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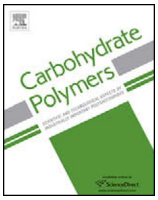

Ferulic acid-coupled chitosan: Thermal stability and utilization as an antioxidant for biodegradable active packaging film

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ABSTRACT

The aim of the present research was to study the thermal stability of ferulic acid after coupling onto chitosan, and the possibility of using ferulic acid-coupled chitosan (FA-CTS) as an antioxidant for biodegradable active packaging film. FA-CTS was incorporated into biodegradable film via a two-step process, i.e. compounding extrusion at temperatures up to 150 °C followed by blown film extrusion at temperatures up to 175 °C. Although incorporation of FA-CTS with a content of 0.02–0.16% (w/w) caused decreased water vapor barrier property and reduced extensibility, the biodegradable films possessed improved oxygen barrier property and antioxidant activity. Radical scavenging activity and reducing power of film containing FA-CTS were higher than those of film containing naked ferulic acid, by about 254% and 94%, respectively. Tensile strength and rigidity of the films were not significantly affected by the addition of FA-CTS with a content of 0.02–0.08% (w/w). The above results suggested that FA-CTS could potentially be used as an antioxidant for active packaging film.

1. Introduction

In recent years, active packaging has attracted much attention from the food industry because of its potential impact on shelf life extension as well as retention of product quality and human health safety. Fabrication of active packaging is not complicated, as the active compounds, such as antioxidants and antimicrobial agents, are incorporated into or coated onto packaging materials. The demand for active packaging systems, comprised of environmentally benign polymers and natural active agents, is growing significantly since these systems could reduce the adverse impacts of food packaging on the environment while simultaneously enhancing consumer safety. As discussed in a number of published research articles, solution casting is a common technique used to create biodegradable active packaging films; however, it is difficult to scale up production to an industrial level.

Although melt extrusion technology has also been developed in the past decade to fabricate biodegradable active packaging (Nobile, Conte, Buonocore, Incoronato, Massaro, & Panza, 2009; Nam, Scanlon, Han, & Izydorczyk, 2007; Pelissari, Grossmann, Yamashita, & Pineda, 2009), the loss of active compounds during processing due to direct contact with heat has been reported to be a major problem. Nam et al. (2007) revealed that 1% (w/w) lysozyme-embedded extruded pea starch exhibited effective antimicrobial activity against *Brochothrix thermosphacta*. However, lysozyme recovery sharply decreased with an increase of extrusion temperature. Another related work concerned the incorporation of lysozyme, thymol and lemon extracts (3–15% w/w) into polylactic acid or polycaprolactone films by a melt extrusion process (del Nobile et al., 2009). The biodegradable films retained only slight antimicrobial activity against *Pseudomonas* spp. because some activity had been lost due to high processing temperature.

Recently, encapsulation has been reported as a promising method to protect active compounds from direct exposure to external stimuli such as heat. The incorporation of chitosan nanoparticles with encapsulated eugenol antioxidant into thermoplastic starch via extrusion at temperatures up to 155 °C could reduce the loss of eugenol or retain its activity (Woranuch & Yoksan, 2013a). However, the eugenol-loaded chitosan nanoparticles could

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Table 1
Composition of biodegradable materials.

Sample	Content (% w/w)		
	Ferulic acid	Chitosan	FA-CTS
BM[*]	–	–	–
BM/CTS	–	0.04	–
BM/FA	0.0003	–	–
BM/FA-CTS1	–	–	0.02
BM/FA-CTS2	–	–	0.04[**]
BM/FA-CTS3	–	–	0.08
BM/FA-CTS4	–	–	0.16

[*] BM stands for biodegradable material composed of poly(lactic acid), thermoplastic starch and poly(butylene adipate-*co*-terephthalate) with a weight ratio of 50:40:10.

[**] 0.04% (w/w) of FA-CTS contains ferulic acid content of 0.0003% (w/w).

not be melted and tended to aggregate during the process, resulting in an unsatisfactory thermoplastic starch sheet/film appearance.

Accordingly, the present research aims to improve the thermal stability of phenolic antioxidants and meanwhile to obtain biodegradable active packaging film with a good appearance. Recently, we succeeded in synthesizing phenolic acid-coupled chitosan, i.e. ferulic acid-coupled chitosan (FA-CTS) and found that this derivative is soluble in water and glycerol (Woranuch & Yoksan, 2013b), which would be advantageous for preparing thermoplastic starch-based film. However, the thermal stability of ferulic acid coupled onto chitosan, and the incorporation of FA-CTS into packaging film, have not yet been fully elucidated. Therefore, the objectives of the present research are: (i) to investigate the thermal stability of ferulic acid coupled onto chitosan, compared with that of naked ferulic acid, through incorporation into biodegradable plastic films by extrusion; and (ii) to study the effects of FA-CTS on the morphology, tensile properties, barrier properties and antioxidant activity of biodegradable films.

2. Materials and methods

2.1. Materials

Chitosan (degree of deacetylation of 0.90 and molecular weight of ~200 kDa) was purchased from Seafresh Industry Public Co. Ltd., Thailand. Ferulic acid (99%) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC) were obtained from Sigma-Aldrich, Germany. Cassava starch was supplied by Tong Chan, Thailand. Poly(lactic acid) (PLA; Ingeo™ 4043D) was a product of NatureWorks LLC, USA. Poly(butylene adipate-*co*-terephthalate) (PBAT; Ecoflex® F Blend C1200) was purchased from BASF, Germany.

2.2. Preparation of ferulic acid-coupled chitosan

Ferulic acid-coupled chitosan was prepared by a carbodiimide-mediated coupling reaction, according to our previous report (Woranuch & Yoksan, 2013b). A mole ratio of chitosan to ferulic acid of 1:1 was used in the present study. The reaction was carried out in 1% v/v aqueous acetic acid solution at 60 °C for 3 h.

2.3. Preparation of biodegradable materials containing ferulic acid-coupled chitosan

Seven different formulations of biodegradable materials were prepared, as shown in Table 1. Mixtures of cassava starch, glycerol (35 parts per hundred parts of starch) and other additives (Table 1) were blended in a twin-screw extruder (LTE 20-40; Labtech Engineering Co., Ltd., Thailand), using a barrel temperature ranging from 90 °C to 150 °C and a screw speed of 135–140 rpm. The extrudates were cut into 3-mm-long pellets by a pelletizer. The obtained thermoplastic starch (TPS) pellets were dried in a hot-air oven at 45 °C for 12 h and then mixed with PLA and PBAT resins in a weight ratio of PLA:TPS:PBAT of 50:40:10. The mixed resins were subsequently blown into a film by a blown film extruder (LE 25–30/C; Labtech Engineering) through an annular die using a temperature range of 140–175 °C, a screw speed of 28–29 rpm and a nip roll speed of 3.2 rpm.

2.4. Characterization of biodegradable materials containing ferulic acid-coupled chitosan

X-ray diffraction (XRD) patterns were recorded by a JDX-3530 X-ray diffractometer (JEOL, Japan) over a 2θ range from 3° to 40° using a scan rate of 0.04°/s. SEM micrographs were taken by a Hitachi S-4700 (Hitachi High-Technologies Corp., Japan) at an accelerating voltage of 5 kV. Dried samples were fixed on individual specimen stubs and then coated with a thin layer of gold prior to SEM observation.

2.5. Determination of remaining content of ferulic acid in biodegradable materials

Each sample (50 g) was mixed with chloroform (10 mL) and then centrifuged at 9000 rpm, 25 °C, for 10 min. The insoluble part was then stirred in hydrochloric acid solution (2 M, 2 mL) and boiled at 95 °C for 30 min. After cooling down to room temperature, the homogeneous solution was centrifuged at 9000 rpm, 25 °C, for 10 min. The supernatant was analyzed by a UV–Vis spectrophotometer over a wavelength range from 250 nm to 350 nm. The remaining content of ferulic acid was calculated from a standard curve ($y = 171.4x$, $r^2 = 0.998$).

2.6. Evaluation of radical scavenging activity of biodegradable materials containing ferulic acid-coupled chitosan by DPPH method

Radical scavenging activity of biodegradable materials was determined by a DPPH method modified from the one reported by Parejo et al. (2002). A sample (1 mg) was mixed with an ethanolic solution of stable DPPH radicals (100 μM, 1 mL) and incubated in the dark for 8 h. The absorbance of the DPPH solution was measured at 517 nm. The radical scavenging activity was defined as a decrease in the absorbance of DPPH, and was calculated using the following equation:

$$\%\text{DPPH}\quad\text{decoloration} = \left(\frac{\left[A_{\text{control}} - A_{\text{sample}}\right]}{A_{\text{control}}}\right) \times 100 \qquad (1)$$

where A_{control} is the absorbance of the supernatant in a control tube and A_{sample} is the absorbance of the supernatant in a sample tube.

2.7. Evaluation of antioxidant activity of biodegradable materials containing ferulic acid-coupled chitosan by reducing power assay

The reducing power of biodegradable materials was determined according to the method of Oyaizu (1986). A sample (1 mg) was added to a mixture of phosphate buffer (2.5 mL) and potassium ferricyanide (1% w/v, 2.5 mL) and then heated to 50 °C for 20 min. After cooling to ambient temperature, this was mixed with trichloroacetic acid (10% v/v, 2.5 mL) and centrifuged at 9000 rpm for 10 min. The supernatant (2.5 mL) was then mixed with distilled water (2.5 mL) and freshly prepared ferric chloride solution (0.1% w/v, 0.5 mL). The absorbance at 700 nm of the mixture was recorded as a function of time.

2.8. Determination of peroxide value of potato chips stored in biodegradable films containing ferulic acid-coupled chitosan

Each film sample was cut into a rectangular shape (20 cm × 10 cm) and then sealed at the rim and bottom to form a bag, using a heat sealer (Santo, Thailand) set at a heat level of 5. Potato chips (Lay's Brand, 70 g) were put into the bag before sealing the top. The assembly was stored at 25 °C for 14 days. Lipid composition of potato chips was then extracted by a soxtec analyzer (Tecator Soxtec System HT 1043 Extraction Unit, Tecator, USA) before determining peroxide value using AOCS Official Method Cd 8–53. Lipid (3.0 g) was dissolved in a solvent mixture of glacial acetic acid and chloroform with a ratio of 3:2 (50 mL). Saturated potassium iodide solution (1 mL) was then added and the mixture was shaken thoroughly. Distilled water (100 mL) was added to the mixture before titration with sodium thiosulfate solution (0.001 N), using starch solution as an indicator. A blank titration was carried out using the same protocol as a sample. Peroxide value was calculated using the following equation:

$$\text{Peroxide value (mEq peroxide per kg of sample)} = \left[\frac{([S-B]\times N)}{W}\right]\times 1000 \tag{2}$$

where S is the volume of titrant (mL) for the sample, B is the volume of titrant (mL) for the blank, N is the normality of the sodium thiosulfate solution (mEq/mL), 1000 is for conversion of units (g/kg), and W is the sample mass (g).

2.9. Tensile testing of biodegradable films

Tensile properties of the samples were examined according to ASTM D882 standard test method, using a Hounsfield H50KS testing machine (UK). The sample films were cut into a rectangular shape (15 cm × 2.5 cm) and conditioned in a closed chamber containing saturated sodium nitrite solution at 25 °C (65% RH) for 3 days prior to testing. The test was performed with a load cell of 50 kN, a crosshead speed of 50 mm/min and a grip separation of 100 mm. Ten replicates were tested for each sample to obtain an average value. The thickness of films was measured at five positions on the perimeter and at the center of the film by a digital caliper (model ID-C112BS; Mitutoyo, Japan) to obtain an average value.

2.10. Determination of water vapor permeability of biodegradable films

Water vapor transmission rate (WVTR) was determined according to ASTM E96 using a cup method. The test cup was filled with 20 g of silica gel (desiccant) to produce 0% RH below the film. A film sample was placed between the cup and the ring cover coated with paraffin wax sealant, to leave a test area of 28.29 cm^2. The test was performed at 50% RH and 25 °C. Five replicates were tested for each sample. WVTR was determined as the initial slope of the relationship between weight changes and time divided by the absorption surface area. Water vapor permeability (WVP) was calculated from the following equation:

$$\mathrm{WVP} = \frac{(\mathrm{WVTR}\times L)}{\Delta P} \tag{3}$$

where WVP is the water vapor permeability (g mil/m^2 day atm), WVTR is the measured water vapor transmission rate through a film (g/m^2 day), L is the mean film thickness (mil) and ΔP is the water vapor partial pressure difference between the two sides of the film (atm).

2.11. Determination of oxygen permeability of biodegradable films

Oxygen transmission rate (OTR) was determined according to ASTM D3985 using an oxygen permeation analyzer (model 8501; Systech Illinois, USA). The sample films were cut into a square shape (7 cm × 7 cm) and sandwiched between two aluminum foil rings leaving an exposed area of 28.26 cm^2 for measurement. The films were kept in a closed chamber containing saturated calcium chloride solution at 25 °C (50% RH) for at least 48 h prior to measurement. The OTR test was performed at 0% RH and ambient temperature. Five replicates were tested for each sample. Oxygen permeability (OP) of samples was calculated using the following equation:

$$\mathrm{OP} = \frac{(\mathrm{OTR}\times L)}{\Delta P} \tag{4}$$

where OP is the oxygen permeability (cm^3 mil/m^2 day atm), OTR is the measured oxygen transmission rate through a film (cm^3/m^2 day), L is the mean film thickness (mil) and ΔP is the oxygen partial pressure difference between the two sides of the film (atm).

3. Results and discussion

Ferulic acid-coupled chitosan (FA-CTS) was successfully synthesized, as confirmed by UV–Vis spectroscopy, FTIR, ^{1}H NMR and ninhydrin assay, in our previous report (Woranuch & Yoksan, 2013b). Ferulic acid was coupled onto chitosan at the C-2 positions via amide bonds (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004) with a degree of substitution of 0.37. In the present work, we investigated whether the thermal stability of ferulic acid was improved after coupling onto chitosan. The prepared FA-CTS was thus incorporated into biodegradable plastic through thermal processes: i.e. compounding extrusion at temperatures up to 150 °C to prepare pellets, followed by blown film extrusion at temperatures up to 175 °C to form film. The thermal stability of ferulic acid was determined from the remaining content of ferulic acid in the biodegradable materials, as well as the antioxidant activities of those biodegradable materials in the form of both pellets and films.

3.1. Remaining content of ferulic acid in biodegradable materials

The amount of ferulic acid remaining in both forms of biodegradable materials, i.e. pellets and films, was determined by UV–Vis spectroscopy at a $\lambda_{\max}$ of 323 nm. Biodegradable material without any additives (BM) was used as a control, while biodegradable material containing naked ferulic acid with a content of 0.0003% w/w (BM/FA) was used for a comparison study. Four formulations of biodegradable materials containing ferulic acid-coupled chitosan (BM/FA-CTS1, BM/FA-CTS2, BM/FA-CTS3 and BM/FA-CTS4) were prepared with different ferulic acid-coupled chitosan concentrations, i.e. 0.02, 0.04, 0.08 and 0.16% w/w, respectively (corresponding to ferulic acid amounts of 0.00015, 0.0003, 0.0006 and 0.0012% w/w, respectively). Considering the remaining content of ferulic acid in BM/FA-CTS2 and BM/FA that possessed an equal initial content of ferulic acid, BM/FA-CTS2 pellets showed higher remaining content of ferulic acid (63.3%, 1.9 mg/kg) than BM/FA pellets (26.7%, 0.8 mg/kg) (Fig. 1). Similarly, BM/FA-CTS2 film also showed higher remaining content of ferulic acid (13.3%, 0.5 mg/kg) than BM/FA film (n.d.). These results implied that the coupling of ferulic acid onto chitosan could reduce the loss of ferulic acid during thermal processes. In addition, BM/FA-CTS2 and BM/FA films had lower remaining content of ferulic acid than their corresponding pellets due to the increased number of thermal exposures.

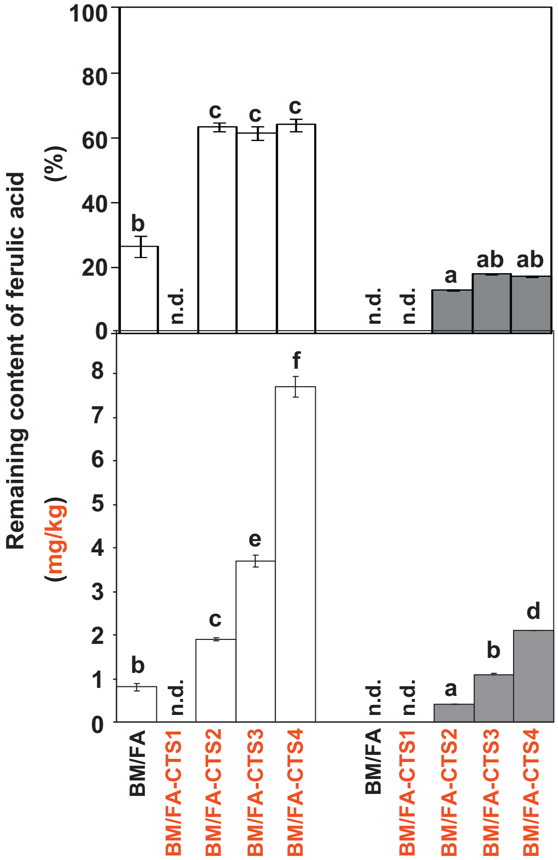

Fig. 1. Remaining content of ferulic acid in different BM pellets (□) and films (■). Data are reported as mean ± SD, $n = 3$. Different small letters indicate a significant difference at $p < 0.05$ (Duncan's new multiple range test); n.d. = not detected.

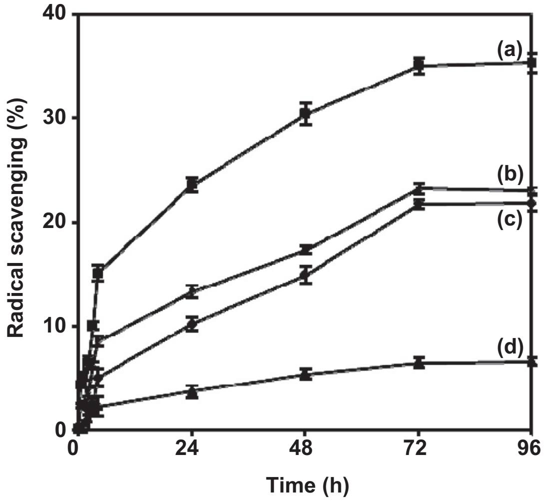

Fig. 2. Radical scavenging percentages as a function of time of different BM pellets and films: (a) BM/FA-CTS2 pellet, (b) BM/FA-CTS2 film, (c) BM/FA pellet and (d) BM/FA film. Each point represents the mean ± SD ($n = 3$).

It should be pointed out that BM/FA-CTS1 contains too small an amount of FA-CTS (0.02% w/w), or in other words a tiny amount of ferulic acid (0.00015% w/w), to be detected by UV–Vis spectroscopy. Although the remaining content of ferulic acid in the biodegradable materials increased with increasing initial content of FA-CTS, the remaining percentage of ferulic acid in the biodegradable pellets and films was not significantly influenced by the initial content of FA-CTS. In BM/FA-CTS2, BM/FA-CTS3 and BM/FA-CTS4 pellets, about 60% of ferulic acid remained, while their corresponding films contained only 20%. These results reflected that the loss of ferulic acid from biodegradable materials containing FA-CTS was about 40% after undergoing each thermal process. The loss of ferulic acid was more severe in the case of the material containing naked ferulic acid when it passed through the first thermal process (compounding extrusion) and the second thermal process (blown film extrusion), i.e. ~75% and 100%, respectively.

3.2. *Antioxidant activity of biodegradable materials containing ferulic acid-coupled chitosan*

Antioxidant efficiency of the biodegradable materials was evaluated by the radical scavenging percentage as determined by DPPH method. In general, freshly prepared DPPH solution is a deep purple color, with a maximum absorption at 517 nm. The antioxidants can quench DPPH free radicals and convert them into a colorless product, resulting in a reduction of absorbance at 517 nm. Fig. 2 illustrates that the radical scavenging percentage of BM/FA and BM/FA-CTS2 increases as a function of time for the first 72 h and then levels off. The marked increase of radical scavenging percentage at the initial stage might be explained by the high reaction rate of ferulic acid hydroxyl groups and DPPH free radicals. BM/FA-CTS2 pellets and film exhibited higher radical scavenging percentages (35.3% and 23.0%, respectively) (Fig. 2a and b) than the corresponding BM/FA (21.8% and 6.5%, respectively) (Fig. 2c and d), indicating that the materials containing coupled ferulic acid exhibited higher antioxidant activity than those containing naked ferulic acid. This result was related to the amount of ferulic acid remaining in the biodegradable materials (see Section 3.1). As compared with biodegradable pellets, the corresponding films possessed lower radical scavenging percentages as indicated by the smaller amount of ferulic acid remaining in the films as the number of thermal exposures increased.

Antioxidant activity of the biodegradable materials was also evaluated by reducing power assay based on the chemical reaction of Fe(III) → Fe(II). The resulting Fe(II) could then react with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Fig. 3 shows that the absorbance values at 700 nm

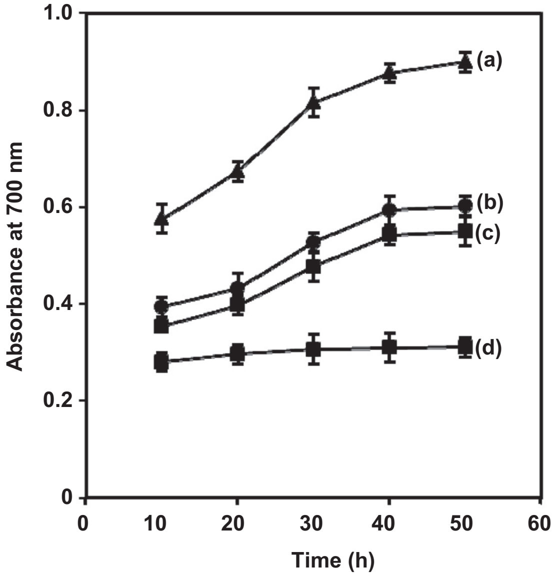

Fig. 3. Reducing power as a function of time of different BM pellets and films: (a) BM/FA-CTS2 pellet, (b) BM/FA-CTS2 film, (c) BM/FA pellet and (d) BM/FA film. Each point represents the mean ± SD ($n = 3$).

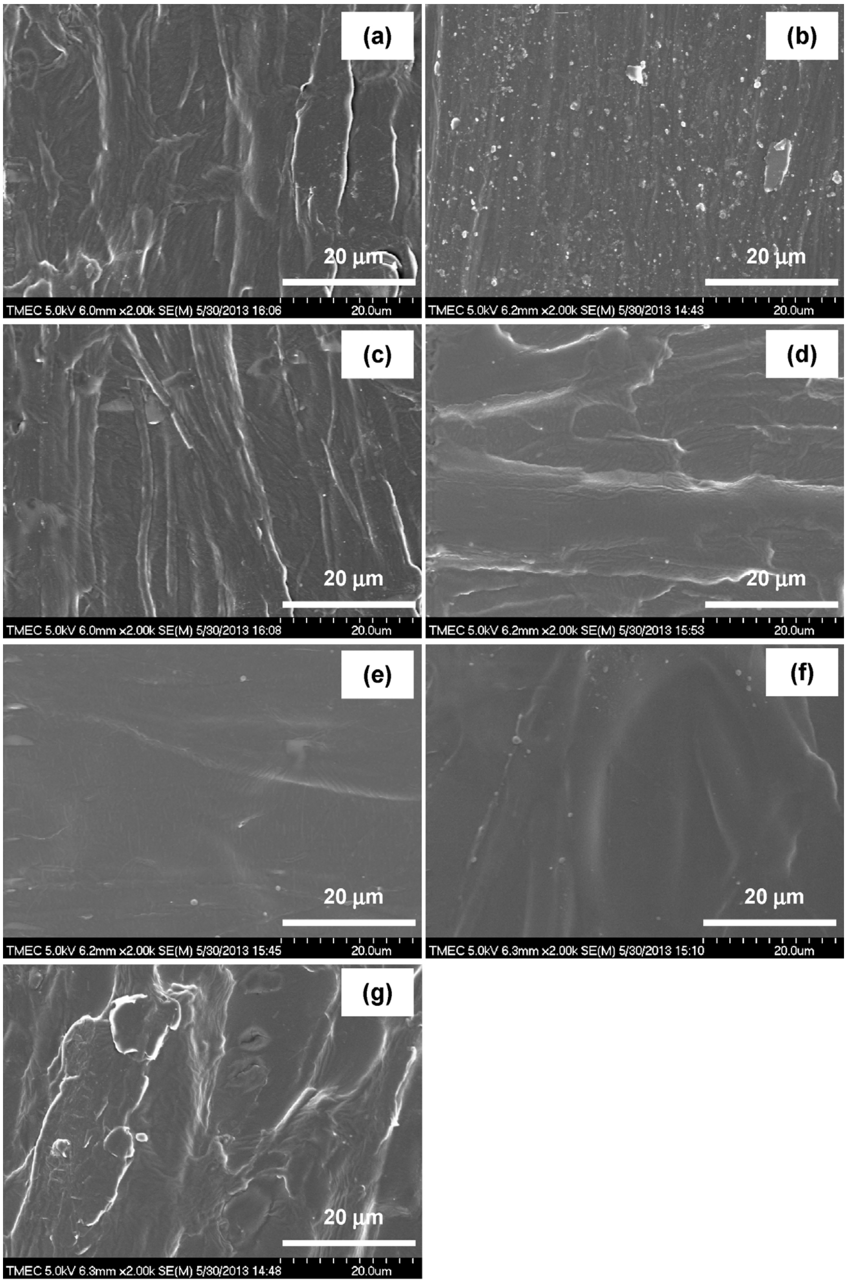

Fig. 4. SEM micrographs at 5 kV at the film surface of different BM films: (a) BM, (b) BM/CTS, (c) BM/FA, (d) BM/FA-CTS1, (e) BM/FA-CTS2, (f) BM/FA-CTS3 and (g) BM/FA-CTS4.

of BM/FA-CTS2 and BM/FA increase as a function of complex formation time, implying increased antioxidant activity. Ferric ferrous complex completely formed at 50 min for all samples. BM/FA-CTS2 pellets and film possessed higher absorbance values at 700 nm, or about 100% greater antioxidant efficiency than BM/FA pellets and film. In addition, the films showed lower absorbance values at 700 nm than the pellets, reflecting the inferior reducing ability of the biodegradable films as compared with their corresponding pellets.

It could be concluded from the above findings that coupling of ferulic acid onto chitosan could reduce the loss of ferulic acid or improve its thermal stability during extrusion; as a result, the materials containing FA-CTS possessed higher remaining content of ferulic acid and showed better antioxidant activities than those containing naked ferulic acid. This might be a result of the molecular immobilization of ferulic acid after coupling onto macromolecule or polymer, i.e. chitosan.

The efficacy of BM/FA-CTS2 film in inhibiting the rancidity of food containing lipids was also investigated in comparison with that of BM film. Potato chips were selected as the food because of their high fat content and ease of oxidation. Peroxide value (PV) of the potato chips stored at 25 °C for 14 days in the BM/FA-CTS2 and BM films was determined to assess the food deterioration, i.e. oxidative rancidity. The result showed that potato chips stored in BM film possessed a PV of 3.76 ± 0.09 mEq peroxide per kg of sample, while those kept in BM/FA-CTS2 film had a PV of 2.56 ± 0.12 mEq peroxide per kg of sample. The lower PV (about 31.8%) of the potato chips stored in BM/FA-CTS2 suggested that the low remaining content of ferulic acid in the film had a positive effect on protecting food from deterioration.

Furthermore, we also studied the influences of FA-CTS on the morphology and crystallinity of the biodegradable films because they are relevant to the mechanical and barrier properties of the material. In addition, tensile properties and barrier properties of the

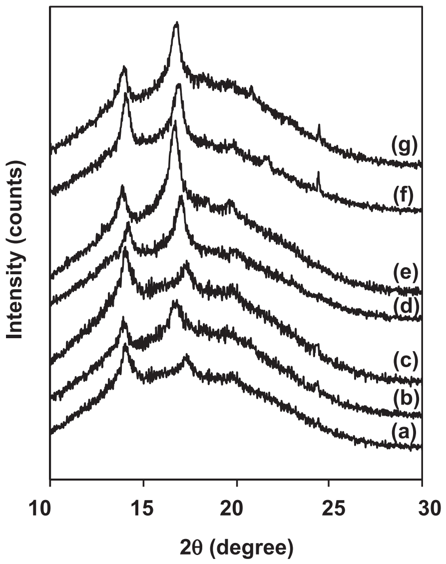

Fig. 5. X-ray diffractograms of different BM films: (a) BM, (b) BM/CTS, (c) BM/FA, (d) BM/FA-CTS1, (e) BM/FA-CTS2, (f) BM/FA-CTS3 and (g) BM/FA-CTS4.

films were also investigated to define the appropriate applications. The amount of ferulic acid was varied in the range of 0.02–0.16% w/w. The properties of the biodegradable films containing ferulic acid-coupled chitosan (BM/FA-CTS) were compared with those of naked biodegradable films (BM), biodegradable films containing chitosan (BM/CTS), and biodegradable films containing naked ferulic acid (BM/FA). It should be pointed out that chitosan was incorporated into BM/CTS film in the form of solid flakes because it was insoluble in any film components and could not be melted. On the other hand, FA-CTS, which is a chitosan derivative obtained by coupling with ferulic acid, could be dissolved in glycerol; as a result, BM/FA-CTS films exhibited more homogeneity than BM/CTS film.

3.3. *Effect of ferulic acid-coupled chitosan on morphology of biodegradable film*

Surface morphology of BM films was observed by SEM. Naked BM film showed a rough surface, implying less homogeneity of components in the film matrix due to the differences in hygroscopicity of starch (hydrophilic) and PLA (hydrophobic) (Bolay, Lamure, Leis, & Subhani, 2012; Mansor, Ibrahim, Yunus, & Ratnam, 2011; Wang, Yu, Chang, & Ma, 2008) (Fig. 4a). For BM/CTS film, small particles were observed throughout the film matrix (Fig. 4b). These particles are remnants of chitosan flakes. Similar to BM film, the film containing free ferulic acid exhibited a rugged surface due to a lower content of ferulic acid remaining in the film (Fig. 4c). It is interesting that the incorporation of FA-CTS with a content in the range of 0.02% (w/w) to 0.08% (w/w) gave the films a smoother surface than the naked BM film, implying the homogeneity of materials. Smoothness of the film surface increased with increasing FA-CTS content, reflecting that FA-CTS might act as a compatibilizer for blends of TPS and PLA through hydrogen bonding interaction. However, the film containing FA-CTS with a content of 0.16% (w/w) presented a rough surface owing to the excessive amount of chitosan derivative.

3.4. *Effect of ferulic acid-coupled chitosan on crystallinity of biodegradable film*

Crystal type and crystallinity of starch in the BM films was investigated by XRD technique. It should be noted that PLA and PBAT, which are two of the major components in BM films, did not show any diffraction peaks at the observed 2θ range in the XRD pattern (Gorrasi, Milone, Piperopoulos, Lanza, & Sorrentino 2013; Shen et al., 2012). Naked BM film exhibited diffraction peaks at 2θ of 14°, 17° and 20°, corresponding to the V-type crystal structure of starch (Fig. 5a) (Ma, Yu, & Wan, 2006; Soest, Hulleman, de Wit, & Vliegenthart, 1996; Yokesahachart & Yoksan, 2011). The V-type conformation might involve the formation of amylose–glycerol complexes (Ma et al., 2006). The incorporation of chitosan and ferulic acid did not affect the crystal type and crystallinity of starch in the BM film (Fig. 5b and c). Although the crystal type of starch in BM/FA-CTS film did not change as compared with that in BM film, the crystallinity percentage of starch in the BM/FA-CTS film increased significantly, implying an increased amount of amylose–hydrophobic substance complexes. The additional V-type crystals might arise from the formation of amylose–ferulic acid complexes. Putaux, Cardoso, Dupeyre, Morin, Nulac, and Hu (2008) reported that some phenolic compounds, e.g. thymol, could form helical complexes with amylose.

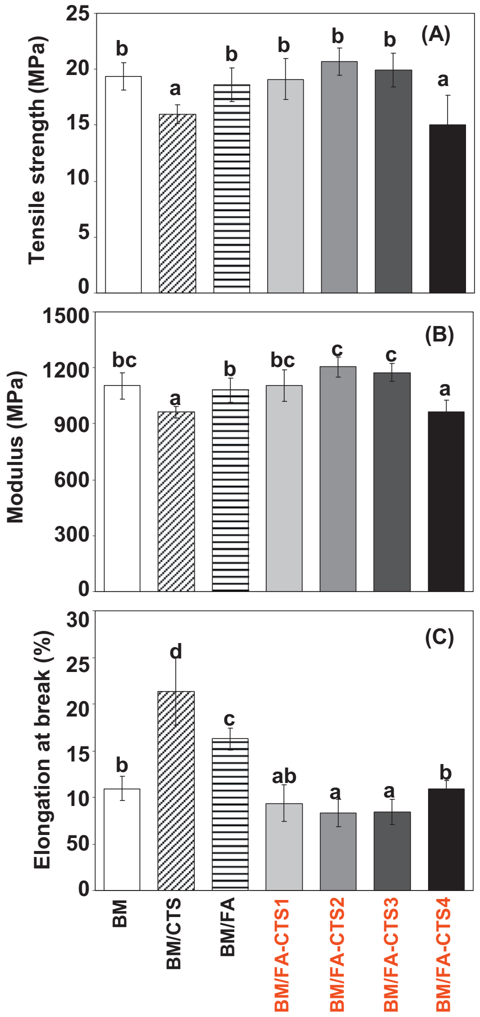

Fig. 6. (A) Tensile strength, (B) modulus and (C) elongation at break of different BM films. Data are reported as mean ± SD, $n = 10$. Different small letters indicate a significant difference at $p < 0.05$ (Duncan's new multiple range test).

3.5. *Effect of ferulic acid-coupled chitosan on tensile properties of biodegradable film*

Tensile strength, modulus and elongation at break of biodegradable films were determined as the stress at peak, the slope at the elastic (linear) portion, and the strain at break of a stress-strain curve, respectively. BM film showed tensile strength, modulus and elongation at break of 19.09 MPa, 1103.72 MPa and 10.95%, respectively (Fig. 6). BM/CTS film exhibited lower tensile strength (15.99 MPa) and modulus (962.28 MPa), but higher elongation at break (21.39%) than BM film. The reduction of tensile strength and stiffness, as well as the augmentation of extensibility of BM film by incorporating chitosan flakes, might result from the discontinuity of the film matrix (see Section 3.3). The incorporation of ferulic acid hardly altered tensile strength (18.61 MPa) and modulus (1080.16 MPa), but increased elongation at break (16.24%) of the BM film, implying that the film became more flexible when FA was added. This might be explained by the plasticizing effect of small molecules of phenolic acid. Pelissari et al. (2009) also reported that oregano phenolic compound could act as a plasticizer to reduce tensile strength and rigidity of starch–chitosan films, but enhance their extensibility. BM/FA-CTS films containing FA-CTS with a content in the range of 0.02% to 0.08% (w/w) showed slightly increased tensile strength (19.10–20.71 MPa) and modulus (1105.51–1206.50 MPa), but trivially decreased elongation at break (8.33–9.34%) as compared with BM film. These results indicated that FA-CTS could function as a compatibilizer to enhance homogeneity of the material, corresponding to the smooth surface of the BM/FA-CTS film observed by SEM (Fig. 4d–f). The tensile properties of BM/FA-CTS film were not significantly affected by the FA-CTS content. However, tensile strength and modulus of BM/FA-CTS film declined to 15.05 MPa and 966.52 MPa, respectively, when FA-CTS with a content of 0.16% (w/w) was loaded. This amount of FA-CTS might be too high to facilitate compatibilization, a supposition which was supported by SEM results (Fig. 4g).

3.6. Effect of ferulic acid-coupled chitosan on barrier properties of biodegradable film

Water vapor permeability (WVP) and oxygen permeability (OP) represent the volume of water vapor and oxygen passing through a material per unit area per unit time per unit barometric pressure. These properties are important information for deciding the appropriate food products for each packaging material. BF film exhibited WVP of 5641 g mil/m^2 day atm (Fig. 7A). The incorporation of chitosan caused a reduction of WVP to 4697 g mil/m^2 day atm, indicating the improved water vapor barrier property of BM film by the incorporation of chitosan. This improvement might be due to the high crystallinity of chitosan, which obstructed water vapor adsorption of the film. The decreased water vapor adsorption or lower wettability of the film was also confirmed by the higher water contact angle (Fig. 7). WVP of BM/FA film was not significantly different from that of BM film (Duncan's multiple range test, $p < 0.05$) (Fig. 7A) because a small content of ferulic acid remained in the film matrix. However, the WVP values of BM/FA-CTS films were 12–28% higher than that of BM film. The deterioration of water vapor barrier properties of BM/FA-CTS films was related to the hydrophilicity of FA-CTS (Woranuch & Yoksan, 2013b). WVP of BM/FA-CTS films tended to increase with increasing FA-CTS content, while their water contact angle values decreased.

The OP values of the films are illustrated in Fig. 7B. BM film possessed OP of 1236 cm^3 mil/m^2 day atm, while BM/CTS film showed higher OP (1560 cm^3 mil/m^2 day atm). This result indicated that the oxygen barrier property of BM film was reduced by adding chitosan, due to discontinuity of the film matrix resulting from dispersed chitosan flakes. BM film exhibited slightly increased OP when ferulic acid was loaded (1287 cm^3 mil/m^2 day atm). The diminished oxygen barrier property of BM/FA film might be explained by the plasticizing effect of ferulic acid, as mentioned above. On the contrary, BM/FA-CTS films showed decreased OP (i.e. 981–1235 cm^3 mil/m^2 day atm) compared with BM film; OP of the film decreased with increasing FA-CTS content. The results indicated that incorporation of FA-CTS could improve the oxygen barrier property of BM film due to the hydrophilicity of FA-CTS and the good water wettability of BM/FA-CTS film surfaces, as confirmed by reduced water contact angle (Fig. 7).

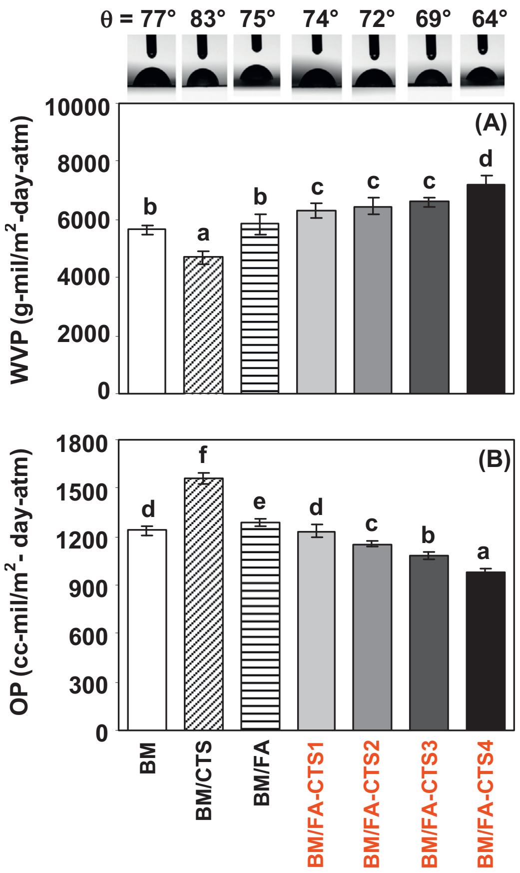

Fig. 7. (A) Water vapor permeability and (B) oxygen permeability of different BM films. Data are reported as mean ± SD, $n = 5$. Different small letters indicate a significant difference at $p < 0.05$ (Duncan's new multiple range test).

4. Conclusions

The objectives of the present research were: (i) to prove whether coupling of ferulic acid onto chitosan was an effective method of improving the thermal stability of ferulic acid; and (ii) to study the effect of ferulic acid-coupled chitosan (FA-CTS) on the properties of biodegradable film. Biodegradable films containing FA-CTS with a content varying from 0.02% to 0.16% (w/w) were prepared by blown film extrusion. Incorporation of FA-CTS with a content of 0.02–0.08% (w/w) did not significantly alter tensile strength and stiffness of the biodegradable films, but decreased film extensibility by 0.6–23.9%. Water vapor permeability of the biodegradable films increased by 11.7–29.8%, while oxygen permeability decreased by 0.1–20.6% when FA-CTS was loaded. The reduction of water vapor barrier property and the improvement of oxygen barrier property of the biodegradable films progressed as a function of FA-CTS content due to its hydrophilicity. The films containing FA-CTS exhibited ~3-fold superior radical scavenging activity compared with film containing naked ferulic acid. The results also confirmed that the thermal stability of ferulic acid was improved after coupling onto chitosan, as indicated by the higher remaining content of ferulic acid in the biodegradable plastic films containing FA-CTS and the greater antioxidant activities of those films as compared with those of the film containing naked ferulic acid.

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