received 26 December 2012
Received in revised form 10 March 2013
Accepted 13 March 2013
Available online 21 March 2013

Keywords:
Laccase-mediator treatment
Natural fibers
Lignin
Cassava starch biocomposite
Water resistance
Flexural strength and modulus

ABSTRACT

Treatment of cellulose fibers and lignin by laccase-mediator system was conducted to enhance the binding efficiency of natural fibers and lignin compounds into cassava starch composite matrix. Violuric acid (VA) was tested for its effect as a mediator for laccase treatment. Influence of different fiber, lignin and water contents of biocomposite was statistically investigated. The results showed that adding 15% (w/w) fibers into biocomposite at 44% (w/w) water content increased flexural strength and modulus for 4 times compared with the control. A combination of fibers + VA gave the greatest enhancement of modulus for 1140% and flexural strength for 375.8% as much as neat starch biocomposite. The presence of fibers, lignin and VA as mediator for laccase treatment substantially enhanced water resistance of starch biocomposite detected by a change in water drop contact angle on biocomposite surface.

1. Introduction

Starch based composite has become interesting for non-food packaging or other short-lived material applications. Apart from its biodegradability, it is promising for production in commercial scale as starch is renewable resource having potential to replace petroleum based plastic packaging. However, mechanical and physical properties of starch based composite are rather poor in terms of low flexibility, high brittleness as well as high water and moisture absorption. Recent uses of this material still need to be blended with petroleum based plastic such as polypropylene (Bagheri, 2009; Mulbry, Reeves, & Millner, 2012), polystyrene (Kiatkamjornwong, Sonsuk, Wittayapichet, Prasassarakich, & Vejjanukroh, 1999; Pushpadass, Weber, Dumais, & Hanna, 2010), polymethylmethacrylate (Bhatnagar & Hanna, 1995), polyvinylchloride (Viña et al., 2007), and polyvinyl alcohol (Sin, Rahman, Rahmat, & Mokhtar, 2011; Tang & Alavi, 2011). To enhance the mechanical properties of starch based composite, addition of cellulose fibers into composite has been demonstrated that the mechanical properties of composite was improved (Alemdar & Sain, 2008; Kaushik, Singh, & Verma, 2010). Previous research of our group found that addition of micro- or nano-scale fibers isolated from non-wood plants such as rice straw and sugarcane bagasse into biocomposite foam significantly increased its flexural strength and modulus comparative to starch foam without fibers when glycerol was used as plasticizer (Srithongkham, Vivitchanont, & Krongtaew, 2012). Although its mechanical properties were improved, the water resistance was additionally required to be increased. Blending with plastics and natural rubber latex has been suggested to enhance the water resistance of biocomposite, however biodegradability of the material significantly decreased (Glenn & Hsu, 1997; Shey, Imam, Glenn, & Orts, 2006).

To solve the mentioned problems, in the present work we were interested to use lignin, a lignocellulosic waste from pulping process, as an additive in starch based biocomposite due to its hydrophobic structure. Influence of isolated micro-/nano-fibers from rice straw were furthermore studied on mechanical properties of the material. An improvement of adhesive properties between lignin, cellulose and resin for particleboard production by laccase treatment has been intensively studied (González-García, Feijoo, Heathcote, Kandelbauer, & Moreira, 2011; Hashim et al., 2011). Laccase-mediator concept based on stable radicals generated in a combination with low-molecular weight redox mediator has been widely implemented in delignification and bio-bleaching process in pulp and paper industry (Aracri & Vidal, 2011; Camarero et al., 2004). Moreover, laccase treatment for fiber modification and improvement of adhesive properties in particle board manufacturing was extensively investigated (Fillat, Roncero, & Vidal, 2012; González-García et al., 2011). It was found that chemical

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http://dx.doi.org/10.1016/j.carbpol.2013.03.046

Table 1
The two-level full factorial design for starch biocomposite formation.

Experiment	Coded level (actual level)				Response value	
	X_1 Water (%, w/w)	X_2 Fibers (%, w/w)	X_3 Lignin (%, w/w)	X_4 VA (mM)	Z_1 Modulus (MPa)	Z_2 Flexural strength (MPa)
1	0 (22)	0 (0)	0 (0)	0 (0)	17.09	4.37
2	1 (44)	0 (0)	0 (0)	0 (0)	19.18	6.66
3	0 (22)	1 (15)	0 (0)	0 (0)	16.06	5.44
4	1 (44)	1 (15)	0 (0)	0 (0)	74.20	27.45
5	0 (22)	0 (0)	1 (10)	0 (0)	13.31	3.77
6	1 (44)	0 (0)	1 (10)	0 (0)	13.49	3.95
7	0 (22)	1 (15)	1 (10)	0 (0)	52.16	12.14
8	1 (44)	1 (15)	1 (10)	0 (0)	39.96	8.41
9	0 (22)	0 (0)	0 (0)	1 (10)	57.37	7.49
10	1 (44)	0 (0)	0 (0)	1 (10)	26.15	6.04
11	0 (22)	1 (15)	0 (0)	1 (10)	127.75	11.76
12	1 (44)	1 (15)	0 (0)	1 (10)	168.05	27.47
13	0 (22)	0 (0)	1 (10)	1 (10)	9.63	3.32
14	1 (44)	0 (0)	1 (10)	1 (10)	7.60	1.80
15	0 (22)	1 (15)	1 (10)	1 (10)	124.80	30.32
16	1 (44)	1 (15)	1 (10)	1 (10)	237.87	31.69

bonding strength between cellulose fibers and phenolic resin was promoted after laccase treatment resulting in significant increases in the interfacial strength of composites (Peng, Zhong, Ren, & Sun, 2010). In previous works, the successful application of the synthetic mediator violuric acid (VA) in combination with laccase from the white-rot fungus *Pycnoporus cinnabarinus* (PcL) and the study of laccase acting as delignification agent with VA to biobleach flax pulp with high delignification efficiency were reported (Fillat, Roncero, & Vidal, 2011; Fillat et al., 2012). However, there was no previous report on laccase-mediator system activated lignin/cellulose/starch based biocomposite. Thus, the present work focused on eco-friendly process for lignin and cellulose modification and subsequently investigated for its application in starch biocomposite formation. The attempt of our study was to introduce laccase-mediator treatment of isolated fibers and lignin prior to starch based biocomposite formation. The results found suggestively broadened the application of laccase enzyme in composite formation for non-food packaging.

2. Materials and methods

Rice straw (*Oryza sativa*) was supplied from a field in Nakornchaisri, Nakhon Pathom province, Thailand. Cassava starch (0.05% protein, 0.14% lipid, 0.15% ash, and 17.44% amylose (dry basis) was purchased from Chuthin Co., Ltd., Thailand. Sodium hydroxide, hydrochloric acid, ethanol, laccase enzyme, violuric acid (VA) and other analytical grade chemicals were purchased from Sigma–Aldrich, Merck, and Ajax Finechem. Alkali lignin (low sulfonate content) with approximate molecular weight of 10,000 g/mol was from Sigma–Aldrich.

2.1. Cellulose micro-/nano-fibers separation from rice straw

Rice straw was cut and milled to 2 mm length. Then, 20 g of milled rice straw was subjected to three consecutive steps: (1) soaking in concentrated NaOH solution based on straw dry weight and then washed with 1 L deionized water, (2) hydrolysis by dilute HCl and then washed with 1 L deionized water, and (3) treatment in dilute alkaline solution (2% (w/w) NaOH). During the first alkaline soaking step, the optimum condition from our previous work was performed. Rice straw was treated in 20% (w/w) NaOH and 38% (v/v) ethanol for 15 h at room temperature (Narkchamnan & Krongtaew, 2012). After the final step of dilute alkaline treatment, isolated fibers was washed several times with 1 L deionized water and finally homogenized to micro-/nano-fibers using high shear homogenizer for 15 min.

2.2. Preparation of starch feedstock for biocomposite production

Cassava starch was dried at 60 °C for 24 h. A suspension of 10% (w/w) cassava starch in deionized water was heated with a constant stirring in a boiling water bath for 25 min to get a constant viscosity. The suspension was covered and chilled (5 °C) overnight to allow gelation. Starch gel-to-dry starch powder ratio of 1:1.8 was subsequently mixed by overhead mixer for 10 min. After mixing, the batter was dried in oven (60 °C) for 24 h and ground to powder.

2.3. Laccase mediator system treatment of lignin and cellulose fibers

First, lignin was dissolved at 5% consistency in a suspension of a 1:1 solution of p-dioxane: 50 mM succinic acid buffer pH 4.8 when dry weight of lignin was calculated from the experimental design for biocomposite composition (Table 1). Never-dried cellulose fibers were weighed according to the design of experiment for biocomposite formation and added into 50 mM succinic acid buffer pH 4.8 with 5% consistency. Simultaneously, violuric acid was added in the prepared solution according to the biocomposite formulation from the design and mixed for 5 min at 45 °C. Subsequently, the lignin solution was added into cellulose fibers suspension. After mixing, laccase was added at a dosage of 3 U/g cellulose fibers (dry basis) and the suspension was stirred for a few minutes for homogeneous cellulose fiber suspension. Temperature of reaction was controlled at 50 °C. After enzyme treatment, solid part was separated by centrifugation. Solid slurry was used for biocomposite formation.

2.4. Experimental design for biocomposite formation

Biocomposite was prepared by mixing all solid ingredients including starch feed stock and cellulose fiber + lignin + VA suspension prepared from the laccase-mediator system treatment step in a blender. A certain amount of distilled water was finally added into the batter according to the experimental design (Table 1) and mixed thoroughly. All ingredients were calculated based on starch feedstock dry weight. The mixture was poured into 3-mm depth stainless steel mold. The biocomposite was thermo-molded using

a compression molding machine (LabTech, Thailand) at 210 °C for 3 min.

For the experimental design of biocomposite formation, four factors taken into account for the two-level full factorial design were water content (%, w/w), fiber content (%, w/w), lignin content (%, w/w) and violuric acid (VA) concentration (mM) represented by X_1, X_2, X_3 and X_4, respectively. Thus, the numbers of factorial runs at high (+1) and low (−1) coded values were 2^4 or 16 runs. Coded and actual values of each parameter were shown in Table 1. Biocomposite formed according to the experimental design were tested for its mechanical and physical properties. Flexural strength and modulus were experimental responses used to build the multiple regression models. Multiple regression coefficients, R^2, R^2 (adj) of the models, were computed and ANOVA was analyzed by Microsoft Excel 2010 using a regression toolbox.

2.5. Physical and mechanical properties of cellulose fibers and biocomposite

2.5.1. Holo-cellulose, alpha-cellulose and lignin contents of isolated fibers

Alpha-cellulose, hemi-cellulose (Zobel & McElvee, 1966) and lignin contents (TAPPI standard T222 om-02 and TAPPI standard 250UM-06) of untreated and chemically treated rice straw fibers were analyzed by wet-lab analysis.

2.5.2. Analysis of fiber morphology by scanning electron microscope (SEM) and transmission electron microscope (TEM)

The morphology of the cellulose fibers was examined using a scanning electron microscope (SEM). 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 5 kV using SEM (Hitachi S4800, Japan).

Transmission electron micrographs of the cellulose nanofibres were taken with a Jeol 1200Ex transmission electron microscope (Japan). An aqueous dilute fiber solution was dropped on a micro grid. To increase the microscopic resolution of the image staining, the deposited fibers were stained by a 2% uranyl acetate solution. Identification of fiber morphology was based on size, shape and appearance of the fibers.

2.5.3. Thermogravimetric analysis (TGA)

Thermogravimetric analyzer (Pyris Diamond TG/DTA, Perkin Elmer, USA) was used to determine thermal stability of biocomposite specimen with a heating rate of 10 °C/min in nitrogen atmosphere and the temperature ranging between 30 and 500 °C.

2.5.4. Mechanical testing of the composite

Biocomposite specimen with dimension of 4 cm × 2 cm × 0.3 cm were prepared by cutting mold with hydraulic pressing apparatus and kept in a control relative humidity of 50% RH at 25 °C for 1 day prior to mechanical test. Flexural modulus, flexural stress and strain of the composite specimens were determined by using a universal testing machine (Instron 4467, USA) by 3-point bending fixture with a load cell of 0.5 kN. The crosshead speed was set to 5 mm/min.

2.5.5. Water drop test analysis

Water resistance analysis was determined by drop shape instrument (FTA1000, Brand, Canada). A dynamic change of contact angle between biocomposite surface and water drop was calculated at 1 s and 60 s by image analyzer equipped with the water drop test apparatus.

3. Results and discussion

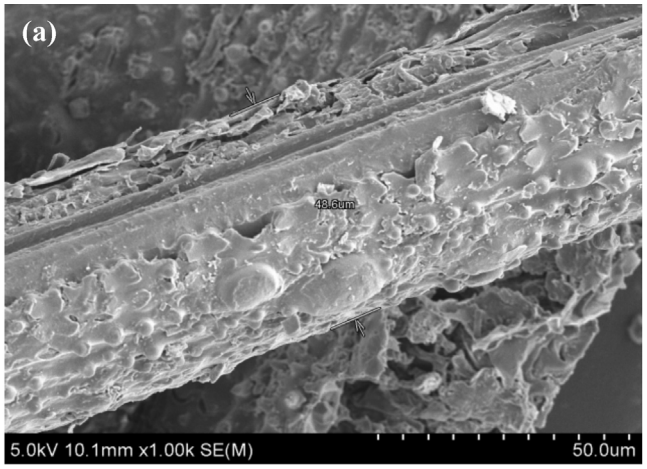
3.1. Cellulose micro-/nano-fibers and lignin isolation from rice straw

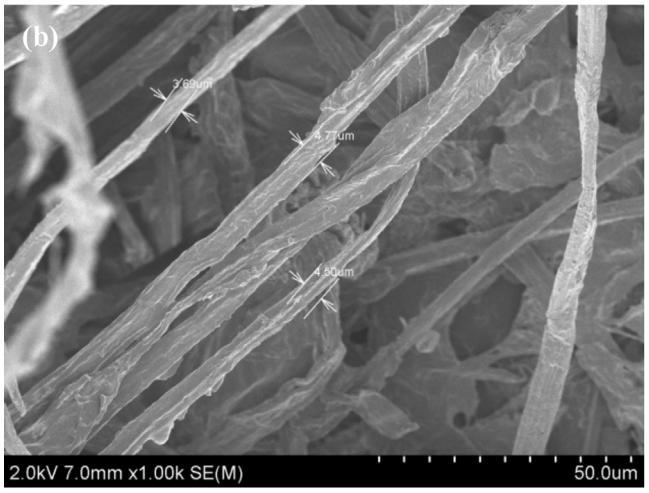
Alkali treatment of lignocellulosic substance disrupts the cell wall by dissolving hemicelluloses, lignin, and silica by hydrolyzing uronic and acetic ester and by swelling cellulose (Krongtaew, Messner, Ters, & Fackler, 2010; Xio, Sun, & Sun, 2001). This results in decrease of cellulose crystallinity while addition of solvent into the system considerably protected hemicelluloses from degradation. According to our previous work, an optimum condition for cellulose separation from rice straw was first step organosolv pretreatment in 20% (w/w) NaOH and 38% (v/v) ethanol at room temperature for 15 h (Narkchamnan & Krongtaew, 2012) and the subsequent step was hydrolysis in 1 M HCl solution under ultrasonication for 2 h at 80 °C. The final step was treatment in 2% (w/w) NaOH for 2 h at 80 °C prior to high shear homogenization. From wet-lab analysis, this condition yielded the cellulose nanofibers containing the maximum alpha-cellulose content of 87% (w/w) and minimum weight loss of 45% (w/w).

Crystallinity of isolated cellulose was detected by intensity at $2\theta = 22.5^\circ$ compared with intensity at $2\theta = 16.5^\circ$ which is amorphous phase from X-ray diffraction (XRD) chromatogram (data not shown). All isolated cellulose fibers showed the same XRD pattern with cellulose I_β characteristic peaks at $2\theta = 16.8^\circ$ and 22.7° representing the 110 and 200 crystallographic planes of the monoclinic cellulose I lattice, respectively. The crystallinity index (CrI) of isolated cellulose fibers from the present work (73% CrI) was lower than sulfuric acid/mechanical defibrilated nanocellulose from rice straw (91% CrI) from Jiang and Hsieh's work (Jiang & Hsieh). This was possibly due to higher hemicellulose contamination from the lower acid concentration applied during the acid hydrolysis step for nanocellulose isolation.

Additionally, we found that purified cellulose isolated from low and high concentrations of NaOH up to 40% (w/w) did not give different patterns of FTIR and XRD chromatograms. The crystalline structure of cellulose was monitored by the ratio of I-alpha at 750 cm^{-1} and I-beta at 710 cm^{-1} of FTIR chromatograms (Debzi, Chanzy, Sugiyama, Tekely, & Excoffier, 1991; Sakdaronnarong & Jonglertjunya, 2012). For vascular plant such as rice, the cellulose has primarily in I-beta structure. In the present work, it was observed that no transformation of two crystalline cellulose phases of native cellulose (cellulose I), monoclinic (I_β) and triclinic (I_α) structures indicated by ratio of A_{710}/A_{750} of FTIR absorbance since the A_{710}/A_{750} ratio of untreated rice straw and isolated cellulose fibers were 1.1 and 1.2, respectively which was not significant different (data not shown). However, it was observed that higher concentration of NaOH facilitates the size reduction of cellulose to micro- or nano-scale as the cellulose was better swelling.

The changes in size and surface of chemically isolated fibers compared with untreated ones were shown in Fig. 1. For untreated rice straw, the SEM image illustrated that its average diameter was 48.6 μm (Fig. 1 (a)). After the physico-chemical treatment with 20% (w/w) NaOH and 38% (v/v) ethanol for 15 h as shown in Fig. 1(b). The SEM image analysis depicted that the surface of the isolated fibers after the physico-chemical treatment from which lignin and hemicelluloses were removed (Fig. 1(b)) was considerably cleaner than that of the untreated rice straw fibers (Fig. 1(a)). The cleaner and smoother fibers surface was suitable for cross linking reaction of chemical binding as well as a better distribution of microfibers for enzymatic radical coupling of fibers for the purpose





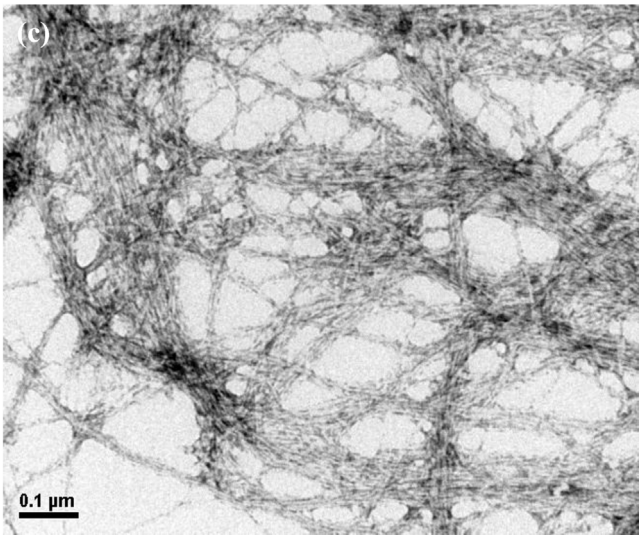


Fig. 1. SEM images (1000× magnifications) of (a) untreated rice straw, (b) cellulose fibers isolated by an optimal condition at15-h soaking time, 38% (v/v) ethanol, 20% (w/w) NaOH and (c) TEM image (100,000× magnifications) of cellulose nanofibers.

of biocomposite reinforcement. Cellulose fibrils can be enlarged for better illustration to approximate the diameter, morphology and distribution of fibers by TEM image as depicted in Fig. 1(c). Therefore, the diameter of fibers from TEM image ranged from 15 to 30 nm similar to our previous report (Narkchamnan & Krongtaew, 2012).

Table 2
Regression coefficients of the quadratic models for modulus and flexural strength of starch biocomposite from the two-level full factorial design.

Regression coefficients of the equation (Eq)	Modulus (MPa) Eq. (1)	Flexural strength (MPa) Eq. (2)
Equation $Z = a + bX_1 + cX_2 + dX_3 + eX_4 + fX_1^2 + gX_2^2 + hX_3^2 + iX_4^2 + jK_1X_2 + kX_1X_3 + lX_1X_4 + mX_2X_3 + nX_2X_4 + oX_3X_4$		
a	50.37	2.34
b	−0.76	–
c	–	−0.32
d	−1.90	1.19
e	−1.76	−0.38
f	–	0.004
g	−0.33	–
h	–	–
i	–	–
j	0.17	0.03
k	–	−0.05
l	0.08	–
m	0.24	–
n	0.73	0.08
o	–	0.08
R^2	0.93	0.86
R^2 (adj)	0.70	0.55
P-Value	0.004	0.027

Note: The dependent variables (Z) were modulus (MPa) and flexural strength (MPa) represented as Z_1 and Z_2, respectively. X_1, X_2, X_3, X_4 were actual values of water, fiber, lignin contents and VA concentrations, respectively. An interception coefficient represented by a when the coefficients b, c, d, e, f, g, h, i, j, k, l, m, n, o were regression coefficients.

3.2. Experimental design for biocomposite formation

For starch biocomposite formation, the influence of fibers and lignin coupled by laccase mediator system was investigated by 2-level full factorial design. Adding lignin into starch biocomposite formed by film casting, extrusion and injection molding was proposed for increasing its moisture resistance and improving mechanical properties (Baumberger, Lapierre, Monties, & Valle, 1998; Le Digabel & Avérous, 2006). Laccase enzyme was used to generate either lignin radicals or phenolic radicals and subsequent coupling reaction to cellulose fibers. It was found that laccase significantly increased the grafting efficiency of the copolymerization (Fillat et al., 2012). Influence of VA as a mediator on laccase treatment of natural fibers and lignin for biocomposite formation was also studied.

From the experimental design, the responses which were modulus and flexural strength of starch biocomposite made of different proportions of water, fiber, lignin contents and VA concentration were shown in Table 1. Two multiple regression models for modulus and flexural strength were computed from experimental data demonstrated in Table 2. Both models were significant at 95% confidence level ($P < 0.05$) and high R^2 of the models predicting modulus and flexural strength were attained at 0.93 and 0.86, respectively. The computational results indicated good fitness between predicting and experimental data. The adjusted R^2 were 0.70 and 0.55 from modulus and flexural strength models indicated that 70% and 55% of sample variation were able to be explained by the factors and interactions of these factors from the models.

3.2.1. Mechanical properties of biocomposite

3.2.1.1. Modulus. At low fiber content in the composite from 0 to 5% (w/w), there was no significant effect of different water contents on modulus as illustrated in Fig. 2(a). However, an increase of fiber content from 5 to 15% (w/w) required more water content up to 44% (w/w) to achieve the highest modulus of composite at 168.05 MPa as demonstrated in Table 1. The modulus was increased from 19.18 MPa to 74.20 MPa when adding 15% (w/w) fibers into the composite at 44% (w/w) water content. Thus, 287%

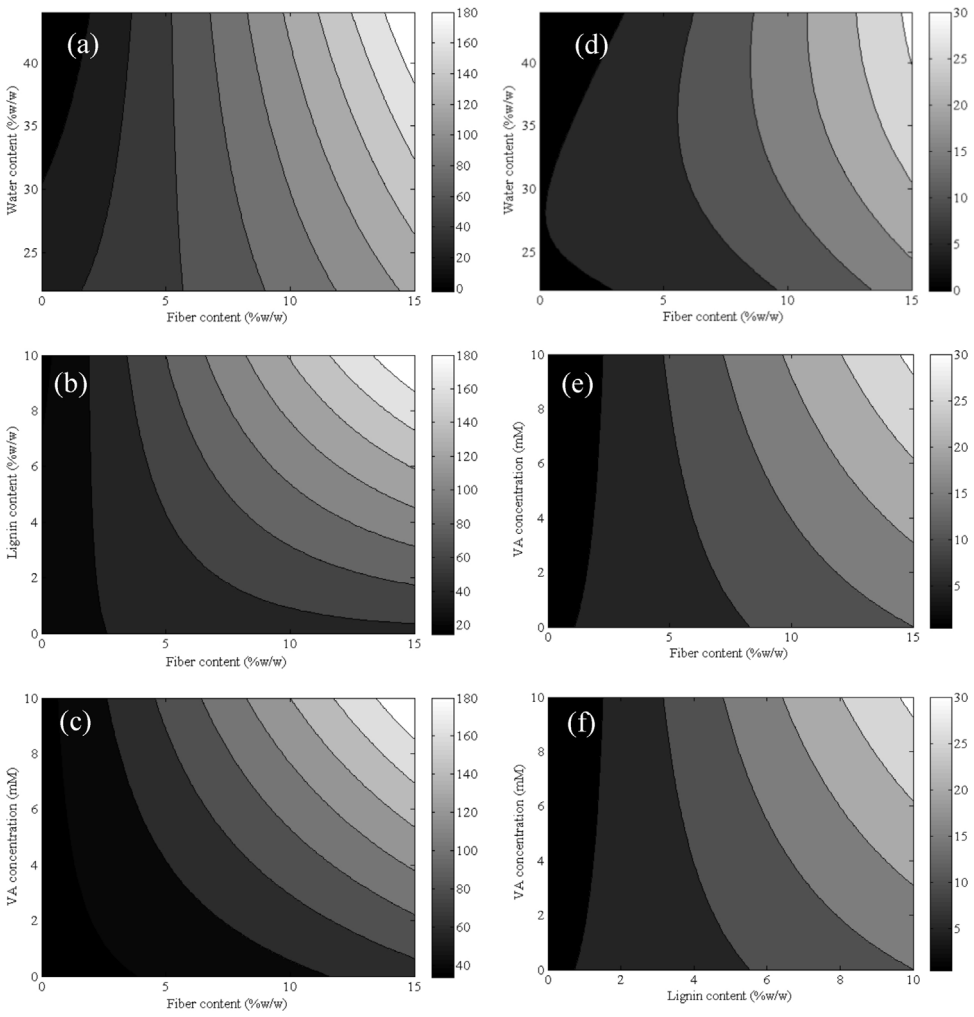


Fig. 2. Interaction of factors on modulus of biocomposite: (a) influence of fiber and water contents, (b) influence of fiber and lignin contents and (c) influence of fiber content and VA concentration; and interaction of factors on flexural strength of biocomposite: (d) influence of fiber and water contents, (e) influence of fiber contents and VA concentration and (f) influence of lignin content and VA concentration.

increase of modulus was obtained relative to neat starch composite. An increase of water content gave significantly smaller effect on modulus comparative to fiber content. Similar results were previously reported that adding cellulose fibers increased modulus of nanocomposite (Alemdar & Sain, 2008). From this study, the modulus of 10% nanocomposite loading into thermoplastic starch (TPS) showed a 145% increase compared to the pure thermoplastic starch. The modulus of TPS increased from 111 MPa to 271 MPa. Recently, there was a report on green nanocomposite based on thermoplastic starch (Kaushik et al., 2010). The nanocomposite with 15% fiber loading showed an improvement of 195% modulus over pure TPS. Tensile test results indicated good bonding and efficient stress transfer from matrix to fibers. The increase in modulus at fracture resulted in improvement in ultimate strength of the composites. In cellulose based composite, the usual reinforcement effect is attributable to the formation of rigid network of fibers connected by hydrogen bonds.

Adding lignin into composite containing starch and water did not affect the modulus of the composite for both 22% (w/w) and 44% (w/w) water contents. Combination of adding fibers and lignin substantially improved the modulus of biocomposite when fiber content was more than 10% (w/w) and lignin content was added from 5% (w/w) to 10% (w/w) as shown in Fig. 2(b). However, when compared with the effect of addition of fibers + VA, addition of fibers + lignin had significantly smaller effect. Addition of only fibers gave small influence on modulus similar to addition of VA alone that only slightly improved modulus of the composite as illustrated in Fig. 2(c). In contrast, the combination of adding fibers + VA considerably increased modulus of starch biocomposite up to 127.75 MPa and 168.05 MPa when the water content was 22% and 44% (w/w), respectively. These modulus values were 7.5–8.8 folds as much as that of starch biocomposite without additives. The highest modulus of biocomposite was achieved at 237.87 MPa from the biocomposite made of 15% (w/w) fibers, 10% (w/w) lignin and 10 mM VA when the water content was 44% (w/w). Comparison was done between neat starch composite without additive, the result showed that 1140% increase of modulus was achieved as the modulus was enhanced from 19.18 MPa for neat starch composite to 237.87 MPa.

Consequently, it was able to conclude that higher modulus was achieved respectively for composite specimens containing fibers + lignin + VA > fibers + VA > lignin + fibers > neat starch composite. The reason was because laccase-VA system generated phenoxy radicals produced by enzymatic oxidation of the mediator, which include depletion through homopolymerization and cross-coupling in the lignin structure or fragmentation of lignin compounds (d'Acunzo & Galli, 2003; Moldes & Vidal, 2008). As a consequence, treating lignocellulosic fibers and phenolic compounds with laccase is likely to produce a variety of oxidation and coupling products that are difficult to predict owing to the

complexity of the lignocellulosic matrix and the nature of free radical reactions (Kenealy & Jeffries, 2003).

3.2.1.2. Flexural strength. According to Eq. (2) from Table 2, interactions of all factors including water content, fiber content, lignin content and VA concentration significantly affected flexural strength of biocomposite. As illustrated in Fig. 2(d), at low content of fibers (0–5%, w/w) there was no substantial influence of water content variation from 22% to 44% (w/w) on flexural strength of biocomposite. To achieve a high level of flexural strength of biocomposite, it required to increase the fiber content and water content in an appropriate proportion. A high value of flexural strength at 27.45 MPa was obtained when adding 15% (w/w) fibers into the starch composite at 44% (w/w) water content (Table 1).

As depicted in Fig. 2(e), in case of addition of fibers lower than 5% (w/w), it was found that VA had no influence on flexural strength at all VA concentrations tested (0–10 mM). Nevertheless, for addition of fibers more than 5% (w/w), an increase of VA concentration significantly enhanced flexural strength of biocomposite. The most suitable proportion for the flexural strength at 27.47 MPa was obtained from biocomposite made of 15% (w/w) fibers and 10 mM VA at 44% (w/w) water content. Comparative to flexural strength of 17.45 MPa from 15% (w/w) fibers at 44% (w/w) water content from Table 1, it was suggested that the VA concentration that suited to 15% (w/w) fiber content was beyond the limit of 10 mM. Therefore, to accomplish the optimum flexural strength, the study on optimal ratio of fibers-to-mediator for the treatment of fibers by laccase-mediator system was critically important.

Similar results were found for lignin treatment by laccase-VA system. As shown in Fig. 2(f), at low lignin content below 3% (w/w), there was no effect of VA concentration on flexural strength of biocomposite, however for addition of lignin higher than 8% (w/w), an increase of VA concentration substantially enhanced its flexural strength. It was found from Eq. (2) that an increase of fiber content in biocomposite from 0 to 15% (w/w) when VA concentration was constant at 5 mM significantly enhanced flexural strength of biocomposite for every concentration of lignin added. The results were in good agreement with a previous study of Pingan and coworkers who studied the effect of lignin incorporated in reactive compatibilization as well as morphological, rheological and mechanical properties of acrylonitrile–butadiene–styrene (ABS) composites (Pingan et al., 2012). It was observed that the presence of lignin increased storage modulus, flexural strength and glass transition temperature. Incorporating small amount of lignin can produce composites with enhanced strength and modulus. In terms of flexural strength from the present study, effect of fibers + lignin + VA > fibers + VA > fibers > fibers + lignin > neat starch composite was found in a respective degree.

In summary, modulus of biocomposite was increased from 19.18 MPa to 237.87 MPa (1140% increase) when adding 15% (w/w) fibers and 10% (w/w) lignin treated by laccase-VA system at VA concentration of 10 mM and 44% (w/w) water content. Flexural strength of biocomposite was enhanced from 6.66 MPa to 31.69 MPa (375.8% increase) at the same ingredients comparative to neat starch composite. Addition of fibers + lignin + VA gave greatest effect on both modulus and flexural strength improvement.

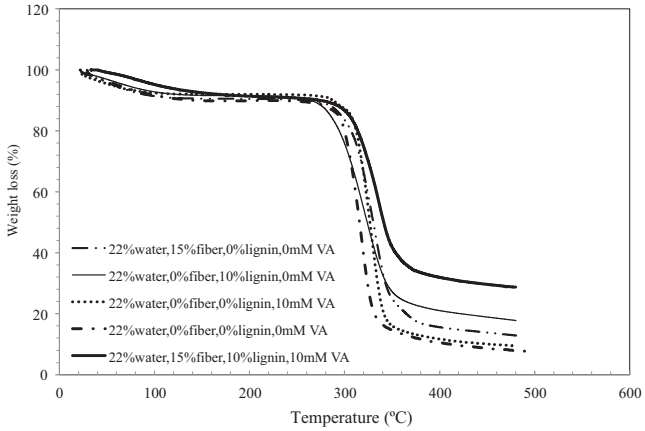


Fig. 3. TGA thermograms of laccase treated starch biocomposites.

3.2.2. Thermogravimetric analysis (TGA) of biocomposite

Five biocomposite specimens formed by (1) 22% (w/w) water, 0% (w/w) fibers, 0% (w/w) lignin and 0 mM VA, (2) 22% (w/w) water, 0% (w/w) fibers, 10% (w/w) lignin and 0 mM VA, (3) 22% (w/w) water, 0% (w/w) fibers, 0% (w/w) lignin and 10 mM VA, (4) 22% (w/w) water, 15% (w/w) fibers, 0% (w/w) lignin and 0 mM VA, and (5) 22% (w/w) water, 15% (w/w) fibers, 10% (w/w) lignin and 10 mM VA were selected to determine their thermal degradation temperature as shown in Table 3 and Fig. 3. Minimum degradation temperature (318.05 °C) was obtained from biocomposite made of only cassava starch at 22% (w/w) water content. Comparison to neat starch composite without any additives, it was observed that when adding 10 mM VA into biocomposite, the degradation temperature was increased from 318.05 to 327.82 °C. Thus, VA increased degradation temperature of biocomposite. The result was probably due to the higher bonding strength of starch composite from the crosslinking reaction induced by laccase-VA system.

Effect of adding fibers (15%, w/w) showed that the degradation temperature increased from 318.05 to 331.28 °C. The degradation temperature at 331.28 °C was obtained from the biocomposite formed by 22% (w/w) water, 15% (w/w) fibers, 0% (w/w) lignin, 0 mM VA as shown in Table 3. The result was in good agreement with a previous work of Carlos and coworkers in 2011. The researchers found that laccase mediator system removes substances adhered to cellulose, alters microfibril surface and increases the proportion of crystalline cellulose as a result (Carlos, Agustin, Amanda, Teresa, & Jose, 2011). These changes affect the thermal degradation of pulp by increasing the production of volatiles, reducing that of char and raising the maximum mass loss rate during volatilization. The results were additionally supported by previous work of Rong and colleagues who reported that cellulose fibers had effect on increasing thermal

Table 3
Thermal degradation characteristics of the starch biocomposites.

Experimental condition					Peak of degradation (°C)	Residue after heated at 500 °C (%, w/w)
No.	Water (%, w/w)	Fiber (%, w/w)	Lignin (%, w/w)	VA (mM)		
1	22	0	0	0	318.05	7.76
2	22	0	10	0	321.12	17.78
3	22	0	0	10	327.82	9.48
4	22	15	0	0	331.28	12.85
5	22	15	10	10	335.73	28.13

Table 4
Contact angle and base width of different starch biocomposites.

Experimental conditions					Contact angel (°) 1 s	Contact angel (°) 60 s	Base width (mm) 1 s	Base width (mm) 60 s
No.	Water (%, w/w)	Fibers (%, w/w)	Lignin (%, w/w)	VA (mM)				
1.	22	15	0	10	63.89	n.d.	2.0548	n.d.
2.	22	15	10	10	75.78	70.80	1.8540	1.8538
3.	44	15	10	0	63.49	53.87	1.9417	1.9588
4.	44	15	10	10	73.39	67.80	1.9895	1.9872

n.d., not detectable.

degradation temperature of biocomposite (Rong, Xiu, Fang, & Yu, 2009).

Addition of lignin (10%, w/w) provided an increase of biocomposite degradation temperature from 318.05 to 321.12 °C. The highest degradation temperature at 335.73 °C was achieved from the biocomposite containing 22% (w/w) water, 15% (w/w) fibers, 10% (w/w) lignin and 10 mM VA. The results were supported by previous work of Rong and coworkers (2009) who reported that lignin had effect on increasing thermal degradation temperature of biocomposite from 309 to 335 °C while lignin itself had the degradation temperature at 240–400 °C (Rong et al., 2009). Moreover, the laccase-mediator treatment of cellulose fibers and lignin filler demonstrated to improve the thermal degradation temperature of the starch biocomposite to the utmost level owing to enhancement of inter-molecular hydrogen bonds among biopolymers.

According to the residue after thermal degradation at 500 °C, the minimum percentage of residue at 7.76% was from the biocomposite containing only starch and water at 22% (w/w) water content. The percentages of residue were increased to 9.48% and 12.85% when 10 mM VA and 15% (w/w) fibers were added, respectively. Addition of 10% (w/w) lignin into starch biocomposite yielded the increase of the residue to 17.78% and addition of every component at 22% (w/w) water, 15% (w/w) fibers, 10% (w/w) lignin and 10 mM VA (Run#15) gave the highest biocomposite residue of 28.13%.

3.2.3. Water resistance

Testing of water resistance was performed when a drop of water was placed on a surface of a biocomposite specimen and the change of water drop shape was subsequently monitored over a period of time. Wettability of tested biocomposite is defined by a certain angle of contact between the water drop and composite surface. If the contact angle is lower than 90°, the biocomposite is wettable, and if the contact angle is wider than 90°, the sample is defined as non-wettable. A contact angle equal to zero indicates the complete wettability of biocomposite. From the experiments, the water drop test results were shown in Table 4. For the biocomposite sample made of only starch at 20% (w/w) water content without additives, i.e. fibers, lignin and VA, the wettability analysis was not able to detect within 60 s because it had rapid absorption of water into the surface of biocomposite. Thus, the neat starch biocomposite was extremely hydrophilic or it was water superabsorbent capacity. For the rest of tested samples, contact angle between a water drop and surface of biocomposite was measurable at 1 s and 60 s as demonstrated in Table 4.

Addition of 10% (w/w) lignin into biocomposite containing 22% (w/w) water, 15% (w/w) fibers and 10 mM VA substantially increased water resistance of biocomposite indicated by a significant increase of contact angle of water drop on the surface of biocomposite. As demonstrated in Table 4 (Nos. 1 and 2) and Fig. 4(a) and (b), the contact angle of a water drop was enhanced from 63.89° to 75.78° at 1 s of measurement for biocomposite made

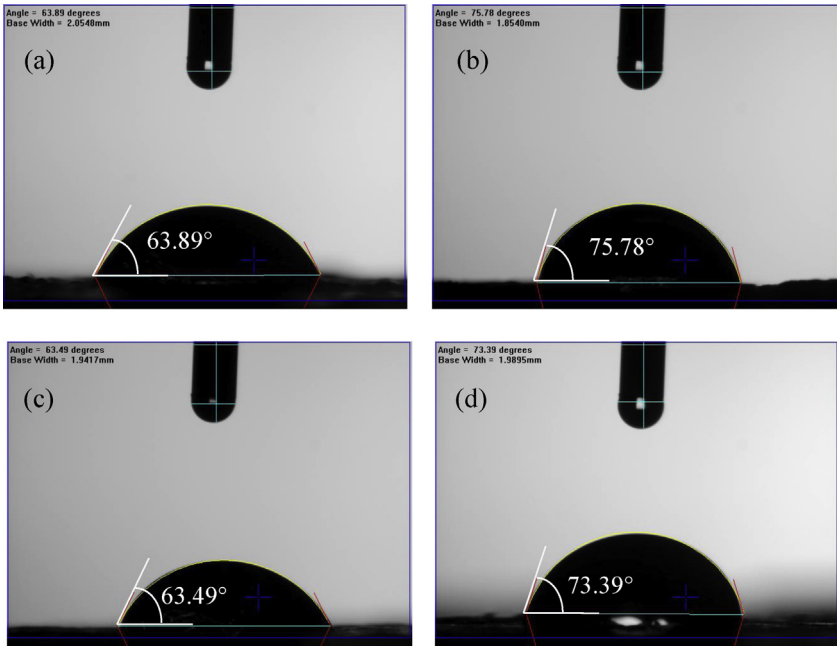


Fig. 4. The water drop contact angle at 1 s of starch biocomposites made of (a) 22% (w/w) water, 15% (w/w) fibers, 0% (w/w) lignin, 10 mM VA, (b) 22% (w/w) water, 15% (w/w) fibers, 10% (w/w) lignin, 10 mM VA, (c) 44% (w/w) water, 15% (w/w) fibers, 10% (w/w) lignin, 0 mM VA, (d) 44% (w/w) water, 15% (w/w) fibers, 10% (w/w) lignin, 10 mM VA.

of 0% (w/w) lignin and 10% (w/w) lignin, respectively. Considerable improvement of water resistance of biocomposite when adding lignin was additionally detected by the change of water contact angle from 1 s to 60 s as shown in Table 4. The results were in good correspondence with Baumberger and coworkers who found that lignin improved water resistance of starch biocomposite film, as long as no plasticizer is used (Baumberger et al., 1998). Similar result was previously reported that lignin significantly decreased the rate of water absorption (Stevents & Klamczynski, 2010).

Table 4 (Nos. 3 and 4) and Fig. 4(c) and (d) showed the effect of adding 10 mM VA into starch biocomposite indicating an increase of the contact angle of water drop from 63.49° to 73.39° at 1 s of angle measurement. The rate of absorption was little reduced from 1 sec to 60 sec or it was suggested that the water resistance of biocomposite was slightly improved when adding 10 mM VA into starch composite containing 44% (w/w) water, 15% (w/w) fibers and 10% (w/w) lignin as shown in Table 4 This was supported by the report of Huttermann and coworkers (1980) who hypothesized that laccase and mediator could be used in combination with spent sulfite liquor as an adhesive for particleboard (Huttermann, Herche, & Haars, 1980). In addition to cross-linking reaction, these authors claimed that the use of less polar milled wood lignin could increase the water resistance of boards in contrast to using more hydrophilic lignosulfonates and hydroxybenzotriazole (HBT) as a mediator required for the laccase mediator system. According to the result, it was determined that VA significantly assisted polymerization by radical coupling reaction and the laccase-VA system seemingly facilitated grafting of the phenolic compound or lignin onto fibers, thus the water resistance of the biocomposite was markedly improved. The results found were different from the results of Fillat and colleagues who found the laccase act as delignification agent with VA and as polymerization agent with p-coumaric acid (PCA) (Fillat et al., 2012).

There was no significant influence of water content between 22% (w/w) and 44% (w/w) on water drop shape for starch biocomposite made of 15% (w/w) fibers, 10% (w/w) lignin and 10 mM VA as shown in Table 4 (Nos. 2 and 4) and Fig. 4(b) and (d). An increase of water content in biocomposite from 22% (w/w) to 44% (w/w) only slightly decreased the contact angle from 75.78° to 73.39°. The rate of water absorption into biocomposite was not different for both composite specimens as demonstrated in Table 4. As a result, adding water in starch biocomposite had no significant effect on the contact angle or water resistance of biocomposite.

Furthermore, the preliminary water dissolution test of biocomposite was performed by immersion of composite from Run#15 (22% water, 15% fibers, 10% lignin and 10 mM VA) in water at 30 °C and 40 °C for 24 h. It was found that no significant appearance change found, however at 40 °C the composite started to absorb water sooner than the specimen tested at 30 °C. The shape of composite was not changed after dry once again at 105 °C and the weight losses were 1.2% and 3.4% for the water soluble test at 30 °C and 40 °C, respectively. Nevertheless, the systematic and intensive research will be carried on to improve composite's mechanical and physical properties such as water resistance by appropriate coating or grafting techniques.

4. Conclusion

Addition of fibers and violuric acid (VA) as mediator for laccase treatment significantly increased both modulus and flexural strength of starch biocomposite. The highest modulus and flexural strength of biocomposite was achieved from the biocomposite consisting of fibers, lignin and VA at a suitable proportion of ingredient and water content. Water resistance of biocomposite was considerably improved when adding fibers, lignin and subsequent treatment with laccase-VA system indicated by changes of water drop shape on the biocomposite surface. This system provided alternative means for production of non-food packaging or other short-lived materials derived from agro-polymers.

Acknowledgements

C. Sakdaronnarong is thankful to the financial support from the grant for new-career researcher, Mahidol University, Thailand. S. Narkchamnan is grateful to the research subsidy from Mahidol University Alumni Association, Thailand.

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