Journal of Applied Polymer Science

Tara tannin as active ingredient in electrospun fibrous delivery system

Jackapon Sunthornvarabhas,[1] Siriluck Liengprayoon,[2] Chahinez Aouf,[3] Walaiporn Rungjang,[4] Kunruedee Sangseethong,[1] Jerome Lecomte,[5] Thongchai Suwonsichon,[6] Chutima Boonreungrod,[6] Eric Dubreucq,[5†] Helene Fulcrand[3†]

[1]National Center for Genetic Engineering and Biotechnology, Cassava and Starch Technology Research Unit, Bangkok 10900, Thailand

[2]Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Bangkok, Thailand

[3]UMR1083, INRA, Montpellier SupAgro, University Montpellier 1, 2 place Viala 34060 Montpellier Cedex 2, France

[4]Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand

[5]UMR 1208 IATE, CIRAD Dept. Persyst, TA B-62/16, 73 Rue JF Breton 34398 Montpellier Cedex 5, France

[6]Department of Product Development, Kasetsart University, Bangkok 10900, Thailand

E. Dubreucq and H. Fulcrandc have equal contribution.

Correspondence to: J. Sunthornvarabhas (E-mail: jackapon.sun@biotec.or.th)

ABSTRACT: This study evaluated the releasing performance of tara tannin, a cocktail of plant polyphenols, incorporated in submicron fiber, produced by the electrospinning process. Polylactic acid was used as a polymer matrix that carried two loading levels of tara tannin, 14.3 and 22.3% dry weight in the final product. The fiber diameter of composite fibers was in the range 500–700 nm. The release of tara tannin was controlled by material attachment as there was no evidence of chemical bonding between materials. This was further confirmed by FTIR and DSC. From the five combinations of acid that were presented in tara tannin, galloylquinic acid, with the smallest molecular weight composition, was released in the largest proportion (%molar) and exhibited antioxidant activity. This was confirmed by 2,2-diphenyl-1-picrylhydrazyl assay and HPLC-MS analyses. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43646.

KEYWORDS: drug delivery systems; electrospinning; fibres; properties and characterization

Received 16 December 2015; accepted 10 March 2016

DOI: 10.1002/app.43646

INTRODUCTION

Tannins are categorized as polyphenols and contain a bioactive compound that has gained considerable attention over the past years. Primary application of this material relates to products that benefit human health because of its antioxidant activity. Many techniques have been developed to preserve its properties and to make it suitable for application due to its sensitivity. Each encapsulation technique has a different process mechanism, loading capacity, and product characteristic. Recently, the electrospinning process has gained popularity in polyphenol encapsulation technology. By implementing the concept of encapsulation, polyphenols can be captured inside the fiber matrix.

Electrospinning[1,2] is a process that utilizes electrical force to stretch a polymer jet into thin, dry fiber. The process consists of three primary components: (i) a high-voltage power supply to create a voltage difference between the fiber collector and the polymer jet nozzle; (ii) a metal nozzle and polymer delivery system; (iii) a fiber collector which is a conductive plate connected to ground or counter polarity to a nozzle that can attract and guide the charged polymer jet. During flight, the continuous-charged liquid jet becomes thinner which promotes solvent evaporation due to an increased jet surface area. At optimum, dry fibers are collected at the collector as either a nonwoven or a woven sheet which can be achieved through modification of the collector. The average fiber diameter can be controlled by multiple factors such as polymer solution properties and fabrication conditions. The effects of these parameters have already been well established.[3] Study of material releasing from electrospun nanofibers has advanced in many aspects. One of the promising directions is the ability to control releasing profile that match to the application. Numbers of current study reported an interesting finding in single fluid electrospinning study.[4–6] These studies use novel modification to fabrication process to control drug model releasing profile. The concept allows ability to prolong releasing period that is suitable for multiple application.

A fibrous delivery system for polyphenol was studied to determine its potential for delivering polyphenol into a targeted area and exhibit antioxidation activity. Varieties of polyphenol were investigated such as green tea extract,[7,8] *Stryphnodendron* astringent bark extract,[9] *Garcinia mangostana*,[10] and anthocyanin-rich, red raspberry.[11] These studies used different polyphenols as the drug model and considered them as a single material and evaluated performance. Unlike other polyphenols, tara tannin consists of multiple combinations and cannot be considered as a single material as a drug model for the investigation. Therefore, it was necessary to treat it as a group or combination and to re-evaluate its performance individually.

This study evaluated the delivery mechanism for each combination of tara tannin and identified the component from the cocktail of polyphenols that exhibited the major contribution to antioxidation activity. We found that the combination with the smallest molecular weight was released in the largest proportion (%molar). Even though all materials were not fully released from the fibrous matrix, the sheet of composite fibers exhibited antioxidation activity.

EXPERIMENTAL

Materials

Polylactic acid (PLA) 200,000 MW in white pellet form (PLA 2002 D, Nature Work LLC), tara tannin (TT) in brown powder form (Silvateam s.p.a, Italy), and analytical grade dichloroethane (DCE) and dimethylsulfoxide (DMSO) were purchased from Sigma Aldrich (Singapore); gallic acid as reference material and other materials to perform DPPH assay using UV–VIS spectroscopy, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and high-performance liquid chromatography (HPLC analysis) were purchased from Sigma Chemical (MO, USA).

PLA/TT Mixed Solution Preparation. Polylactic acid was dissolved in dichloroethane at 15% by weight. Tara tannin was dissolved in dimethylsulfoxide at 10% by weight. Both solutions were prepared separately by shaking at room temperature until a homogeneous solution was observed. Two mixing levels were used in this study by mixing polylactic acid solution at 10 g with tara tannin solution at 2.50 g and 4.29 g. Each mixed solution was prepared by shaking all components at room temperature for 1 h. The final amounts of tara tannin in dry electrospun nanofibers were 14.3% and 22.3% by dry weight, respectively.

PLA/TT Electrospun Nanofibers Preparation. A single-nozzle electrospinning setup was used. A high-voltage power supply (Gamma High Voltage, ES60P-10W, USA) was attached to a metal nozzle to create a voltage difference of 15 kV between the metal nozzle and the grounded collector. A charged metal nozzle with 0.55 mm outer diameter was placed 20 cm directly above the center of a 20 × 20 cm aluminum, flat, grounded collector which was suspended on a frame of polyvinylchloride pipe (PVC). A mechanical pump (Multi-Phaser, NE-4000, USA) was used to deliver the mixed solution from a plastic syringe to the metal nozzle at a rate of 20.0 μL/min. The operating conditions were fixed to avoid complexity in the study.

Sample Characterization

Fiber Size Determination. A scanning electron microscope (SEM) model JSM-6400 (JEOL Ltd., Tokyo, Japan) was used to characterize the electrospun nanofibers characteristics and size. The average fiber diameter and standard deviation (SD) were determined using the log normal length–weight fiber diameter distribution. The fiber diameter and length of fiber were paired in the calculation. A minimum of 100 pairs were used and normalized to obtain the plot between the frequency distribution and the cumulative frequency distribution of fiber diameter for average fiber diameter. Difference between average fiber diameters was determined by two-sample *t*-test.[12]

Material Interaction between PLA and TT Molecules. Fourier transform infrared spectroscopy (FTIR) was used to evaluate the chemical interaction between the molecules within the sheet of electrospun nanofibers. FTIR spectroscopy was performed using a Bruker Alpha-E spectrometer (Bruker Optics Inc., Ettlingen, Germany). Spectral acquisition and instrument control were performed using the OPUS 6.5 (Bruker Optics Ltd, Ettlingen, Germany) software and analyzed using the CytoSpec software (Cytospec Inc., Berlin, Germany). A spectrometer with the detector at 4 cm^{-1} resolution and 64 scans per sample was used.

A differential scanning calorimeter (DSC) was used to evaluate the blending between molecules within the sheet of electrospun nanofibers. Physical interaction was evaluated by investigating the glass transition temperature (T_g) of each component within the sheet of electrospun nanofibers. The glass transition temperature was measured using a PerkinElmer DSC 8000 (PerkinElmer Inc., MA, USA). Analytical and instrument control were performed using the Pyris software 11.0 (PerkinElmer Inc., MA, USA). Sheet of fibers with a weight of 1.8 mg and a minimum of five replicates per sample were heated from 5 to 200 °C at a rate of 10 °C/min. The results were interpreted from the second heating session to avoid traces of impurities. All heating and cooling sessions used the same heating rate. The glass transition temperature was determined from the inflection point.

Identification of Released Compound from Nanofiber. A sheet of PLA/TT electrospun nanofibers (14.3%) with a weight of 5 mg was placed in the Eppendorf concentrator (FisherScientific, USA) and filled with 250 μL of distilled water. Supernatants were collected at different releasing periods and stored at −20 °C for DPPH assay and HPLC-MS analyses.

Antioxidant Activity of Released Compound from Nanofiber. Each sample solution was prepared by mixing 20 μL of supernatant with 80 μL of DMSO. Tara tannins and gallic acid dissolved in DMSO at a concentration of 0.01 mg/mL were used as a reference to evaluate the antioxidant activity of the released compound. To determine the antioxidant power, 100 μL of each sample solution was mixed thoroughly with 100 μL of 0.25 mM DPPH in DMSO. The decrease in the DPPH absorbance was monitored at 517 nm from 1 to 60 min reaction time using a Spectra Max190 microplate reader (Molecular Devices, USA). The antioxidant power of the released tara tannin from each period was expressed as %DPPH remaining at 60 min of reaction time. Analysis was performed three times to ensure accuracy. Tukey's range test[12] was used to identify difference between antioxidant power at each releasing period.

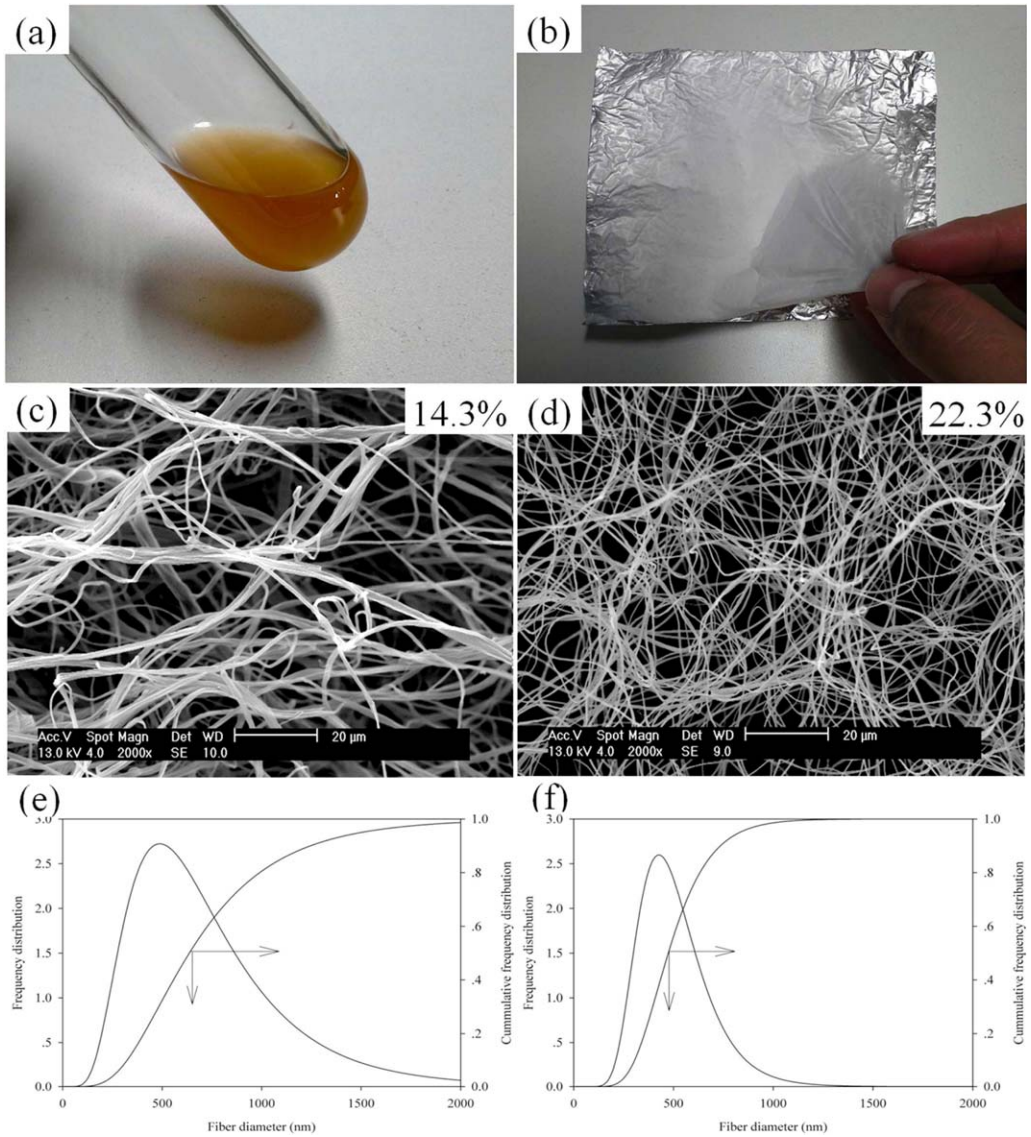

Figure 1. Upper row, images of mixed solution between tara tannins and polylactic acid (a) before and (b) after electrospinning process. Middle row, images of electron micrograph of composite fiber at different loadings: (c) for 14.3% and (d) for 22.3%. Bottom row, images of fiber size distribution and accumulative fiber size distribution calculated by length–weight analysis for each condition: (e) for 14.3% and (f) for 22.3%. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Identification of Tara Tannin. The presence of tara tannin in supernatant obtained after 24 h incubation was confirmed using HPLC-MS. The HPLC (Waters, Milford, MA) equipped with a diode array detector (DAD) and a single quadrupole mass detector was used to analyze the released tara tannin from the fibrous matrix. A sample of 15 μL was injected into the XSelect CSH C18 column (3.5 × 2.1 × 150 mm, Waters, Milford, MA). The gradient conditions of the mobile phase used were: solvent A (H_2O/HCOOH, 99.9/0.1, v/v), solvent B (CH_3CN/HCOOH, 99.9/0.1, v/v); initial 1% B; 0–10 min, 15% B linear; 10–15 min, 20% B linear; 15–22 min, 40% B linear; 22–25 min, and 1% B linear; 25–30 min. The flow rate was set at 0.25 mL/min. The DAD was set at 190–600 nm (λ_{max} of phenolic compounds was 280) and mass spectra were acquired using electrospray ionization in the positive mode and recorded in a mass range of 150–1000. In source, the capillary voltage was set at 4 kV. The nebulizer pressure was 44 psi, and the temperature and flow of drying gas were set at 200 °C and 12 L/min, respectively. Analysis was performed three times to ensure accuracy. Quantitative comparison of the released component was performed by calculating the percentage of the peak area for each component which was released in a specific release period from the fibrous matrix per summation of the overall peak area of the studied component. Tukey's range test[12] was used for determining difference of observed pattern of material release from different releasing period.

RESULTS AND DISCUSSION

Fabrication of Fibrous Composite

Tara tannin was unable to form fibers due to insufficient molecular entanglement similar to other macromolecules.[13,14]

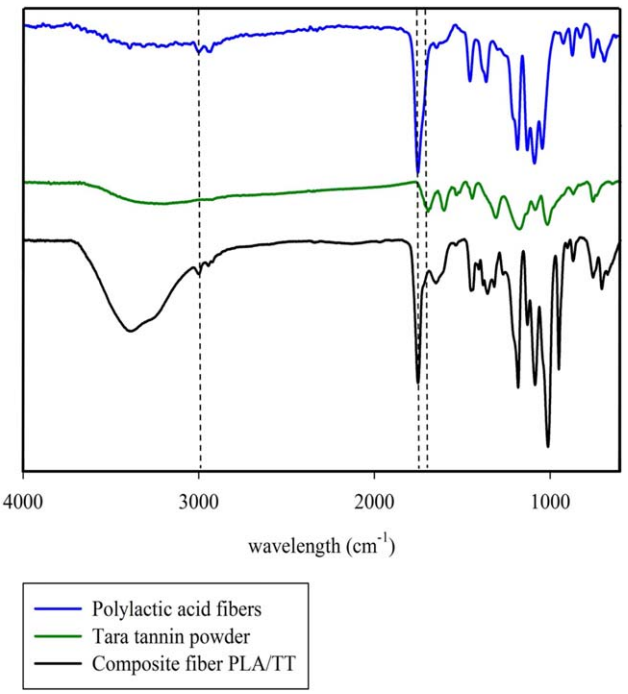

Figure 2. FTIR spectra of materials and sheet of composite nanofibers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Therefore, a high-molecular-weight, long-chain polymer was used as a backbone to assist fiber forming.[15] In this work, polylactic acid was selected as the platform matrix due to its excellent mechanical properties and biodegradation. The morphological characteristics of the composite nanofiber were evaluated under the scanning electron microscope as shown in Figure 1(c,d). These fabrication conditions were optimized to ensure smooth and round fibers for all mixed solutions. An average fiber diameter was determined by the length–weight distribution as shown in Figure 1(e,f). Composite with tara tannin loading at 14.3% and 22.3% had average fiber diameters of 640 ± 100 and 480 ± 100 nm, respectively. Even though wide fiber diameter distribution was observed in Figure 1(e,f), statistical analysis was used to determine difference between average fiber diameters. Two-sample *t*-test was used in calculation; $S^2_{14.3\%}=1.688$, $S^2_{22.3\%}=1.973$, $n_{14.3\%}=102$, and $n_{22.3\%}=104$. It was found that $t_0=0.864 < t_{\alpha=0.20}=1.282$. This implied most of the fibers, 80% of total fiber, were statistically difference even though wide fiber distribution was observed. Differences in the fiber size between the two conditions were caused by different amounts of solvent in the mixed solution. At 22.3% loading, the amount of dimethylsulfoxide was greater than that in the 14.3% formula. The greater liquid portion in the charged liquid jet allowed the jet to stretch further during its flight path and resulted in a fiber diameter reduction. Figure 1 shows the homogeneous mixed solution and fiber characteristics. Weight per unit area was calculated from (i) fabrication period 4 h, (ii) polymer feed rate 20 μL/min, (iii) estimated polymer density (1.4 g/cc), (iv) solid content per volume or porosity (15%),[16] and (v) size of collected sample (10 cm in diameter). Weight per unit area of sheet of fiber was calculated to be 0.013 g/cm^2.

Interaction between Polylactic Acid and Tara Tannin

The FTIR spectra revealed no evidence of new peaks in the spectrum of the blend composite and all dominant peaks were observed in blend composite as shown in Figure 2. Peak CH_3 stretching at 2950 cm^{-1} was observed in pure polylactic acid and blend composite. Peak C═O overtones were observed in the 3500 cm^{-1} region but had low intensity in comparison to other peaks.[17] The broad spectrum in the region 3000–3500 cm^{-1} indicated a hydroxyl group from tara tannin.[18] Strong intensity peaks consisting of the carbonyl group from tara tannin, unsaturated ester (1730 cm^{-1}), and polylactic acid, saturated aliphatic ester (1750 cm^{-1}), appeared in the blend composite. This result suggested that there was no covalent bonding between polylactic acid and tara tannin. Material adsorption was possibly primary attributed by intermolecular interaction.

The DSC thermogram was used to identify the glass transition temperature of the blend composite. For the miscible blend composite, the increasing glass transition demonstrated molecular entanglement between materials. For the immiscible blend composite, two or more distinctive glass transition temperatures could be identified.[19] The thermograph of this study revealed two glass transition temperatures. This result suggested molecules of polylactic acid and tara tannin was not blended miscibly even though the physical appearance suggested a homogeneous blending between the two materials. From these results, molecules of polylactic acid and tara tannin were blended and attached during fiber forming. Therefore, releasing tara tannin from the fibrous was not chemically controlled. Figure 3 presents the glass transition temperature of each material and values are reported in Table I.

Cui *et al.*[20] compared the glass transition of polylactic acid film and electrospun nanofibers. Differences in the glass transition

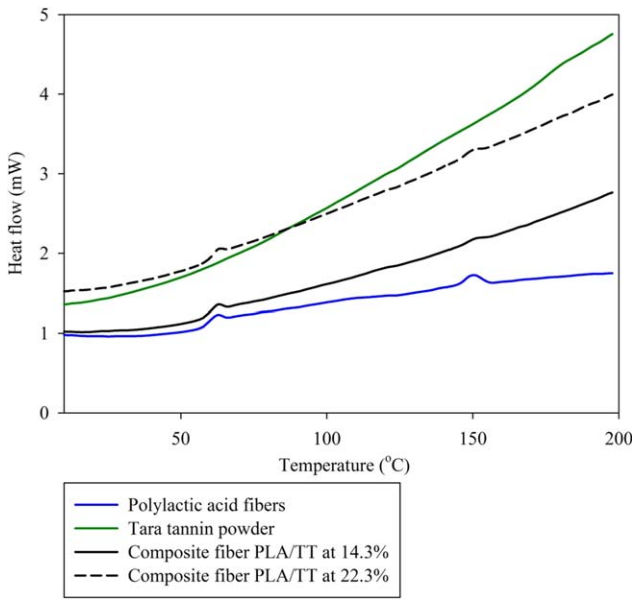

Figure 3. DSC thermograph of glass transition temperature of two materials within sheet of composite nanofibers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. Glass Transition Temperatures (°C)

Glass transition of polylactic acid	
Pure polylactic acid fiber	55.76 ± 0.21
Composite 14.3%	55.52 ± 0.72
Composite 22.3%	56.52 ± 0.48
Glass transition of tara tannin	
Pure tara tannin powder	167.76 ± 2.27
Composite 14.3%	142.02 ± 0.76
Composite 22.3%	142.31 ± 0.94

temperature between the two formats of film ($T_g = 61.7$ °C) and electrospun nanofibers ($T_g = 54.6$ °C) were due to changes in the molecular alignment caused by the inner stress from the electrospinning process. A similar case was reported for the effect of processing on the molecular structure that was detected by DSC. Hossain *et al.*[21] reported that the glass transition of polylactic acid microfiber did not show any significant difference after the fiber forming process or melt drawing process, and concluded the process was insufficient to cause any molecular alteration to the material that could be detected by DSC. These works reported the effect of molecular alignment and orientation on the glass transition temperature for pure material. In the same work, Cui *et al.*[20] reported a change in the glass transition temperature for miscible blend composite between polylactic acid and paracetamol, especially with variation in the paracetamol content. The higher loading content influenced the molecular chain alignment and orientation and ultimately affected the composite glass transition temperature for the miscible blend composite. For tara tannin, the results showed a similar glass transition temperature when the loading of tara tannin was increased from 14.3% to 22.3% by weight. In this work, loading of additional component did not alter the glass transition temperature when different loadings were investigated. This was due to the two materials not forming complete blending as appeared from the two glass transition temperatures. In conclusion, a reduction in the glass transition temperature for tara tannin was caused by inner stress acting upon the tara tannin molecules by electrical force from the electrospinning process and causing molecular alignment with a different molecule orientation.

To date, there have been no available records on the study of the molecular structure of the released components from an electrospun fibrous matrix. Further study is required to identify the limitations of the electrospinning fabrication process that affect the macromolecular properties through changes in the molecular orientation to preserve the performance and properties of the active ingredient in an electrospun fibrous matrix.

Characteristics of Released Tara Tannins from Fibrous Matrix

The tara tannin composition in a supernatant obtained from a fibrous matrix at different releasing periods was determined using HPLC-MS. The UV chromatogram of tara tannin at 280 nm [Figure 4(a)] as well as the molecular ions [Figure 4(b)] detected in the mass detector were used for identification. Instead of sugar as a core unit, tara tannin is based on a quinic acid (Q) linked to a different number of galloy moieties (G).[22] In this fibrous matrix, five combinations between these two units were detected: i.e., galloylquinic acids (Q-G, m/z 345), digalloylquinic acids (Q-2G, m/z 497), trigalloylquinic acids (Q-3G, m/z 649), tetragalloylquinic acids (Q-4G, m/z 801), and

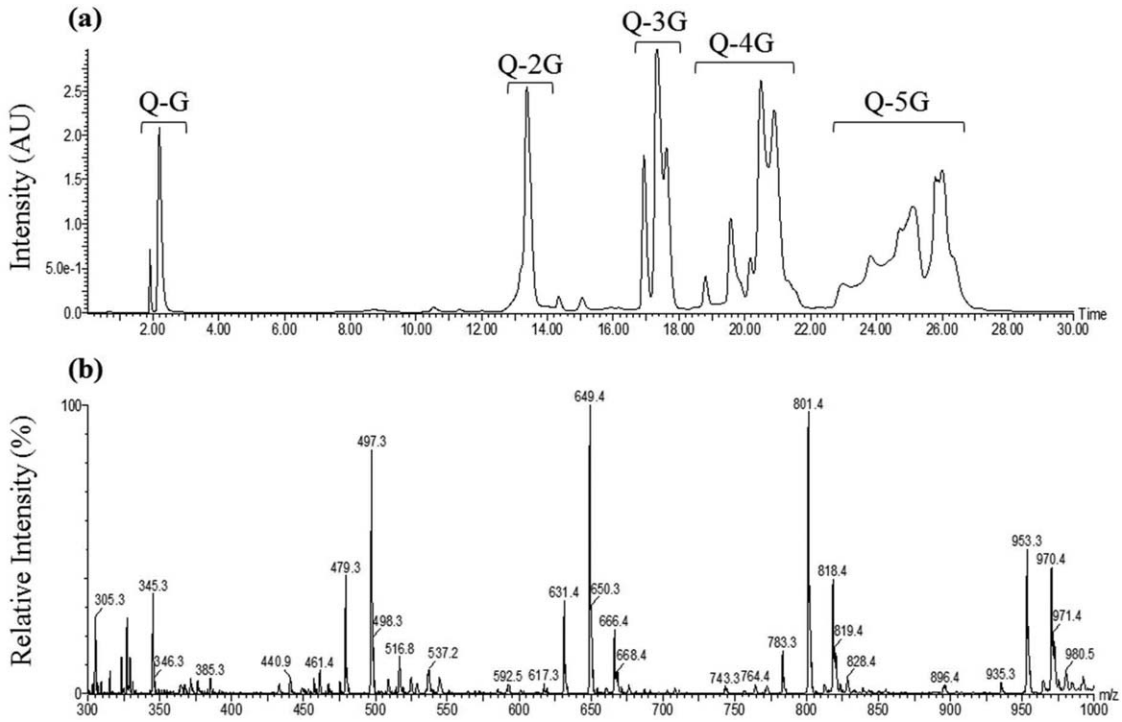

Figure 4. (a) UV chromatogram of released tara tannins at 280 nm after 60 min submerged in deionized water and (b) mass spectrum from positive ESI-MS in 300–1000 mass range.

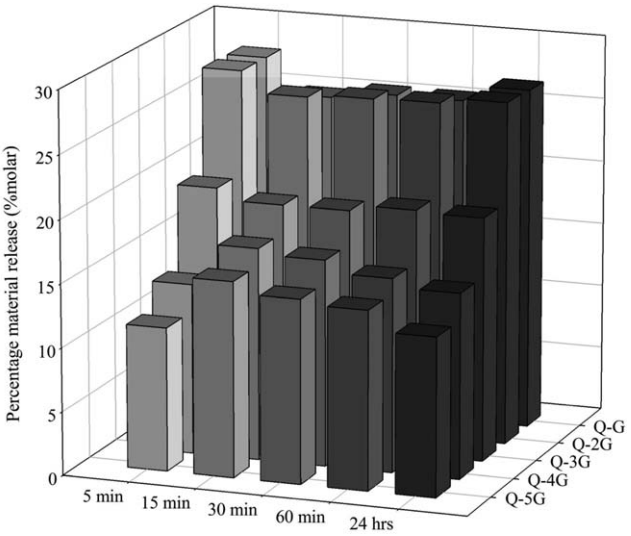

Figure 5. Relative molar proportions of released molecules of tara tannin from fibrous composite at different releasing periods.

pentagalloylquininc acids (Q-5G, m/z 953). These structures were recognized by the 152 amu difference which corresponded to a galloyl unit.[18,22]

Due to the heterogeneity and different degree of polymerization of tara tannin structures, the releasing molecules of tara tannins at different periods were relatively compared using the integrated intensity of each identified tannin structure (Figure 5 and Table II). The results indicated that the most abundant released molecular structure of tara tannin was Q-G (345 m/z 345) while a monomer Q-5G unit (m/z 801) was the smallest proportion observed at all periods (Figure 5). From the results, tara tannin seemed to release from the matrix in the same manner for all releasing periods as confirmed by Tukey's range test which identified difference between molar percentage material release for each combination for all releasing periods.

Antioxidant Activity Assay of Released Tara Tannins from Fibrous Matrix

The antioxidant activity of the released tara tannin solution was determined using DPPH assay. Tara tannin and gallic acid were dissolved in dimethylsulfoxide and used as a reference solution for comparing the antioxidant power between the pure material and the released material. Gallic acid was used in the analysis because it is a basic molecule in tara tannin.[18] The antioxidant activity was recorded as the remaining DPPH every minute for 60 min of reaction time.

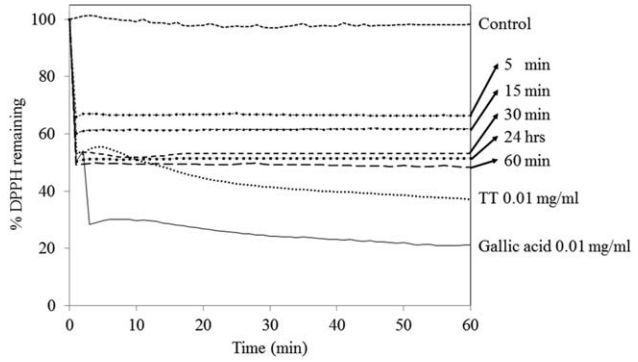

Figure 6. Antioxidant activity of released tara tannin from fibrous matrix.

In Figure 6, $\%DPPH_{remaining}$ decreased at longer releasing periods. Between 30 min, 60 min, and 24 h, Tukey's range test reported no statistically difference between antioxidant power from different releasing periods. This suggested tara tannin was released within 30–60 min and reached stable phase. Nevertheless, the common characteristic of tara tannin from each period was the immediate antioxidant activity. This was confirmed by the sharp decrease in $\%DPPH_{remaining}$ in every sample during the first few minutes of reaction time before becoming stable. Furthermore, the antioxidant activity of the original tara tannin was better than from the fibrous matrix, as expected.

Corresponding to the reduction of the molecular structure of tara tannin that was observed from the DSC thermogram, the released material behaved similarly to small molecules rather than the large molecule of tara tannin. At 60 min releasing period, the $DPPH_{remaining}$, 48.37%, fell to within the performance of gallic acid at a concentration of 0.001–0.010 mg/mL, 91.25–41.38%, or equivalent to an amount of 0.1–1.0 μg, respectively. Similar to other studies that reported the fast release characteristic and the small amount compared to the encapsulated material, it is possible to conclude that the release material at 60 min of releasing period performed similarly when pure material of 0.5 μg was released from the fibrous matrix.

CONCLUSIONS

Tara tannin was incorporated into a polymeric fibrous matrix. Due to the formation between quinic acid and galloy moieties, there were five combinations. A galloylquinic acid combination

Table II. Corresponding Percentage Peak Area (% Molar) to Retention Time from Different Releasing Periods Based on HPLC Analysis

	Tara tannin (RT)	5 min	15 min	30 min	60 min	24 h
Q-G	9.57	26.81 ± 3.1	25.57 ± 0.2	25.59 ± 0.4	25.01 ± 1.4	27.79 ± 1.5
Q-2G	14.52	26.97 ± 0.8	26.53 ± 0.1	26.43 ± 0.2	26.17 ± 0.4	27.85 ± 0.9
Q-3G	18.05	19.17 ± 0.3	19.33 ± 0.3	18.85 ± 0.1	18.84 ± 0.5	19.74 ± 1.7
Q-4G	21.66	14.56 ± 1.5	15.24 ± 0.7	16.22 ± 1.0	16.67 ± 1.7	13.36 ± 0.2
Q-5G	25.37	12.49 ± 2.2	14.09 ± 0.8	14.46 ± 1.7	15.35 ± 2.1	11.26 ± 0.2

Components: Q-G, galloylquinic acid; Q-2G, digalloylquinic acid; Q-3G, trigalloylquinic acid; Q-4G, tetragalloylquinic acid; Q-5G, pentagalloylquininc acid; RT, average retention time in which molecule passed and was detected.

with the smallest molecular weight released the largest molar proportion. This information will be used for future polyphenol screening and for selection to improve the performance and for use as the starting point of a study to control the releasing mechanism where varieties of material are released from a fibrous matrix.

ACKNOWLEDGMENTS

This work was supported by the Thailand Research Organization Network (TRON), grant number, RDG5750030, under the research category "Research of Cane and Sugar" and the PHC Program for Higher Education and Research 2013-2014, titled "Development of Bioplastics Derived from Plant Lipids, Natural Polyphenols and Polysaccharides".

REFERENCES

1. Reneker, D. H.; Yarin, A. L. *Polymer* **2008**, *49*, 2387.
2. Behrouz, C.; Nick, T. *Food Hydrocoll.* **2005**, *51*, 227.
3. Thompson, C. J.; Chase, G. G.; Yarin, A. L.; Reneker, D. H. *Polymer* **2007**, *48*, 6913.
4. Yu, D. G.; Yang, C.; Jin, M.; Williams, G. R.; Zou, H.; Wang, X.; Annie Bligh, S. W. *Colloids Surf. B: Biointerfaces* **2016**, *138*, 110.
5. Yu, D. G.; Li, X. Y.; Wang, X.; Yang, J. H.; Annie Bligh, S. W.; Williams, G. R. *ACS Appl. Mater. Interfaces* **2015**, *7*, 18891.
6. Yu, D. G.; White, K.; Chatterton, N.; Li, Y.; Lia, L.; Wang, X. *RSC Adv.* **2015**, *5*, 9462.
7. Shijun, S.; Long, L.; Guang, Y.; Jingrong, L.; Chao, L.; Tao, G.; Shaobing, Z. *Int. J. Pharma.* **2011**, *421*, 310.
8. Kim, Y. J.; Park, M. R.; Kim, M. S.; Kwon, O. H. *Mater. Chem. Phys.* **2012**, *133*, 674.
9. Costa, L. M. M.; Olyveira, G. M.; Cherian, B. M.; Leão, A. L.; Souza, S. F.; Ferreira, M. *Ind. Crops Products* **2013**, *41*, 198.
10. Charernsriwilaiwat, N.; Rojanarata, T.; Ngawhirunpat, T.; Sukma, M.; Opanasopit, P. *Int. J. Pharma.* **2013**, *452*, 333.
11. Sunan, W.; Massimo, F. M.; Shai, B.; Lim, L. T. *Food Res. Int.* **2013**, *52*, 467.
12. Montgomery, D. C. In Design and Analysis of Experiments; Montgomery, D. C., Eds.; Wiley: New York, **2008**, Chapter 3, p 36.
13. Stijnman, A. C.; Bodnar, I.; Tromp, R. H. *Food Hydrocoll.* **2011**, *25*, 1393.
14. Nieuwland, M.; Geerdink, P.; Brier, P.; Eijnden, P. V. D.; Henket, J. T. M. M.; Langelaan, M. L. P.; Stroeks, N.; Deventer, H. C. V.; Martin, A. H. *Innovative Food Sci. Emerg. Technol.* **2014**, *24*, 138.
15. Sunthornvarabhas, J.; Chatakanonda, P.; Piyachomkwan, K.; Sriroth, K. *Mater. Lett.* **2011**, *65*, 985.
16. Milleret, V.; Simona, B.; Neuenschwander, P.; Hall, H. *Eur. Cells Mater.* **2011**, *21*, 286.
17. Orozco, V. H.; Brostow, W.; Chonkaew, W.; López, B. L. *Macromol. Symp.* **2009**, *277*, 69.
18. Aouf, C.; Benyahya, S.; Esnouf, A.; Caillol, S.; Boutevin, B.; Fulcrand, H. *Eur. Polym. J.* **2014**, *55*, 186.
19. Mudigoudra, B. S.; Masti, S. P.; Chougale, R. B. *Res. J. Recent Sci.* **2012**, *1*, 83.
20. Cui, W.; Li, X.; ; Zhu, X.; Yu, G.; Zhou, S.; Weng, J. *Biomacromolecules* **2006**, *7*, 1623.
21. Hossain, K. M. Z.; Parsons, A. J.; Rudd, C. D.; Ahmed, I.; Thielemans, W. *Eur. Polym. J.* **2014**, *53*, 270.
22. Clifford, M. N.; Stoupi, S.; Kuhnert, N. *J. Agric. Food Chem.* **2007**, *55*, 2797.