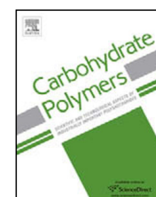
Carbohydrate Polymers 153 (2016) 105–114

Contents lists available at ScienceDirect

Carbohydrate Polymers

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



# Transglutaminase catalyzed hydrolyzed wheat gliadin grafted with chitosan oligosaccharide and its characterization

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ARTICLE INFO

*Article history:*
Received 11 May 2016
Received in revised form 21 July 2016
Accepted 22 July 2016
Available online 25 July 2016

*Keywords:*
Hydrolyzed wheat gliadin
Chitosan oligosaccharide
Transglutaminase
Antibacterial

ABSTRACT

Chitosan oligosaccharide (COS) was grafted on hydrolyzed wheat gliadin (HWG) with microbial transglutaminase (MTGase) as catalyst. The grafting reaction exhibited the best performance when it was done under the optimum temperature 50 °C for 50 min with HWG/COS mass ratio of 40:1, pH 6.00–6.50. The maximum grafting rate of COS was 64.83% at this condition. The chemical structure characterizations of HWG-COS performed by FTIR, $^{13}$C NMR, X-ray diffractometry and TGA-DTG illustrated that amino groups in COS had participated in the formations of the amino band during the synthesis. HPLC and GFC analysis showed that HWG-COS had two main components, which together accounted for 80.64% of the total polymer and the molecular weight of the two components was 61.77 kDa and 27.29 kDa, respectively. HWG-COS was undissolved in water and many organic solvent, slightly soluble in 1% NaOH, with a solubility of 1.84 mg/L. In antibacterial activity test, HWG-COS showed the best antimicrobial properties to *Salmonella enteriditis*, with an antibacterial activity improved by 41.74%.

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## 1. Introduction

Chitin is the most abundant polysaccharide in natural macromolecules, next to cellulose, as the major component of the shells of crustaceans, such as crabs and insects. Although chitin has many functional specialties in various areas, the low water solubility and poor biodegradation performance restricts its applications (Zou et al., 2016). As the degraded products of chitosan or chitin, chitosan oligosaccharide (COS) is a mixture of oligomers of β-1, 4-linked D-glucosamine residues that have better biocompatibility and solubility due to their shorter chain lengths and free amino groups in D-glucosamine units, which is of special interest in agriculture, food industry, environmental engineering, and medicine for it has activities such as anti-inflammatory, antimicrobial (Choi et al., 2001), hypocholesterolemic (Muzzarelli et al., 2006), immunostimulating (Feng, Zhao, & Yu, 2004), and anti-tumour activity (Salah et al., 2013). COS possess the properties of anti-inflammatory, antimicrobial, can inhibit the growth of dozens of species of microorganisms (Choi et al., 2001).

Meanwhile, protein based materials draw people's attention for their good barrier properties against oxygen and aroma compounds (Cuq, Gontard, & Guilbert, 1998; Türe, G€allstedt, & Hedenqvist, 2012). Among them, wheat gluten, a by-product of the wheat starch industry, is known to contain a number of different proteins in abundance. The gliadins provide viscosity and extensibility to dough, whereas the glutenins contribute to dough cohesiveness and elasticity (Kasarda, 1989). Wheat gluten is a good quality and inexpensive protein compared with other vegetable proteins. However, the water soluble of wheat gluten is poor, which affects its application (Agyare, Xiong, & Addo, 2008; Wang, Zhao, Yang, & Jiang, 2006; Wang, Zhao, Bao, Hong, & Rosella, 2008). As raw wheat gluten is widely underutilized as an animal feed, its current applications are limited; therefore, it is desirable to develop new applications for this product in the packaging industry. Moreover, wheat gluten films can be obtained by thermoplastic processing, which consists of mixing proteins and plasticizer by a combination of heat and shear (Hernandez & Krochta, 2008) followed by an additional stage involving further thermo-mechanical treatments (e.g. compression molding) (Pommet, Redl, Guilbrt, & Morel, 2005; Sun, Song, & Zheng, 2008), which, from economic and environmental viewpoints, is the most viable way to produce rigid gluten-based materials since it is fast and requires no solvent (G€allstedt, Mat-

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http://dx.doi.org/10.1016/j.carbpol.2016.07.097
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tozzi, Johansson, & Hedenqvist, 2004; Jansens, Lagrain, Rombouts, Smet, & Delcour, 2011).

Therefore, the objective of this work was to synthesis a novel polymer use COS and wheat gluten as substrates. In order to synthesize the novel polymer, an acyl transfer reaction has to be conducted by microbial transglutaminase (MTGase). Microbial transglutaminase (MTGase, c-glutamyltransferase, EC 2.3.2.13) is an extracellular enzyme with a remarkable characteristic of calcium-independent catalytic property. Furthermore, it exhibits wide substrate specificity over a wide range of temperature and pH values (Raffaele, Loredana, Prospero, Angela, & Giosafatto, 2011). The MTGase is often used to catalyze macromolecular grafting and crosslinking of proteins (Wu, Bentley, & Payne, 2011). MTGase catalyzes cross-linking between the glutamine and lysine side chains on wheat gluten, resulting in the formation of high-molecular-weight polymers and improvement in the network structure and gelation behavior of wheat gluten (Wang, Zhao, Yang, Jiang, & Chun, 2007). However, owing to the high glutamine and proline contents in the interior of its structure and the rarely ionized side chains, wheat gluten exhibits poor solubility (Wieser, 2007), and is thus unsuitable for MTGase catalysis. Limited hydrolysis improves gluten solubility (Agyare et al., 2008; Batey, 1985) and it's foaming and emulsifying properties (Popineau, Blandine, Larre, & Berot, 2002). It is reported that functionality of gluten could also be enhanced by protease digestion or acid hydrolysis (Babiker, Fujisawa, Matsudomi, & Kato, 1996). For the sake of integrating mutual advantages, researchers have tried to conjugate chitosan oligosaccharide with variety of proteins or peptides (Sang, Zhou, Yun, & Zhang, 2010).

In this paper, a novel polymer HWG-COS was synthesized using COS and hydrolyzed wheat gliadin (HWG) as substrates, with microbial transglutaminase (MTGase) as biocatalyst. Under appropriate conditions, the acyl transfer reactions between the COS and HWG by MTGase were explored to synthesize HWG-COS. The characterisation of the polymer, solubility, and antibacterial activity test was executed.

## 2. Materials and methods

### 2.1. Materials

Microbial transglutaminase (MTGase) (1.5 U/mg), was purchased from Yiming Fine Chemical Company (Jiangsu, China). Bovine serum albumin (BSA), d-glucosamine hydrochloride, sephadex G-75 chromatography, lysozyme (14400 Da), trypsin (2500 U/mg) was purchased from Guoyuan Biotechnology Co. Ltd. (Shanghai, China). All the reagents above were of biological grade. Chitosan oligosaccharide (Mw < 5000 Da), with the deacetylation degree of 90.32%, was purchased from Yuhuan Biochemical Co. Ltd. (Zhejiang, China). Gliadin (protein content 10%) was purchased from Haifan Biochemical Co. Ltd. (Chongqing, China). *p*-Dimethylaminobenzaldehyde, *N*,*N*-Dimethylformamide and other chemicals were purchased from Chemical Head Factory (Chongqing, China), and all of them were of analytical grade.

### 2.2. Preparation of HWG-COS copolymer

#### 2.2.1. Purification of MTGase

MTGase was first poured into 0.1 mol/L $KH_2PO_4$/NaOH buffer solution (PBS, pH 6.45). The liquid was centrifuged at the speed of 5000 RPM for 10 min. Then the supernatant was purified by dialysis through the 10,000 molecular weight cut-off dialysis tubing for three days. The dialyzed MTGase was lyophilized for 24 h to obtain the MTGase lyophilized power and stored at 4 °C.

#### 2.2.2. Preparation of hydrolyzed wheat gliadin (HWG)

A moderate amount of wheat gliadin was poured into 0.1 mol/L $Na_2C0_3$/$NaHCO_3$ buffer solution (pH 10.0), 0.5% (w/w) trypsin was added and stirred at 55 °C for 40 min. Then the solution was treated in boiling water for 10 min to inactivate the enzyme. The hydrolysate was centrifuged at the speed of 3000 RPM for 10 min, and then the supernatant was collected to obtain HWG.

#### 2.2.3. Preparation of HWG-COS copolymer

Chitosan oligosaccharide (1 g) was dissolved in 50 mL aqueous solution to prepare 2% (w/v) COS solution. Then 2% COS solution and HWG were mixed together with the mass ratio of 1:40 and the pH was adjusted to 6.00–6.50 with dilute hydrochloric acid to form homogeneous solution. Thereafter, MTGase powder (0.1 g) was added to the above mixture to catalyze the reaction between COS and HWG. Magnetic stirring was continuous for 50 min at 50 °C. Then the solution was treated in boiling water for 10 min and later cooled to room temperature. The solution was centrifuged at the speed of 3000 RPM for 10 min. Subsequently, the supernatant was purified by dialysis through the 10,000 molecular weight cut-off dialysis tubing for three days. The dialyzed product was finally freeze-dried to obtain the purified HWG-COS.

### 2.3. One-factor-at-a-time experiment

To achieve the best grafting performance, the temperature, pH, reaction time and the mass ratio of HWG to COS should be optimized. Due to the optimum temperature and pH of MTGase in acyl transfer reaction was fixed as 50 °C and 6.00–6.50, the other two independent variables were investigated, including the reaction time (10, 20, 30, 40, 50, 60, 70 min), the mass ratio of HWG to COS (10/1, 20/1, 30/1, 40/1, 50/1). Their variables were fixed at a certain value, while changing only one variable in a single experiment.

### 2.4. Measurement of the grafting ratio

The grafting ratio is defined as the number of amine groups substituted per repeating structural unit of the chitosan oligosaccharide. The grafting ratio of HWG-COS was determined by the following equation:

$$G(\%) = \left(\frac{W_1 - W_2}{W_2}\right) \times 100\%$$

where G is the grafting ratio (%), $W_1$ is the mass of HWG-COS, $W_2$ is the mass of COS.

### 2.5. FTIR spectroscopy

Fourier transform infrared (FTIR) analysis was carried out on a Nicolet, 550 II spectrometer (USA) scanning from 4000 $cm^{-1}$ to 500 $cm^{-1}$ at room temperature. Chitosan oligosaccharide, wheat gliadin and HWG-COS samples were, respectively, mixed with KBr and pressed to plates for measurements.

### 2.6. $^{13}C$ NMR spectroscopy

$^{13}C$ NMR spectra of COS, wheat gliadin and freeze-dried HWG-COS were measured on a spectrometer (Agilent, vnmrs 600) at room temperature, among them COS using $D_2O$ as solvent, wheat gliadin and HWG-COS using DMSO as solvent, with tetramethylsilane (TMS) as the internal standard.

### 2.7. X-ray diffractometry

X-ray diffraction patterns of chitosan oligosaccharide and HWG-COS were obtained on a shimadzu model XRD-6000 diffractometer.

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