Carbohydrate Polymers 96 (2013) 495–502

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Preparation, characterization and antioxidant property of water-soluble ferulic acid grafted chitosan

Sarekha Woranuch [a,b], Rangrong Yoksan [a,b,*]

[a] Department of Packaging and Materials Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand
[b] Center for Advanced Studies in Nanotechnology and Its Applications in Chemical, Food and Agricultural Industries, Kasetsart University, Bangkok 10900, Thailand

ARTICLE INFO

Article history:
Received 3 January 2013
Received in revised form 5 April 2013
Accepted 6 April 2013
Available online 12 April 2013

Keywords:
Chitosan
Ferulic acid
Grafting
Antioxidant property
Water-soluble chitosan

ABSTRACT

The objective of the present work was to improve the antioxidant activity and water solubility of chitosan by grafting with ferulic acid through a carbodiimide-mediated coupling reaction. UV–vis spectrophotometry, FTIR, ^{1}H NMR and ninhydrin assay confirmed the grafting of ferulic acid onto chitosan at the C-2 position. Ferulic acid grafted chitosan – prepared using a mole ratio of chitosan to ferulic acid of 1:1, reaction temperature of 60 °C, and reaction time of 3 h – possessed the highest ferulic acid substitution degree, i.e. 0.37. Although ferulic acid grafted chitosan showed reduced crystallinity (~10%) and decreased decomposition temperature (~55 °C) as compared to chitosan, it exhibited greater radical scavenging activity (~55%) and was soluble in water (up to 1.3 mg/mL). The improved antioxidant property and water solubility of this chitosan derivative could open a wide range of applications, particularly its use as an antioxidant in food, food packaging, biomedical, pharmaceutical and cosmetics industries.

1. Introduction

Chitosan is a polysaccharide obtained from the deacetylation of chitin, which is found in crab shells, shrimp shells and squid pens. Until now, chitosan has been used in food packaging applications in the forms of edible film (Fajardo et al., 2010) and active packaging film (Mohamed, Clementine, Didier, Gérard, & Noëlle, 2013) due to its non-toxicity, biodegradability, biocompatibility, and ability to form films, membranes, gels, beads, fibers and particles. Most chitosan films have been fabricated by a solution casting method using dilute acetic acid as a solvent (Fajardo et al., 2010; Mohamed et al., 2013). However, this method involves a multi-step process including neutralization, which is required after film formation, and the residual acetic acid might cause off-odor/flavor of food. In addition, small additive molecules such as active compounds and plasticizers are possibly lost during film neutralization, resulting in decreased functional and mechanical properties of the film (Nakashima et al., 2006).

Water-soluble chitosan is an ideal material in overcoming the solubility limit of chitosan. A series of chitosan derivatives – e.g. carboxymethyl chitosan (Yu et al., 2013), quaternary ammonium derivatives of chitosan (Fernandes et al., 2010), and *N*-(2-hydroxypropyl)-3-trimethylammonium chitosan chloride (Pinto et al., 2012) – has been used to prepare film-forming solution in neutral pH water. Chemical modification is not only an effective method of improving chitosan solubility, but can also impart new functions to chitosan. Grafting certain phenolic compounds, such as caffeic acid and eugenol, onto chitosan has been reported as a way to improve chitosan antioxidant activity (Aytekin, Morimura, & Kida, 2011; Jung, Chung, & Lee, 2006).

Phenolic compounds are attractive due to their multifunctional properties, such as combined antimicrobial and antioxidant activities. Ferulic acid is a naturally occurring phenolic acid widely used in cosmetics, food, and pharmaceutical industries owing to its effective antioxidant, antimicrobial, anti-inflammatory, antithrombotic and anticancer activities (Ou & Kwok, 2004). This bioactive compound also contains hydroxyl and carboxyl groups, which can be reacted with other materials. Although the grafting of ferulic acid onto polymers such as chitosan, flax and sisal pulp fibers has been reported, the synthesis was accomplished by an enzyme-catalyzed modification (Aljawish et al., 2012; Aracri, Roncero, & Vidal, 2011) in which the reaction occurred between the phenol hydroxyl group of ferulic acid and the reactive functional group of the polymer. The loss of the active phenol hydroxyl group might suppress some functional properties, e.g. antioxidation of ferulic acid (Trouillas, Marsal, Siri, Lazzaroni, & Duroux, 2006). The grafting of ferulic acid at the carboxyl group is thus an interesting alternative to preserve its functional properties. At present, water-soluble ferulic acid grafted chitosan with high antioxidant activity has not yet been reported.

* Corresponding author at: Center for Advanced Studies in Nanotechnology and Its Applications in Chemical, Food and Agricultural Industries, Kasetsart University, Bangkok 10900, Thailand. Tel.: +66 2 562 5097; fax: +66 2 562 5046.
E-mail addresses: rangrong.y@ku.ac.th, yyrangrong@yahoo.com (R. Yoksan).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.04.006

Therefore, the aim of the present work was to improve the antioxidant property and water solubility of chitosan by grafting with ferulic acid through a carbodiimide-mediated coupling reaction. The structural characterization (by UV–vis spectroscopy, FTIR, ^{1}H NMR, and ninhydrin assay), crystallinity (by XRD), thermal properties (by TGA and DSC), solubility, and antioxidant property of the obtained ferulic acid grafted chitosan were also investigated.

2. Experimental

2.1. Materials

Chitosan (degree of deacetylation of 0.90 and molecular weight of ~200 kDa) was purchased from Seafresh Chitosan (Lab) (Bangkok, Thailand). Ferulic acid (99%), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma–Aldrich (Munich, Germany). Acetic acid and ethanol (95%) were purchased from Merck (Darmstadt, Germany). Deuterated water (D_2O), deuterated acetic acid (CD_3COOD) and deuterated methanol (CD_3OD) were acquired from Cambridge Isotope Laboratories (Andover, MA, USA). Ninhydrin and trichloroacetic acid were products of Fisher Scientific (Loughborough, UK). Potassium ferricyanide was purchased from Ajax Finechem (Taren Point, NSW, Australia). Ferric chloride was obtained from Loba Chemie (Mumbai, India).

2.2. Preparation of ferulic acid grafted chitosan

Chitosan solution (1.2% w/v) was prepared by agitating chitosan flakes in an aqueous acetic acid solution (1% v/v) at ambient temperature overnight. A solution of ferulic acid, EDAC (1 mol equivalent to ferulic acid) and ethanol (10 mL) was then added dropwise into the chitosan solution (50 mL) while stirring and keeping at a constant temperature. Different mole ratios of chitosan to ferulic acid (i.e. 1.0:0.0, 1.0:0.5, 1.0:1.0, 1.0:1.5, 1.0:2.0 and 1.0:2.5) and various reaction temperatures (i.e. 40, 60 and 80 °C) were used. The reaction was performed at different times (i.e. 1.5, 3.0, 4.5 and 6.0 h). The mixture was subsequently dialyzed against water for a few days to eliminate free acetic acid and isourea by-products (Scheme 1), followed by centrifuging at 10,000 rpm for 30 min to remove residual ferulic acid. The supernatant was lyophilized to provide ferulic acid grafted chitosan.

2.3. Characterization of ferulic acid grafted chitosan

UV–vis absorption spectra were recorded over a wavelength range from 200 nm to 400 nm by a Shimadzu UV-2450 spectrometer (Tokyo, Japan). FTIR spectra were carried out using a Bruker Tensor 27 spectrometer (Bremen, Germany) over a wavenumber range of 4000–400 cm^{-1} with 16 scans at a resolution of 4 cm^{-1}. Samples and KBr were mixed and ground into a fine powder and then hydraulically pressed into individual discs prior to FTIR measurement. ^{1}H NMR spectra were taken at ambient temperature on a Bruker UltraShield (500 MHz) spectrometer. CD_3COOD/D_2O (1% v/v) was used as a solvent for chitosan and ferulic acid grafted chitosan, while CD_3OD was used to dissolve ferulic acid. X-ray diffraction (XRD) patterns were recorded by a JEOL JDX-3530 X-ray diffractometer (Tokyo, Japan) over a 2θ range from 3° to 40° using a scan rate of 0.04° min^{-1}. TGA and DSC thermograms were obtained using a Mettler-Toledo (Schwerzenbach, Switzerland) TGA/DSC 1 STAR^e System thermogravimetric analyzer and differential scanning calorimeter with STAR^e software. For TGA, the sample (5–7 mg) was placed in a crucible and heated from 30 °C to 600 °C under nitrogen atmosphere at a heating rate of 10 °C/min and a nitrogen flow rate of 50 mL/min. For DSC measurement, the sample (5–7 mg) was packed into an aluminum pan and heated from 30 °C to 600 °C under nitrogen atmosphere at a heating rate of 10 °C/min and a nitrogen flow rate of 50 mL/min.

2.4. Determination of amino group content by ninhydrin assay

The amount of amino groups of chitosan was determined by ninhydrin assay using a method modified from Sabnis and Block (2000). Ferulic acid grafted chitosan was dissolved in 1% (v/v) aqueous acetic acid solution (1 mg/mL) under constant stirring at ambient temperature for 24 h. Acetate buffer (pH 5.5, 0.5 mL) and freshly prepared ninhydrin reagent (50 mg/mL of ninhydrin in dimethylformamide, 2 mL) were added to the sample solution (0.5 mL). The mixture was incubated in a boiling water bath for 30 min, and then cooled down to room temperature prior to UV measurement at 570 nm. The remaining content of amino groups was defined from the decrease in absorbance of the solution.

2.5. Solubility test of ferulic acid grafted chitosan

Solubility of ferulic acid grafted chitosan was tested in common organic solvents, i.e. hexane, toluene, chloroform, ethanol, acetone, acetic acid solution (1% v/v), NaOH solution (1% w/v), and water. Sample (1 mg) and each organic solvent (1 mL) were mixed together by vortexing for a few minutes. The mixtures were then agitated at room temperature overnight prior to observation.

2.6. Evaluation of antioxidant activity of ferulic acid grafted chitosan by DPPH assay

The antioxidant activity of ferulic acid grafted chitosan was evaluated by DPPH assay according to the method of Parejo et al. (2002). Sample (1 mg) was mixed with an ethanolic solution of the stable DPPH radical (100 μM, 1 mL) and then 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 Eq. (1):

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

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 ferulic acid grafted chitosan by reducing power assay

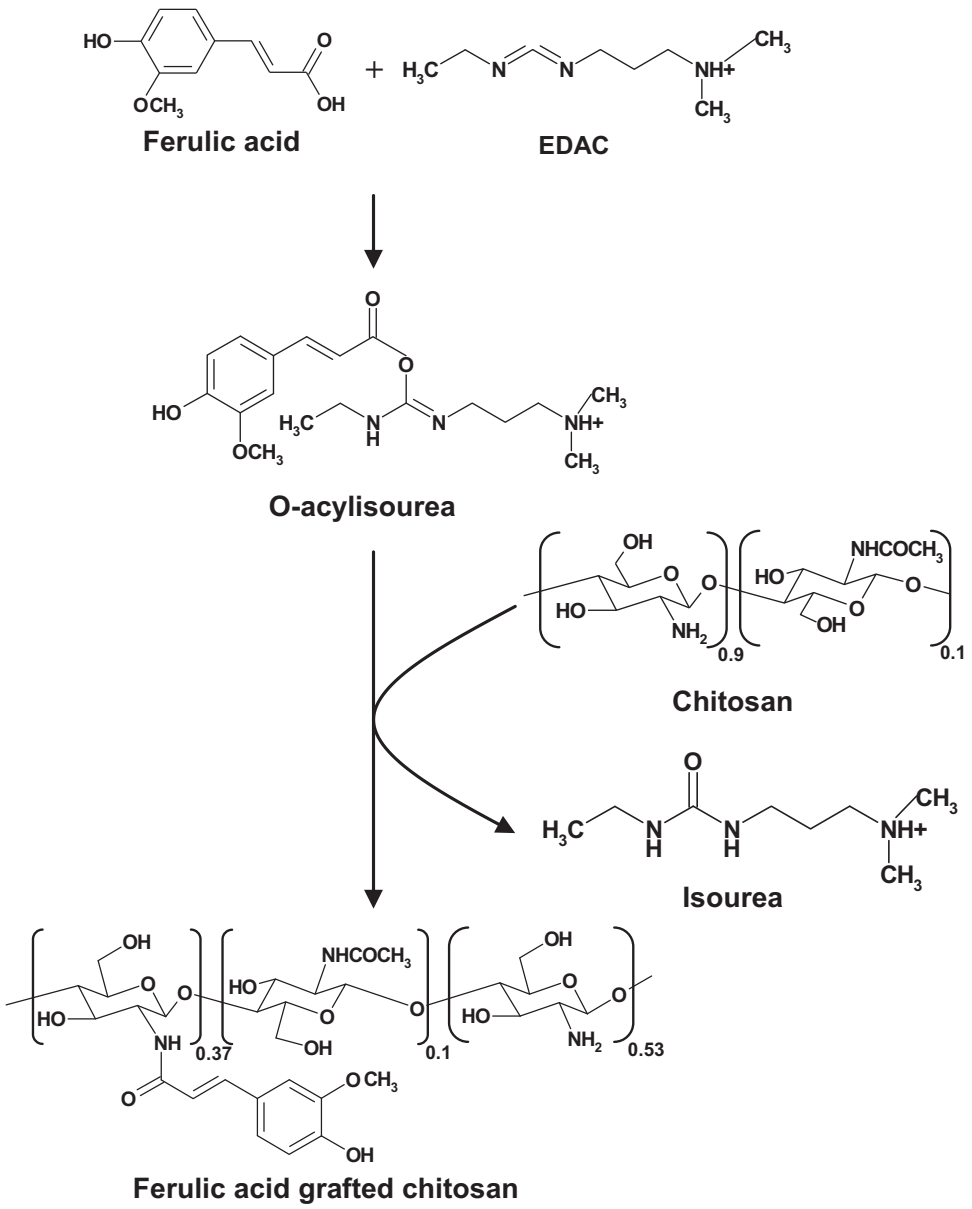
The reducing power of ferulic acid grafted chitosan was determined according to the method of Oyaizu (1986). Briefly, sample (1 mg) was added to a mixture of phosphate buffer (2.5 mL) and potassium ferricyanide (1% w/v, 2.5 mL) and then heated to 50 °C for 20 min. After the mixture was cooled to ambient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

3. Results and discussion

3.1. Characterization of ferulic acid grafted chitosan

Ferulic acid was grafted onto chitosan through a carbodiimide-mediated coupling reaction, as illustrated in Scheme 1.

The successful preparation of ferulic acid grafted chitosan was confirmed by UV–vis spectrophotometry, FTIR and ^{1}H NMR techniques. Ferulic acid (in ethanol solution) gave a maximum



Scheme 1. Synthesis of ferulic acid grafted chitosan.

absorption peak at 323 nm, while chitosan (in 1% (v/v) acetic acid solution) exhibited no absorption peak at any wavelength ranging from 250 nm to 400 nm (data not shown). However, ferulic acid grafted chitosan (in 1% (v/v) acetic acid solution) showed the same maximum absorption peak as ferulic acid at 323 nm (data not shown), reflecting the grafting of ferulic acid onto chitosan.

Fig. 1 shows FTIR spectra of ferulic acid, chitosan and ferulic acid grafted chitosan. Ferulic acid possessed characteristic peaks at 3436 cm^{-1} (OH), 2951–3016 cm^{-1} (C—H stretching) and 1517 cm^{-1} (C=C aromatic ring) (Fig. 1a) (Wang, Cao, Sun, & Wang, 2011). Chitosan exhibited major characteristic bands at 3443 cm^{-1} (OH), 2923 cm^{-1} (C—H stretching), 1638 cm^{-1} (amide I), 1552 cm^{-1} (amide II), 1078 cm^{-1} (C—O—C) and 899 cm^{-1} (pyranose ring) (Fig. 1b) (Bobu, Nicu, Lupei, Ciolacu, & Desbrières, 2011). Compared to chitosan, ferulic acid grafted chitosan showed new peaks at 1517 cm^{-1} (C=C aromatic ring) and increased peak intensity of C—H stretching at 2926 cm^{-1} (Fig. 1c). The result implied that ferulic acid was grafted onto chitosan.

The chemical structure of ferulic acid grafted chitosan was also further confirmed by ^{1}H NMR technique. Ferulic acid showed proton signals at δ = 3.877 ppm (—OCH_3), δ = 6.283–6.315 ppm (H—h), δ = 6.791–6.807 ppm (H—e), δ = 7.038–7.058 ppm (H—f), δ = 7.159 ppm (H—b) and δ = 7.569–7.601 ppm (H—g) (Fig. 2a) (Bunzel, Ralph, Funk, & Steinhart, 2003). The proton signals of chitosan appeared at δ = 3.187 ppm (H-2), δ = 3.710–4.579 ppm (H-3—H-6) and δ = 5.263 ppm (H-1) (Fig. 2b) (Bobu et al., 2011). The grafting of ferulic acid onto chitosan led to the appearance of new strong signals at δ = 6.311–7.672 ppm (methine protons of ferulic acid) (Fig. 2c), suggesting the successful preparation of ferulic acid grafted chitosan.

3.2. Effect of reaction temperature, reaction time and initial content of ferulic acid on degree of substitution

The substitution degree of ferulic acid onto chitosan was determined by ^{1}H NMR technique based on the integral ratio of ferulic

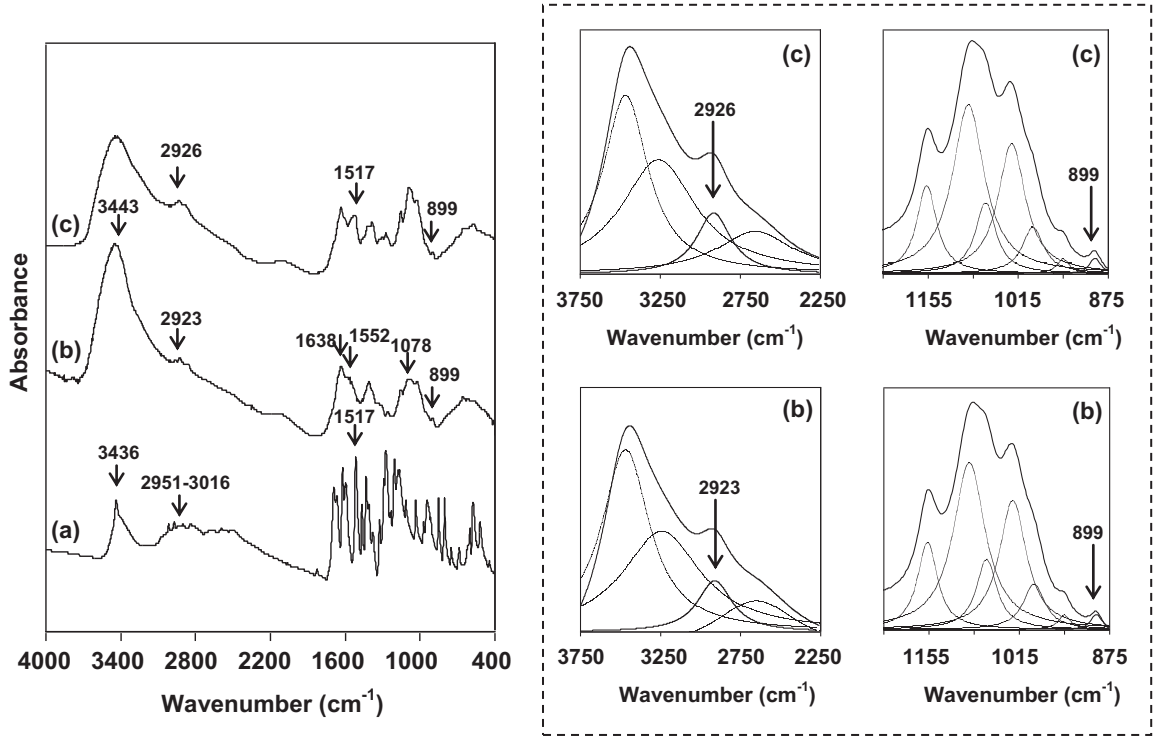


Fig. 1. FTIR spectra and their curve-fitting quantitative bands (right) of: (a) ferulic acid; (b) chitosan; and (c) ferulic acid grafted chitosan, prepared using a mole ratio of chitosan to ferulic acid of 1:1 at 60 °C and 3 h.

acid methine protons to H-2 of chitosan, i.e. DS = (integral of ferulic acid methine protons/5)/(integral of H-2/1). Fig. 3 shows that the substitution degree of ferulic acid was in the ranges of 0.22–0.37, 0.23–0.37 and 0.15–0.37 when the reaction temperature, reaction time and initial content of ferulic acid were varied from 40 to 80 °C, 1.5 to 24 h, and 0.5 to 2.5 mol/mol of chitosan, respectively. The substitution degree tended to increase with increasing reaction temperature (up to 60 °C), reaction time (up to 3 h), and initial content of ferulic acid (up to 1 mol/mol of chitosan). Further augmentation of those factors resulted in decreased substitution. The reduction of substitution level might be a result of partial hydrolysis of the grafted ferulic acid when the reaction temperature was higher than 60 °C and reaction time was longer than 3 h. This assumption is in agreement with the report by Osemeahon, Barminas, Aliyu, and Nkafamiya (2008). In addition, the mobility of ferulic acid might be limited due to high viscosity when the added ferulic acid content was higher than 1 mol/mol of chitosan, causing decreased substitution degree.

Curve-fitting quantitative FTIR technique, using OPUS 6.5 software, is an indirect method of determining the substitution level of ferulic acid onto chitosan. The level of grafting was related to the integral ratio of the C—H stretching band (2926 cm^{-1}, belonging to chitosan and ferulic acid) to the pyranose band (899 cm^{-1}, belonging to chitosan), i.e. I_{2926}/I_{899} (Fig. 1, right). It should be noted that chitosan possessed an I_{2926}/I_{899} value of 19.64 (data not shown). Ferulic acid grafted chitosan showed I_{2926}/I_{899} values higher than those of chitosan: in the ranges of 28.7–55.2, 21.7–55.2 and 36.0–55.2 when the reaction temperature, reaction time and initial content of ferulic acid were varied from 40 to 80 °C, 1.5 to 24 h, and 0.5 to 2.5 mol/mol of chitosan, respectively (Fig. 3). This result reflected the successful grafting of ferulic acid onto chitosan. Similar to ^{1}H NMR results, the value of I_{2926}/I_{899} increased with increasing reaction temperature, reaction time and initial content of ferulic acid up to 60 °C, 3 h, and 1 mol/mol of chitosan, respectively, and their further increase caused a reduction of substitution level (Fig. 3). This result supported the above finding. Therefore, the condition with reaction temperature of 60 °C, reaction time of 3 h and mole ratio of chitosan to ferulic acid of 1:1 was suggested for preparing ferulic acid grafted chitosan. The obtained ferulic acid grafted chitosan possessed degree of ferulic acid substitution of 0.37, i.e. 100 chitosan pyranose units contained 37 molecules of ferulic acid.

3.3. Remaining amino group content of ferulic acid grafted chitosan

Ninhydrin assay is an indirect method of confirming whether the grafting of ferulic acid occurred at amino groups of chitosan. This method is based on the reaction of the primary amino groups of chitosan with ninhydrin reagent to form chitosan–ninhydrin complexes, which have a deep blue color, resulting in an increase in absorbance at 570 nm (Sabnis & Block, 2000). Fig. 4 shows that the absorbance at 570 nm of chitosan was 0.88. The grafting of ferulic acid onto chitosan resulted in decreased absorbance values, ranging from 0.41 to 0.75. The reduction of absorbance implied the decreased free amino group content of ferulic acid grafted chitosan. This result reflected the grafting of ferulic acid onto chitosan at amino groups. However, the absorbance of ferulic acid grafted chitosan tended to decrease with increasing initial content of ferulic acid up to 1 mol/mol of chitosan, and then tended to increase as a function of initial ferulic acid content (Fig. 4). This result confirmed that an initial content of ferulic acid of 1 mol/mol of chitosan provided the derivative with the highest substitution degree of ferulic acid. This was in agreement with the result illustrated in Fig. 3c.

3.4. Packing structure of ferulic acid grafted chitosan

The packing structure of ferulic acid grafted chitosan was examined by X-ray diffractometry. Chitosan showed characteristic crystalline peaks at 2θ of 9°, 15° and 20° (Tripathi, Mehrotra,

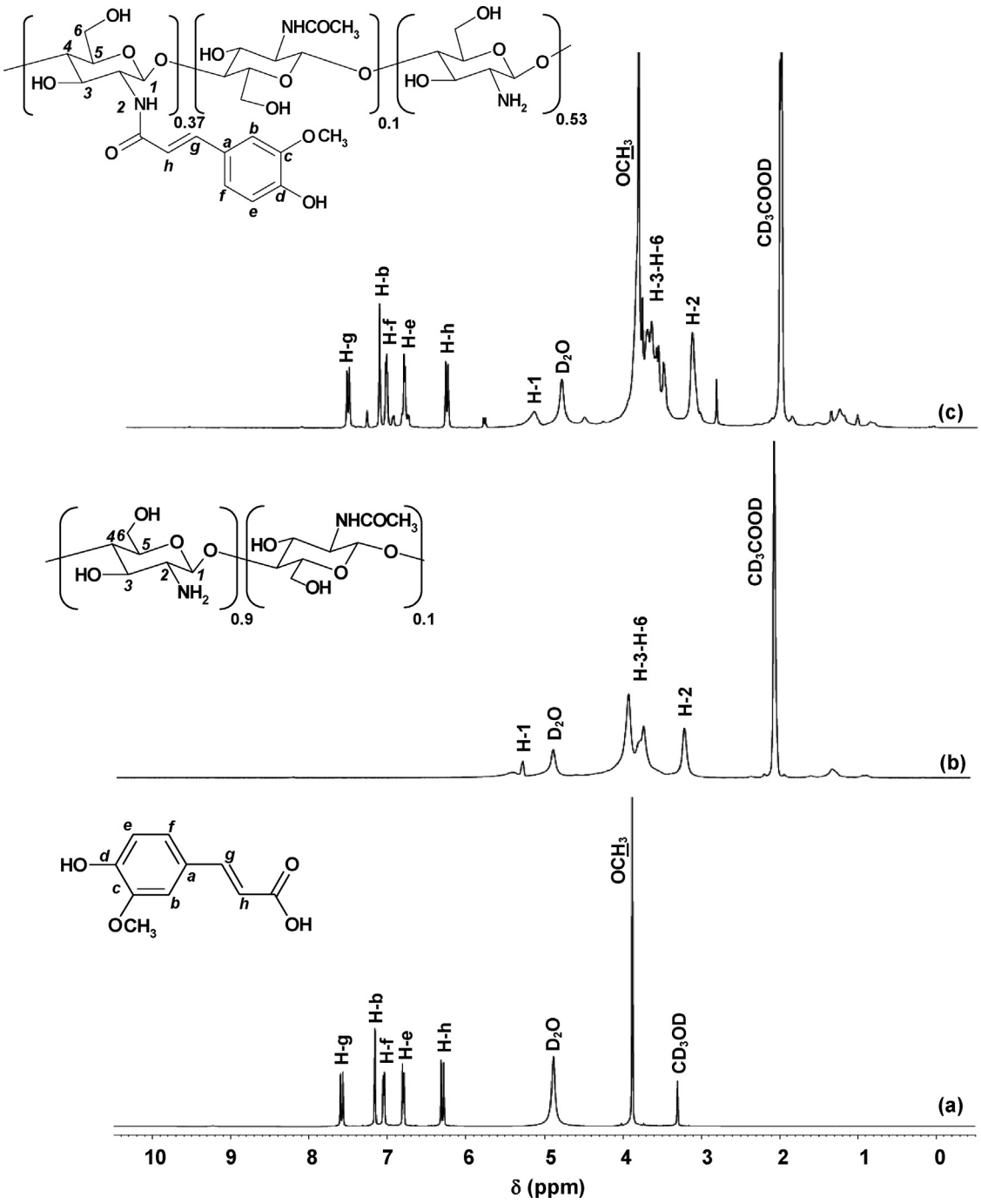


Fig. 2. ^{1}H NMR spectra of: (a) ferulic acid; (b) chitosan; and (c) ferulic acid grafted chitosan, prepared using a mole ratio of chitosan to ferulic acid of 1:1 at 60 °C and 3 h.

& Dutta, 2009), with crystallinity of 31.6% (Fig. 5a). The high crystallinity of chitosan is generally caused by inter- and intramolecular hydrogen bonds. Compared with chitosan, ferulic acid grafted chitosan exhibited broader XRD patterns (Fig. 5b–f) and lower crystallinity, i.e. 18.4–21.9%. The crystallinity reduction of ferulic acid grafted chitosan might be attributed to the destruction of inter- and intramolecular hydrogen bonds of chitosan by bulky ferulic acid. Ferulic acid grafted chitosan prepared using an initial content of ferulic acid of 1 mol/mol of chitosan showed the lowest crystallinity level (18.4%) due to its possessing the highest substitution degree of ferulic acid, i.e. 0.37 (Fig. 3C). Crystallinity percentages of other ferulic acid grafted chitosan (prepared using the initial contents of ferulic acid of 0.5, 1.5, 2.0 and 2.5 mol/mol of chitosan) were not significantly different (20.4–21.9) because they possessed the same degree of ferulic acid substitution, i.e. 0.15–0.22 (Fig. 3C).

3.5. Thermal properties of ferulic acid grafted chitosan

Thermal properties of chitosan and ferulic acid grafted chitosan were determined by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). TGA is a useful technique to investigate the mass loss of a sample upon heating at a given heating rate. The temperature for maximum rate of weight loss was determined as decomposition temperature, which is clearly observed as a peak in the derivative thermogravimetric (DTG) thermogram. Fig. 6A shows that the mass loss of chitosan and ferulic

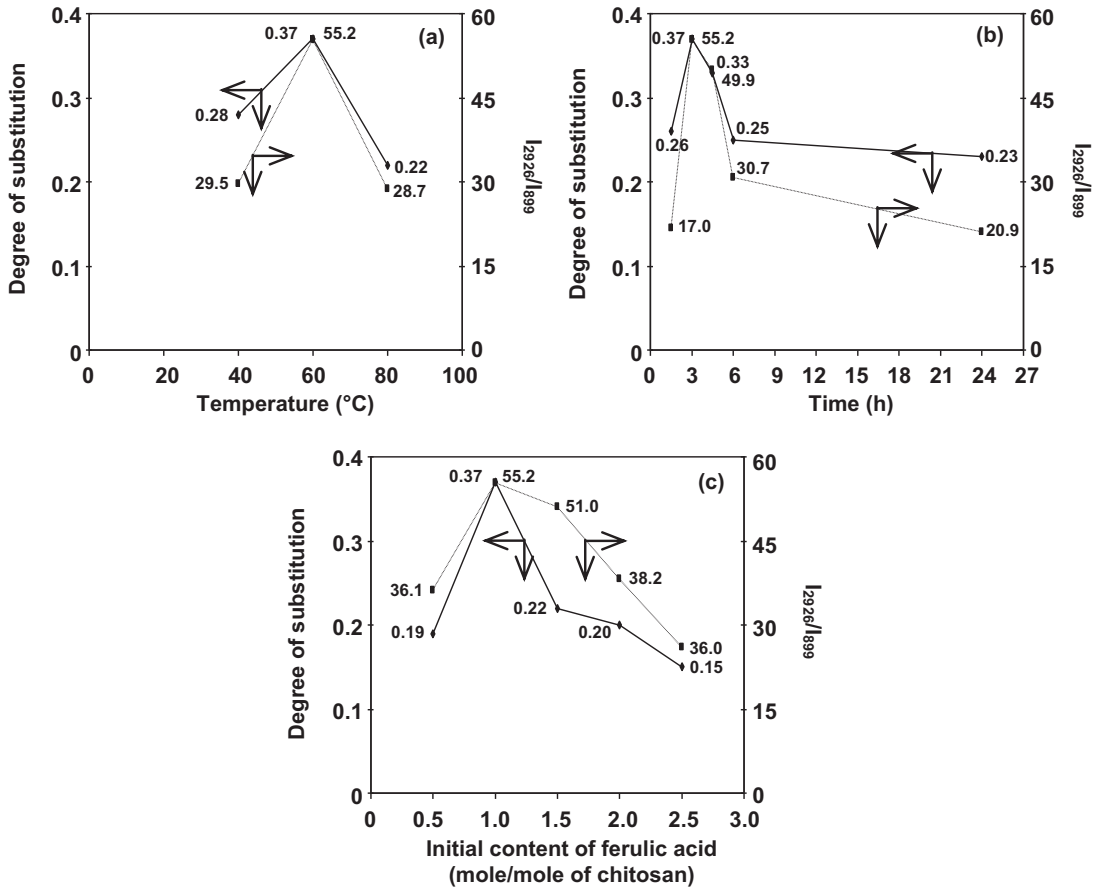


Fig. 3. Substitution degree of ferulic acid onto chitosan (—) and I_{2926}/I_{899} (—) as a function of: (a) reaction temperature (reaction time = 3 h, and initial content of ferulic acid = 1 mol/mol of chitosan); (b) reaction time (reaction temperature = 60 °C, and initial content of ferulic acid = 1 mol/mol of chitosan); and (c) initial content of ferulic acid (reaction temperature = 60 °C, and reaction time = 3 h).

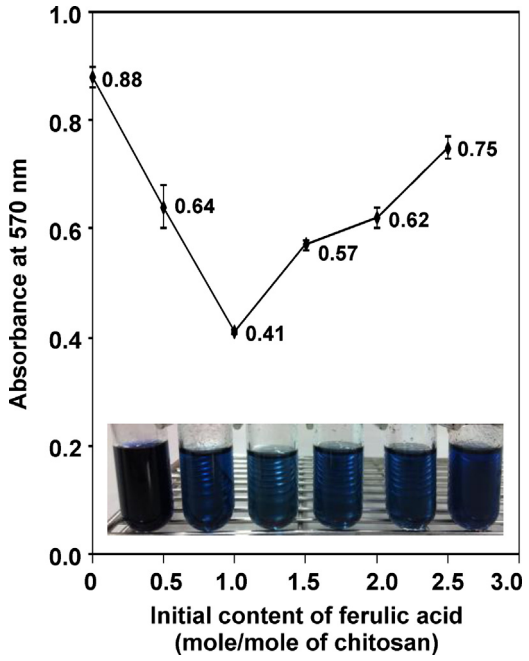


Fig. 4. Absorbance at 570 nm of chitosan and ferulic acid grafted chitosan prepared using different initial contents of ferulic acid. Each point represents the mean ± SD (n = 3). The inset represents the color of the sample–ninhydrin complex.

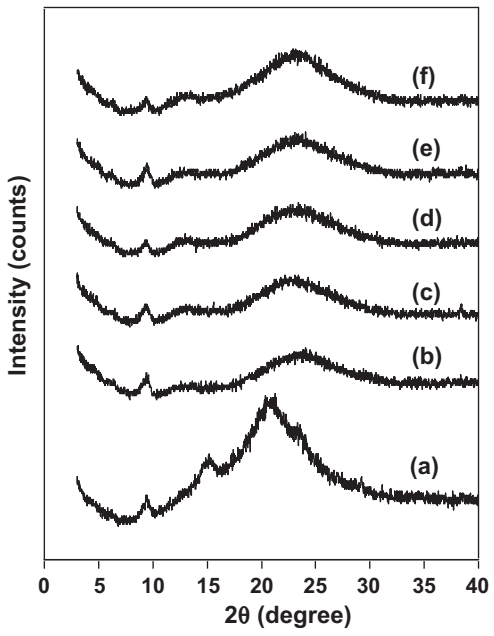


Fig. 5. X-ray diffractograms of: (a) chitosan; and (b)–(f) ferulic acid grafted chitosan, prepared using different mole ratios of chitosan to ferulic acid – (b) 1.0:0.5, (c) 1.0:1.0, (d) 1.0:1.5, (e) 1.0:2.0, and (f) 1.0:2.5.

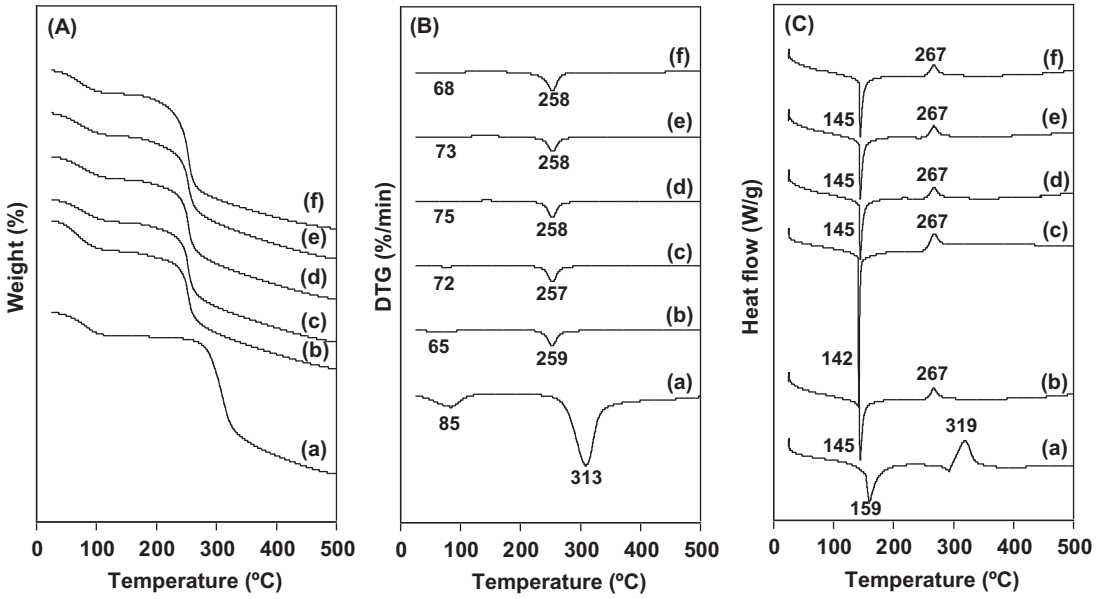


Fig. 6. (A) TGA, (B) DTG and (C) DSC thermograms (exo up) of: (a) chitosan; and (b)–(f) ferulic acid grafted chitosan, prepared using different mole ratios of chitosan to ferulic acid – (b) 1.0:0.5, (c) 1.0:1.0, (d) 1.0:1.5, (e) 1.0:2.0, and (f) 1.0:2.5.

acid grafted chitosan progresses as a function of temperature. Chitosan exhibited two-step mass loss at temperatures ranging from 50 to 100 °C (peak at 85 °C) and from 250 to 350 °C (peak at 313 °C) (Fig. 6Aa and Ba), attributed to the evaporation of moisture and the decomposition of chitosan, respectively (Zeng, Qin, Wang, & Li, 2011). Similarly, ferulic acid grafted chitosan also possessed two-step mass loss. The grafting of ferulic acid resulted in decreased decomposition temperature of chitosan, to a temperature range of 257–259 °C (Fig. 6Ab–f and Bb–f). This might be due to the crystallinity reduction of chitosan after grafting with ferulic acid. However, the substitution degree of ferulic acid had a negligible effect on the decomposition temperature of ferulic acid grafted chitosan.

DSC is a thermoanalytical technique used to study the thermal transitions of a polymer. Chitosan exhibited an endothermic peak at 159 °C and an exothermic peak at 319 °C (Fig. 6Ca). The endothermic reaction might involve the evaporation of water bound to the chitosan polymeric chain, while the exothermic event at higher temperature is likely related to the cross-linking reactions of the decomposed compositions of chitosan (Zeng et al., 2011). It should be pointed out that the cross-linking reactions of the decomposed compositions of ferulic acid grafted chitosan took place at lower temperatures (Fig. 6Cb–f) than those of chitosan (Fig. 6Ca). This might be related to the lower decomposition temperature of chitosan after grafting with ferulic acid.

3.6. Solubility in common organic solvents of ferulic acid grafted chitosan

The solubility of chitosan and ferulic acid grafted chitosan were tested at ambient temperature in common organic solvents. Chitosan and ferulic acid grafted chitosan could not be dissolved in hexane, toluene, chloroform, ethanol, acetone, dimethylformamide, or NaOH solution (1% w/v); however they were well soluble in acetic acid solution (1% v/v) due to the amino group protonation. Unlike chitosan, ferulic acid grafted chitosan could be soluble in water at a concentration of up to 1.3 mg/mL. The water solubility of ferulic acid grafted chitosan might be explained by the reduction of chitosan crystallinity and the hydrophilicity of the ferulic acid hydroxyl group (Nardini, Natella, Scaccini, & Ghiselli, 2006).

3.7. Antioxidant properties of ferulic acid grafted chitosan

Antioxidant efficiency of ferulic acid grafted chitosan was evaluated by DPPH method and reducing power assay. The DPPH radical scavenging percentage of samples was determined by the decrease in absorbance at 517 nm. Chitosan possessed radical scavenging of 41.1% (Fig. 7, left). The antioxidant activity of chitosan might be related to the reaction of free radicals with the hydroxyl group at the C-6 position through H-abstraction, and the reaction of free radicals with the amino group at the C-2 position to form

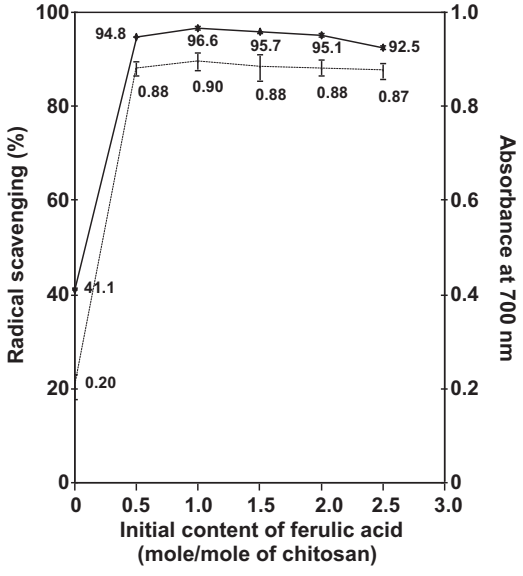


Fig. 7. Radical scavenging percentage (—) and reducing power (—) of ferulic acid grafted chitosan prepared using different initial contents of ferulic acid. Each point represents the mean ± SD ($n = 3$).

stable macromolecule radicals (Xie, Xu, & Liu, 2001). Compared to chitosan, ferulic acid grafted chitosan showed increased radical scavenging of up to 92.5–96.6% (Fig. 7, left). The augmentation of reduction capability of DPPH radicals induced by ferulic acid grafted chitosan might be an effect of ferulic acid, which has been reported to be an antioxidant (Ou & Kwok, 2004). Proton transfer from the phenolic group to the active radicals is a major mechanism for the antioxidant activity of ferulic acid. However, the radical scavenging percentage of ferulic acid grafted chitosan was hardly dependent on the initial content of ferulic acid (Fig. 7, left) or the degree of ferulic acid substitution. This might be attributed to the effective antioxidant property of ferulic acid.

The antioxidant activity of ferulic acid grafted chitosan was also investigated by a reducing power assay based on the chemical reaction of Fe(III) → Fe(II), in which the as-formed Fe(II) then reacts with ferric chloride to form ferric–ferrous complex that has an absorption maximum at 700 nm. The higher absorbance at 700 nm indicates the stronger reducing power. Chitosan possessed an absorbance at 700 nm of 0.20, while ferulic acid grafted chitosan showed an absorbance in the range of 0.87–0.90 (Fig. 7, right). This result implied that both chitosan and its derivative could reduce Fe(III) to Fe(II); however, the reducing power of ferulic acid grafted chitosan was significantly higher than that of chitosan. The antioxidant power of ferulic acid grafted chitosan was not markedly affected by ferulic acid content (Fig. 7, right). These results corresponded to those from the DPPH method.

4. Conclusions

Ferulic acid was grafted onto chitosan through a carbodiimide-mediated coupling reaction. Successful grafting was confirmed from the appearance of a maximum UV–vis absorption peak at 323 nm, the appearance of a FTIR absorption band at 1517 cm^{-1}, the increase of C–H stretching band intensity, and the appearance of proton signals at δ = 6.311–7.672 ppm. Ferulic acid grafted chitosan prepared using a mole ratio of chitosan to ferulic acid of 1:1, reaction temperature of 60 °C and reaction time of 3 h possessed the highest ferulic acid substitution degree of 0.37. Ninhydrin assay confirmed that ferulic acid was grafted onto chitosan at amino groups. Compared to chitosan, ferulic acid grafted chitosan showed reduced crystallinity (about 10%), decreased decomposition temperature (about 55 °C), and improved radical scavenging activity (about 55%), as well as water solubility (1.3 mg/mL). The results suggested that ferulic acid grafted chitosan could potentially be used as an antioxidant in food, food packaging, cosmetics, biomedical and pharmaceutical applications.

Acknowledgements

The authors would like to acknowledge the Thailand Research Fund (TRF) through the Royal Golden Jubilee Ph.D. program (PHD/0050/2553); the Commission on Higher Education, Ministry of Education, Thailand (National Research University of Thailand); and the National Science and Technology Development Agency (NSTDA), Thailand (2009 NSTDA Chair Professor Grant funded by the Crown Property Bureau). The authors are also grateful to Mettler-Toledo (Thailand) Co., Ltd., for permission to use TGA and DSC instruments.

References

Aljawish, A., Chevalot, I., Piffaut, B., Rondeau-Mouro, C., Girardin, M., Jasniewski, J., et al. (2012). Functionalization of chitosan by laccase-catalyzed oxidation of ferulic acid and ethyl ferulate under heterogeneous reaction conditions. *Carbohydrate Polymers*, *87*, 537–544.

Aracri, E., Roncero, M. B., & Vidal, T. (2011). Studying the effects of laccase-catalysed grafting of ferulic acid on sisal pulp fibers. *Bioresource Technology*, *102*, 7555–7560.

Aytekin, A. O., Morimura, S., & Kida, K. (2011). Synthesis of chitosan–caffeic acid derivatives and evaluation of their antioxidant activities. *Journal of Bioscience and Bioengineering*, *111*, 212–216.

Bobu, E., Nicu, R., Lupei, M., Ciolacu, F., & Desbrières, J. (2011). Synthesis and characterization of N-alkyl chitosan for papermaking applications. *Cellulose Chemistry and Technology*, *45*, 619–625.

Bunzel, M., Ralph, J., Funk, C., & Steinhart, H. (2003). Isolation and identification of a ferulic acid dehydrotrimer from saponified maize bran insoluble fiber. *European Food Research and Technology*, *217*, 128–133.

Fajardo, P., Martins, J. T., Fuciños, C., Pastrana, L., Teixeira, J. A., & Vicente, A. A. (2010). Evaluation of a chitosan-based edible film as carrier of natamycin to improve the storability of Saloio cheese. *Journal of Food Engineering*, *101*, 349–356.

Fernandes, S. C. M., Freire, C. S. R., Silvestre, A. J. D., Neto, C. P., Gandini, A., Berglund, L. A., et al. (2010). Transparent chitosan films reinforced with a high content of nanofibrillated cellulose. *Carbohydrate Polymers*, *81*, 394–401.

Jung, B. O., Chung, S. J., & Lee, S. B. (2006). Preparation and characterization of eugenol-grafted chitosan hydrogels and their antioxidant activities. *Journal of Applied Polymer Science*, *99*, 3500–3506.

Mohamed, C., Clementine, K. A., Didier, M., Gérard, L., & Noëlle, D. C. M. (2013). Antimicrobial and physical properties of edible chitosan films enhanced by lactoperoxidase system. *Food Hydrocolloids*, *30*, 576–580.

Nakashima, T., Matsuo, M., Bin, Y., Nakano, Y., Kobayashi, T., Komemushi, S., et al. (2006). Mechanical properties and antibacterial efficacy of chitosan films. *Biocontrol Science*, *11*, 27–36.

Nardini, M., Natella, F., Scaccini, C., & Ghiselli, A. (2006). Phenolic acids from beer are absorbed and extensively metabolized in humans. *Journal of Nutritional Biochemistry*, *17*, 14–22.

Osemeahon, S. A., Barminas, T. J., Aliyu, B. A., & Nkafamiya, I. I. (2008). Development of sodium alginate and konkoli gumgrafted-polyacrylamide blend membrane: optimization of grafting conditions. *African Journal of Biotechnology*, *7*, 1309–1313.

Ou, S., & Kwok, K. C. (2004). Ferulic acid: pharmaceutical functions, preparation and applications in foods. *Journal of the Science of Food and Agriculture*, *84*, 1261–1269.

Oyaizu, M. (1986). Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*, *44*, 307–315.

Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Flerlage, N., Burillo, J., et al. (2002). Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilled Mediterranean herbs and aromatic plants. *Journal of Agricultural and Food Chemistry*, *50*, 6882–6890.

Pinto, R. J. B., Fernandes, S. C. M., Freire, C. S. R., Sadocco, P., Causio, J., Neto, C. P., et al. (2012). Antibacterial activity of optically transparent nanocomposite films based on chitosan or its derivatives and silver nanoparticles. *Carbohydrate Research*, *348*, 77–83.

Sabnis, S., & Block, L. H. (2000). Chitosan as an enabling excipient for drug delivery systems. I. Molecular modifications. *International Journal of Biological Macromolecules*, *27*, 181–186.

Tripathi, S., Mehrotra, G. K., & Dutta, P. K. (2009). Physicochemical and bioactivity of cross-linked chitosan–PVA film for food packaging applications. *International Journal of Biological Macromolecules*, *45*, 372–376.

Trouillas, P., Marsal, P., Siri, D., Lazzaroni, R., & Duroux, J. L. (2006). A DFT study of the reactivity of OH groups in quercetin and taxifolin antioxidants: the specificity of the 3-OH site. *Food Chemistry*, *97*, 679–688.

Wang, J., Cao, Y., Sun, B., & Wang, C. (2011). Characterisation of inclusion complex of trans-ferulic acid and hydroxypropyl-β-cyclodextrin. *Food Chemistry*, *124*, 1069–1075.

Xie, W., Xu, P., & Liu, Q. (2001). Antioxidant activity of water-soluble chitosan derivatives. *Bioorganic & Medicinal Chemistry Letters*, *11*, 1699–1701.

Yu, S. H., Hsieh, H. Y., Pang, J. C., Tang, D. W., Shih, C. M., Tsai, M. L., et al. (2013). Active films from water-soluble chitosan/cellulose composites incorporating releasable caffeic acid for inhibition of lipid oxidation in fish oil emulsions. *Food Hydrocolloids*, *32*, 9–19.

Zeng, L., Qin, C., Wang, L., & Li, W. (2011). Volatile compounds formed from the pyrolysis of chitosan. *Carbohydrate Polymers*, *83*, 1553–1557.