Preparation and Characterization of Cassava Leaves/ Cassava Starch Acetate Biocomposite Sheets

Noor Fadzliana Ahmad Sharif,[a] Saiful Izwan Abd Razak,[b,]* Wan Aizan Wan Abdul Rahman,[a] Nadirul Hasraf Mat Nayan,[c] Abdul Razak Rahmat,[a] and Mohd Yazid Yahya[d]

Biocomposite packaging sheets made of cassava leaves (CL) and cassava starch acetate (CSA) were successfully prepared in this study, and using the surface impregnation method, the sheets were able to obtain desirable properties. The CL sheets were impregnated with CSA at various concentrations to improve the sheets' performance. This newly developed packaging material exhibited low moisture uptake and had a viable tear index value when the CSA impregnation level was 6%. Moreover, the sheets' properties were comparable to that of available paper or plastic sheets, having low moisture uptake, good wetting time and tear strength, smooth sheet formation, and enhanced thermal stability. Using agro-based materials from cassava plants for packaging materials could reduce the dependency on paper- and plastic-based packaging. Suitable utilization of this material includes as bag, carton and wrap.

Keywords: Cassava leaves; Tapioca; Packaging; Starch acetate; Impregnation; Mercerization; Moisture absorption

*Contact information: a: Polymer Engineering Department, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia; b: IJN-UTM Cardiovascular Engineering Centre, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia; c: Department of Chemical Engineering, Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia, 86400 Batu Pahat, Johor, Malaysia; d: Centre for Composites, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia; *Corresponding author: sfl_izwan@yahoo.com*

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the most agronomically important cyanogenic crop and is particularly important as a food source. Cassava is a very rustic crop that grows well under marginal conditions that other crops could not survive in. Its flexible harvesting time and vegetative propagation *via* stem cutting make it an excellent food reserve. A large proportion of cassava varieties are drought tolerant, can be produced in degraded soils, and are resistant to the most important diseases and pests (Ceballos *et al.* 2004).

The roots of cassava are edible, and in some countries they are used as the main source of carbohydrates, especially in Africa. In addition to being used for food, the roots can be processed to produce starch and flour, which are useful in the fermentation industry for biotechnology (Jiang *et al.* 2013).The roots are also used in edible films and coatings (Tongdeesoontorn *et al.* 2012), as well as in biodegradable polymeric material (Regina and Andrade 2013). The importance of cassava leaves (CL) is comparable to its roots. In African countries, CL is the most consumed leaf and has become a highly valued, basic vegetable (Achidi *et al.* 2005). On the other hand, in Asian countries, the leaves are regarded as waste or just as a by-product of the harvested roots. Nonetheless, statistics from the Food and Agricultural Organization (FAO) revealed that the cassava plant was one of the top 10 most produced commodities in the world (Asante-Pok 2013).In Southeast Asia, the production of CL exceeded 70,000 metric tons/year, but only 7.0 of those metric tons were processed for utilization in the industrial field.

Certain applications of CL are associated with its hydrophobic, waxy surface, which could help form a water-repellent component that prevents the formation of water film. The amount of wax has been determined for several species of cassava and was found to be between 10 and 30 µg/cm^2 (Zinsou *et al.* 2006). Furthermore, to alleviate the enormous amount of waste, our current work was directed toward utilizing CL for industrial applications.

With the arrival of new technological approaches, the focus has shifted to widening the use of CL for newer applications with the aim of adding value; one of the interesting applications is for food packaging material. For a long time, most of the common food packaging materials were made of petroleum-based plastics. However, the increased use of synthetic packaging has led to serious ecological problems because of their non-biodegradability.

Although their complete replacement is near impossible to achieve, specific applications of agricultural resources for packaging could be feasible. Our previous study shows the potential use of CL for packaging materials. Initial treatment of the CL yields sheets with good mechanical integrity and improves the sheet making capability - without significant loss of the waxy components. Furthermore, our previous work also revealed that processed CL sheets contain very low levels of cyanoglucosides (<0.1 ppm) (Sharif *et al.* 2014).

It is encouraging to further enhance the CL sheets with conventional film-forming bio-based material such as cassava starch (CS). CS can be produced from the roots of cassava plants, and it consists of 17% amylose, which is responsible for its strong film-forming characteristics (Bangyekan *et al.* 2006). However, its strong hydrophilic nature prevents its application with other plastic products. In addition, unmodified CS film is brittle and weak, which leads to inadequate mechanical properties (Xu *et al.* 2004). However, the modification of CS through the acetylation process achieves hydrophobic cassava starch acetate (CSA) by the alteration of its molecular structure (Shogren 1996; Adebowale *et al.* 2006). According to the FAO/WHO Expert Committee on Food Additives, starches that have a degree of substitution (DS) that is low (0.01 to 0.2) do not cause any health-related effects and have been approved by the Food and Drug administration (FDA) for safe use in food applications (Murúa-Pagola *et al.* 2009). Interestingly, CSA has gained wide commercial importance in the fields of binding (Argüello-Guevara and Molina-Poveda 2013), film forming (Yun *et al.* 2008), thickening (Ashogbon and Akintayo 2014), stabilizing (Udomrati and Gohtani 2014), and texturing (Eguchi *et al.* 2014).

There have been no reported studies on biocomposite sheets made of cassava leaves/cassava starch acetate (CL/CSA). It was envisioned that the properties of processed CL sheets could be enhanced by the surface impregnation of CSA. Therefore, our primary goal was to impede the moisture activity of CL while preserving its mechanical properties. The CL was chemically treated using a conventional mercerization process, formed into sheets, and then impregnated with CSA at various concentrations. Our study showed that at a certain CSA concentration, the CL and CSA materials were able to form packaging sheets with desirable properties.

EXPERIMENTAL

Preparation of CL/CSA Sheets

Fresh picked, Malaysian-grown CL were cleaned by running tap water over them, and then they were stored at 4 °C in a refrigerator prior to use. Their mercerization and the sheet-making process are described in detail in our prior study (Sharif *et al.* 2014). Briefly, the CL were mercerized using a NaOH concentration of 15% for 24 h under ambient conditions. (Sharif *et al.* 2014). The CSA had a low DS (0.020) and was provided by Thanh Vu Co. Ltd, Vietnam. The CSA was mixed into methylene chloride (Merck) at various concentrations (2%, 4%, 6%, and 8% w/v), and the solution was mechanically stirred overnight at 50 rpm. The initially prepared CL were immersed into the solutions containing CSA for 5 s at under ambient conditions. The impregnated CL were then placed in a fume hood under ambient conditions to allow evaporation of the organic solvent. The steps for making the CL/CSA biocomposite sheets are depicted in Fig. 1.

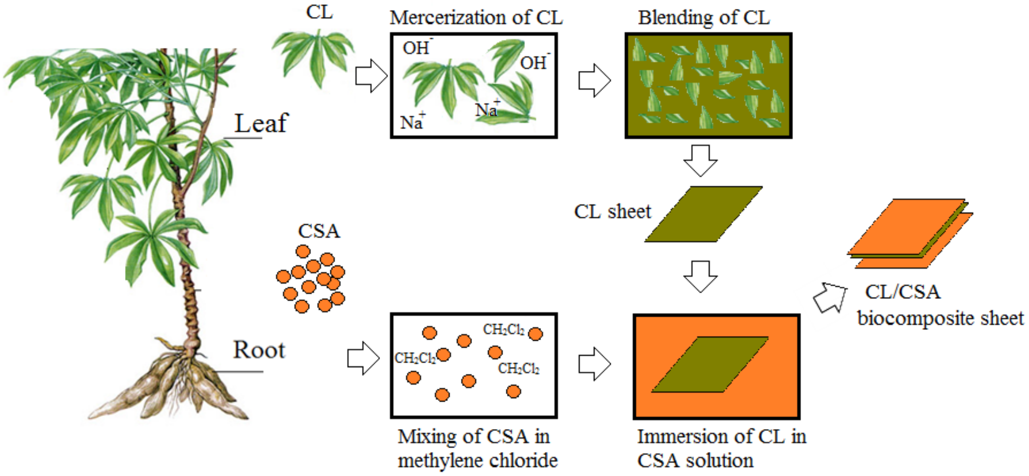

Fig. 1. Schematic diagram for the preparation of CL/CSA biocomposite sheets

Characterization

A moisture absorption test was done on triplicate CL/CSA biocomposite samples of 2 x 2 cm, in which the samples were placed on top of a wire mesh in five different relative humidity (RH) environments that were conditioned in desiccators in accordance with ASTM E-104-02 (2012) standards. The RH was controlled using saturated salt solutions of LiCl (11%), KCH_3CO_2 (25%), $Mg(NO_3)_2$ (53%), NaCl (75%), and K_2SO_4 (97%). Samples were weighed at intervals of six hours until they were saturated. The equilibrium moisture content at each water activity was calculated on a dry basis. The *moisture absorption* (%) was calculated using Eq. 1,

$$Moisture\ absorption\ (\%) = \frac{W_f - W_i}{W_i} \times 100\% \qquad (1)$$

in which W_f represents the final weight of the sample and W_i represents its initial weight (A samples was conditioned at a temperature of 80 °C before the measurement of its initial weight).

The water wetting test was performed on square CL/CSA biocomposite sheets with dimensions of 1 × 1 cm. A drop of water was allowed to fall from a burette with an approximate height of 1.0 cm from the sample. The time required for the water droplet to spread over the sample of 1 cm length was recorded with a stopwatch. At least three replications were done for each CL/CSA type.

A tear test was performed to evaluate the tearing properties of the processed sheets because it is the most common and important testing used in evaluating paper or sheet properties. The test was conducted using the Elmendorf Tear method according to ASTM D-1922 (2009). The capacity of the pendulum used in the Elmendorf tear tester was 12.3 g. The sample sheets were cut according to the standard's required dimensions and were dried at 60 °C for 24 h before testing. A slit was made at the center of the edge perpendicular to the tested direction. At least five replicates were done for each CL/CSA type. The tear index was calculated using Eq. 2,

$$Tear\ index = \frac{Average\ tearing\ forces\ (mN)}{Average\ grammage\ (\frac{g}{m^2})} \quad (2)$$

in which the *average tearing force* was calculated with Eq. 3.The number 16 refers to 1600-gf of instrument constant with a 0 to 100 scale. Number of plies is one.

$$Average\ tearing\ force\ (mN) = \frac{16 \times 9.81 \times Average\ scale\ \ reading}{Number\ of\ plies} \quad (3)$$

Fourier transform infrared (FTIR) spectra were obtained with KBr discs of the samples using a model 2000 Perkin Elmer (USA) spectrometer that had a resolution of 4.0 cm^{-1}. Scanning electron microscope (SEM) images were obtained using a Leo Supra 50VP (Carl Zeiss, Germany). Thermogravimetric analyses (TGA) were performed using a Perkin Elmer Pyris TGA7 (USA). The samples were heated from 30 °C up to 600 °C under nitrogen flow to observe the degradation steps of the samples at a heating rate of 10 °C /min.

RESULTS AND DISCUSSION

Moisture Absorption

Figure 2 indicates that the moisture absorption of all the biocomposite samples, including the control CL, increased almost linearly with increasing RH. Moisture absorption isotherms (moisture content vs. RH) normally have a sigmoidal shape. This shape can also be recognized from the experimental data in Fig. 2. Nevertheless, the maximum moisture uptakes of the control CL sheets were much lower than that of many reported cellulosic fiber-based kraft paper (>8%, reaching 16% for kapok) (Liu and Wang 2011). The low moisture uptake was attributed to the presence of wax on the CL, which provided an effective barrier that prevented the absorption of water molecules from the surrounding environment. Thickness of the control CL was measured to be around 226.6 microns. Meanwhile thickness of the CSA coating layers on the biocomposites were measured to be 7.8, 20.3, 42.6, 28.7 microns for 2, 4, 6, 8% CSA coating, respectively. The moisture gain decreased with increasing CSA concentrations up to 6%. The reason for this is that the CSA provided additional surface coverage on the CL surface, which interacted favorably with the adhered wax component. This reduced the environmental moisture uptake by its acetyl groups, thus increasing the CL's hydrophobic capability. These two stages of moisture activity barrier (CSA and wax) are preferable for packaging applications, mostly for replacing synthetic plastic films or sheets. The CL/8%CSA biocomposite sample, on the other hand, achieved a higher moisture uptake than the control CL. This might be due to non-uniform formation of CSA on the CL, which led to clusters and agglomerates. These CSA clusters and agglomerates might cause the CSA coating to detach of from the CL surface, as indicated by the reduction of the measured thickness for CL/8%CSA sample. Furthermore, the high CSA content decreased the CSA solubility in the methylene chloride, which eventually made it difficult to impregnate and form uniform layers on the CL. Plus, the immersion of CL into a solvent rich solution might have removed the adhered wax on the CL. Thus, more exposed CL surfaces and damage led to the opening up of new hydroxyl groups, which increased the moisture uptake through hydrogen bonding with surrounding water molecules (Razak *et al.* 2012).

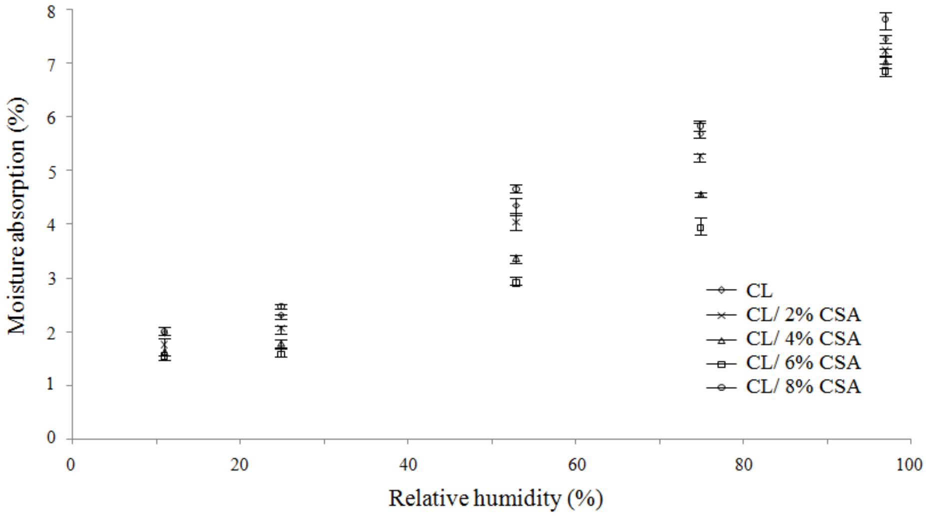

Fig. 2. Moisture absorption of CL/CSA biocomposite sheets with increasing RH

Wetting

The wetting times of the CL/CSA Biocomposite sheets are represented in Fig. 3.

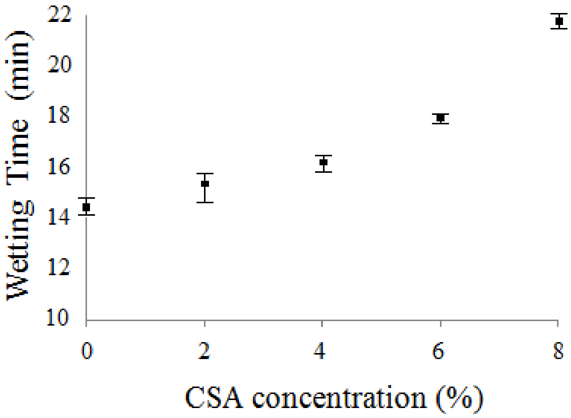

Fig. 3. Wetting time of CL/CSA biocomposite sheets with increasing CSA concentration

The control CL took about 14 min to wet. The wetting time needed for water to wet the CL/CSA sheets increased as the CSA concentration level increased. This corresponds to the presence of hydrophobic CSA, which decreased the surface tension of the CL by providing a high mismatch of surface tension between it and the water, thus making it more difficult for water molecules to wet the CL surface.

The significant jump of wetting time, up to 22 min (CL/8%CSA), can be explained by the relatively large CSA particles on the surface, which provided further hydrophobicity to the sheet. This can be observed in the SEM image shown in Fig. 5d. Some degree of hydrophobicity is preferable for packaging applications that require water retention.

Tear Index and Morphology

Figure 4 depicts the tear index trend of CL sheets impregnated with various concentrations of CSA. The control CL sheet exhibited a tear index value of about 784.7 mN·m^2/g. This can be attributed to the mercerization process of the CL, which renders a better crystalline structure (Bledzki and Gassan 1999) compared to the raw leaves. The SEM image of the control CL is shown in Fig. 5a.

With mercerization *via* sodium hydroxide, the CL became less dense and less rigid, thereby making the cellulose chains more capable of rearranging themselves and undergoing reorientation and recrystallization along the direction of the tear. Because waxes consist of very long-chain aliphatic mixtures with a broad chain length distribution, it is plausible that the processed CL was amorphous in large parts and exhibited properties of a soft material.

The tear index of the sheets showed marginal reduction from the control upon mercerization with 2% and 4% concentrations of CSA. This slightly poor resistance to crack propagation was due to the non-continuous formation of CSA, which could have introduced weak spots during the mechanical testing. However, the sample of CL/6%CSA revealed a higher tear index than the unimpregnated CL. At this CSA concentration, the CSA formed a continuous layer on the CL surface, which reinforced the sheet by impeding the tear propagation. Its corresponding electron micrograph (Fig. 5b) exhibited a relatively flat and smooth surface, which represents the CSA component.

Further magnification on the surface (Fig. 5c) revealed a uniform and packed CSA component on the CL, which supports the increased tear index. The minor tear index reduction of the CL/8%CSA sample compared to that of CL/6%CSA was caused by the detachment of the CSA particles from the surface, which conferred slightly poorer tear properties on the sheet. This is shown in the SEM image in Fig. 5d.

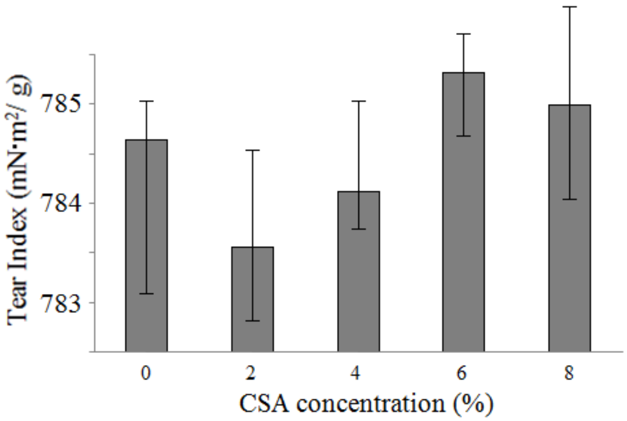

Fig. 4. Tear index of CL/CSA biocomposite sheets with increasing CSA concentration

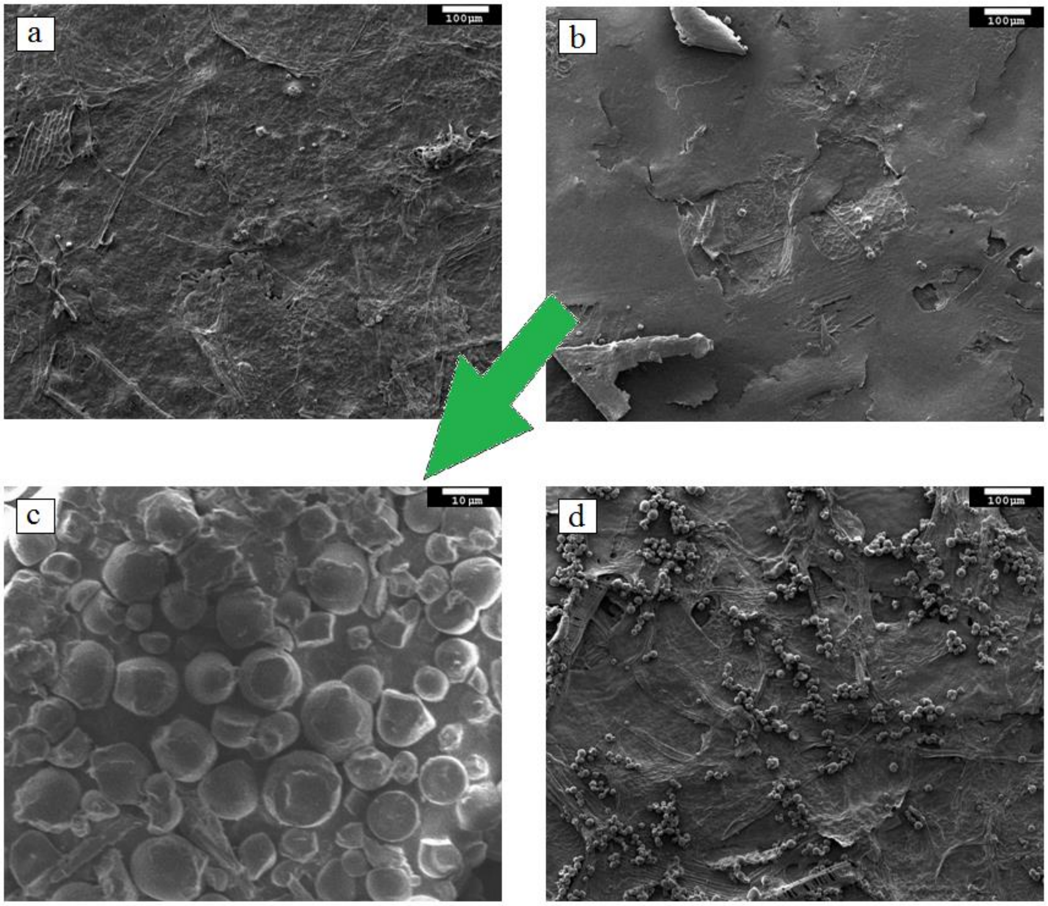

Fig. 5. SEM images of a) control CL sheet, b) CL/6%CSA, c) higher magnification of CL/6%CSA, and d) CL/8%CSA

FTIR

Green leaves are primarily composed of lignocellulosic components. The two most significant peaks for the FTIR analysis were at 3400 cm^{-1} and 1700 cm^{-1}. The spectrum of the control CL sheet in Fig. 6a showed a band at 3400 cm^{-1}, which represents the intermolecular and intramolecular H bonds of free OH in cellulose (Razak *et al.* 2013). The pronounced peak at 1700 cm^{-1} was the characteristic peak of the carbonyl (C=O) stretching vibration from the free carboxylic acid, esters, and fatty acids, which represents the constitutive compounds of leaf wax (da Luz 2006). The existence of this wax-associated peak was a sign that the wax was still present in the CL after being processed into sheets. The hydrophobic waxes on the CL surfaces formed a water repellent surface and thereby prevented the formation of a water film.

It can be seen that impregnation with 6% CSA (Fig. 6b) resulted in lower detection of the OH band as compared to the control CL. This is due to the CSA, which further limited the exposure of the sheets' cellulose surfaces. On the other hand, the peak at 1700 cm^{-1} (representing the acetyl groups of the CSA) was magnified with increasing impregnation level. This indicated that the CL surfaces were dominated by the hydrophobic functional group of the CSA.

The CL/8%CSA sample showed a similar IR pattern, except for the noticeable intensity of the OH band. This re-emergence of the hydroxyl vibration might have been due to processing difficulties, which may have led to the opening up of the cellulose surface. The proposed CL-CSA interactions are illustrated in Fig. 7. The functional group of the wax compound (methyl and carboxyl) provided a good interaction with the acetyl group of the CSA through H-bonding.

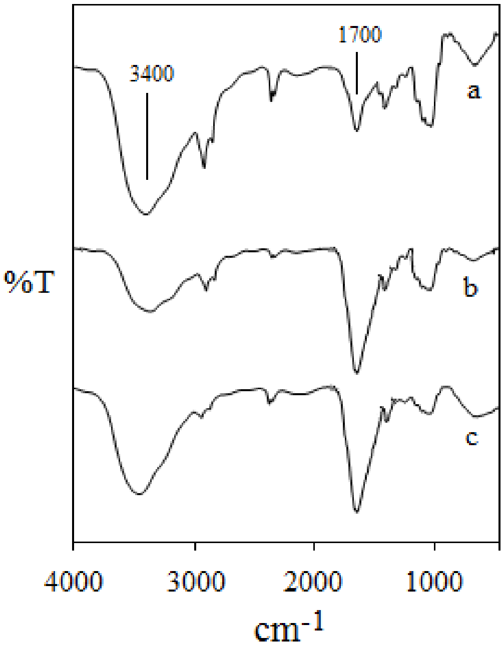

Fig. 6. FTIR spectra of a) control CL, b) CL/6%CSA, and c) CL/8%CSA

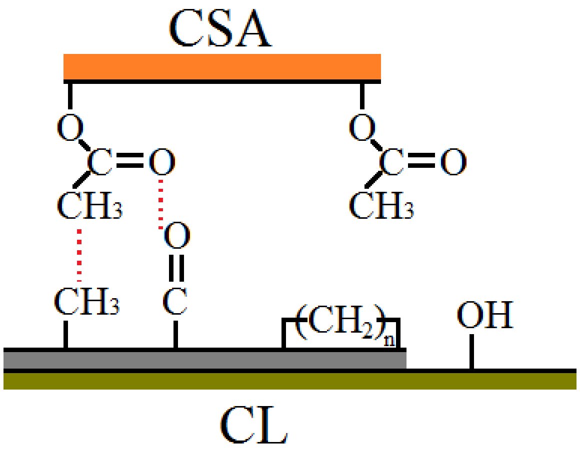

Fig. 7. Interactions between CL and CSA

TGA

Figure 8 shows the thermogravimetric profiles of the control CL and of the CL/6%CSA. Both of the samples showed a first step at 100 °C, which corresponded to the water evaporation and degradation of the short chain wax compound. The CSA component of the impregnated CL (Fig. 8b) degraded at around 340 °C (Parvathy and Jyothi 2012), followed by the relatively slow degradation of the lignocellulosic components of the leaves. It is believed that this delay in decomposition temperature was caused by the strong interaction between the CL and CSA. Thus, the CSA had a positive effect on the thermal stability of the resulting CL/CSA biocomposite sheets.

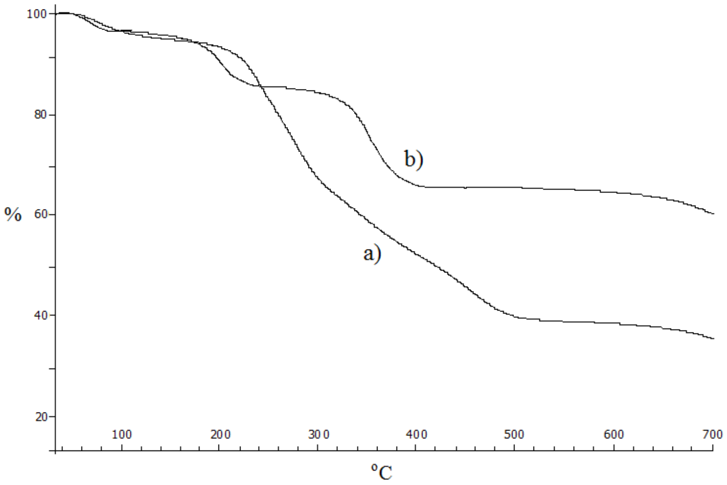

Fig. 8. TGA curves of a) control CL and b) CL/6% CSA

CONCLUSIONS

1. Processed CL has the potential to be used as a packaging material when it is impregnated with CSA. A mild CSA concentration (6%) proved to be suitable for the preparation of CL/CSA biocomposite sheets. This was established by the CL/6%CSA samples' low moisture uptake, reasonable wetting time, good tear strength, uniform sheet formation, feasible CL-CSA interaction, and enhanced thermal stability.
2. The ease of forming these sheets, as well as their economic value, present high potential for these sheets being produced on a large scale. This new approach shows promise for packaging applications that require the utilization of bio-derived resources and must be economically cost-effective. Even so, more studies should be done to investigate the viability of replacing plastic or paper packaging.

ACKNOWLEDGMENTS

The authors would like to thank Universiti Teknologi Malaysia and Ministry of Education Malaysia for providing financial assistance for this research.

REFERENCES CITED

Achidi, A. U., Ajayi, A. O., Bokanga, M., and Maziya-Dixon, B. (2005). "The use of cassava leaves as food in Africa," *Ecol. Food Nutr.* 44(6), 423-435. DOI: 10.1080/03670240500348771

Adebowale, K. O., Afolabi, T. A., and Olu-Owolabi, B. I. (2006). "Functional, physicochemical and retrogradation properties of sword bean (*Canavalia gladiata*) acetylated and oxidized starches," *Carbohyd. Polym.* 65(1), 93-101. DOI: 10.1016/j.carbpol.2005.12.032

Argüello-Guevara, W., and Molina-Poveda, C. (2013). "Effect of binder type and concentration on prepared feed stability, feed ingestion and digestibility of

Litopenaeus vannamei broodstock diets," *Aquacult. Nutr.* 19(4), 515-522. DOI: 10.1111/anu.12003

Ashogbon, A. O., and Akintayo, E. T. (2014). "Recent trend in the physical and chemical modification of starches from different botanical sources: A review," *Starch-Stärke* 66(1-2), 41-57. DOI: 10.1002/star.201300106

ASTM D1922-09 (2009). "Standard test method for propagation tear resistance of plastic film and thin sheeting by pendulum method," ASTM International, West Conshohocken, PA. DOI: 10.1520/D1922-09

ASTM E104-02 (2012). "Standard practice for maintaining constant relative humidity by means of aqueous solutions," ASTM International, West Conshohocken, PA. DOI: 10.1520/E0104-02R12

Bangyekan, C., Aht-Ong, D., Srikulkit, K. (2006). "Preparation and properties evaluation of chitosan-coated cassava starch films," *Carbohyd. Polym.* 63(1), 61-71. DOI: 10.1016/j.carbpol.2005.07.032

Bledzki, A. K., and Gassan, J. (1999). "Composites reinforced with cellulose based fibres," *Prog. Polym. Sci.* 24(2), 221-274. DOI: 10.1016/S0079-6700(98)00018-5

Ceballos, H., Iglesias, C. A., Perez, J. C., and Dixon, A. G. (2004). "Cassava breeding: Opportunities and challenges," *Plant Mol. Biol.* 56(4), 503-516. DOI: 10.1007/s11103-004-5010-5

da Luz, B. R. (2006). "Attenuated total reflectance spectroscopy of plant leaves: a tool for ecological and botanical studies," *New Phytol*. 172(2), 305-318. DOI: 10.1111/j.1469-8137.2006.01823.x

Eguchi, S., Kitamoto, N., Nishinari, K., and Yoshimura, M. (2014). "Effects of esterified tapioca starch on the physical and thermal properties of Japanese white salted noodles prepared partly by residual heat," *Food Hydrocolloid*. 35, 198-208. DOI: 10.1016/j.foodhyd.2013.05.012

Jiang, L., Zhu, L., Xu, X., Lin, M., Li, Y., Li, X., and Huang, H. (2013). "Bioproduction of hydrogen by simultaneous saccharification and fermentation of cassava starch with 2-deoxyglucose-resistant mutant strains of *Clostridium tyrobutyricum*," *Int. J. Hydrogen Energ*. 38(15), 6349-6356. DOI: 10.1016/j.ijhydene.2013.02.109

Liu, J., and Wang, F. (2011). "Influence of mercerization on micro-structure and properties of kapok blended yarns with different blending ratios," *J. Eng. Fiber Fabr*.6(3), 63-68.

Murúa-Pagola, B., Beristain-Guevara, C. I., and Martínez-Bustos, F. (2009). "Preparation of starch derivatives using reactive extrusion and evaluation of modified starches as shell materials for encapsulation of flavoring agents by spray drying," *J. Food Eng*. 91(3), 380-386. DOI: 10.1016/j.jfoodeng.2008.09.035

Parvathy, P. C., and Jyothi, A. N. (2012). "Synthesis, characterization and swelling behaviour of superabsorbent polymers from cassava starch-*graft*-poly(acrylamide)," *Starch-Stärke* 64(3), 207-218. DOI: 10.1002/star.201100077

Razak, S. I. A., Rahman, W. A. W. A., Hashim, S., and Yahya, M. Y. (2012). "In situ surface modification of natural fiber by conducting polyaniline," *Compos. Interf.* 19(6), 365-376. DOI: 10.1080/15685543.2012.733178

Razak, S. I. A., Rahman, W. A. W. A., Hashim, S., and Yahya, M. Y. (2013). "Enhanced interfacial interaction and electronic properties of novel conducting kenaf/polyaniline biofibers," *Polym-Plast. Technol*. 52(1), 51-57. DOI: 10.1080/03602559.2012.718401

Regina, F. O., and Andrade, C. T. (2013). "Processing and properties of cassava starch/maleated polybutadiene/organoclay hybrids," *Int. J. Polym. Mater.* 62(6), 362-368. DOI: 10.1080/00914037.2011.641650

Sharif, N. F. A., Razak, S. I. A., and Rahman, W. A. W. A. (2014). "Cassava leaves as packaging materials," *Cell. Chem. Technol*. 48(5-6), 585-590.

Shogren, R. L. (1996). "Preparation, thermal properties and extrusion of high-amylose starch acetates," *Carbohyd. Polym*. 29(1), 57-62. DOI: 10.1016/0144-8617(95)00143-3

Tongdeesoontorn, W., Mauer, L. J., Wongruong, S., Sriburi, P., and Rachtanapun, P. (2012). "Mechanical and physical properties of cassava starch-gelatin composite films," *Int. J. Polym. Mater*. 61(10), 778-792. DOI: 10.1080/00914037.2011.610049

Udomrati, S., and Gohtani, S. (2014). "Enzymatic esterification of tapioca maltodextrin fatty acid ester," *Carbohyd. Polym.* 99, 379-384. DOI: 10.1016/j.carbpol.2013.07.081

Xu, Y., Miladinov, V., and Hanna, M. A. (2004). "Synthesis and characterization of starch acetates with high substitution," *Cereal Chem*. 81(6), 735-740.

Yun, Y., Wee, Y., Byun, H., and Yoon, S. (2008). "Biodegradability of chemically modified starch (RS4)/PVA blend films: Part 2," *J. Polym. Environ.* 16(1), 12-18. DOI: 10.1007/s10924-008-0084-9

Zinsou, V., Wydra, K., Ahohuendo, B., and Schreiber, L. (2006). "Leaf waxes of cassava (*Manihot esculenta* Crantz) in relation to ecozone and resistance to Xanthomonas blight," *Euphytica* 149(1), 189-198. DOI: 10.1007/s10681-005-9066-3

Article submitted: January 27, 2015; Peer review completed: April 19, 2015; Revised version received: April 26, 2015; Accepted: May 12, 2015; Published: May 28, 2015.
DOI: 10.15376/biores.10.3.4339-4349