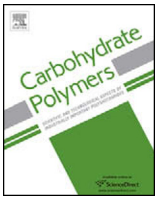
Carbohydrate Polymers xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Carbohydrate Polymers

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



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

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ARTICLE INFO

Article history:
Received 7 March 2014
Received in revised form 22 June 2014
Accepted 23 June 2014
Available online xxx

Keywords:
Ferulic acid
Chitosan
Thermal stability
Antioxidant
Active packaging
Biodegradable film

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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http://dx.doi.org/10.1016/j.carbpol.2014.06.074

Please cite this article in press as: Woranuch, S., et al. Ferulic acid-coupled chitosan: Thermal stability and utilization as an antioxidant for biodegradable active packaging film. *Carbohydrate Polymers* (2014), http://dx.doi.org/10.1016/j.carbpol.2014.06.074

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^{TM} 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:

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

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.

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