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Evaluation of structural and functional properties of chitosan–chlorogenic acid complexes

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ABSTRACT

The objectives of the present study were to first synthesize chitosan–chlorogenic acid (CA) covalent complex and then compare structural and functional properties between chitosan–CA covalent complex and physical complex. First, chitosan–CA covalent complex was synthesized and its total phenolic content was as high as 276.5 ± 6.2 mg/g. Then structural and functional properties of chitosan–CA covalent and physical complexes were analyzed. The covalent reaction induced formation of both amide and ester bonds in chitosan. Data of X-ray diffraction (XRD) and scanning electron microscopy (SEM) indicated that the complexations of CA changed crystallinity and morphology of chitosan, and covalent complexation induced a larger change of physical structure than physical complexation. In terms of functional properties, chitosan–CA covalent complex exhibited better thermal stability than physical complex in terms of antioxidant activity, and the viscosity of chitosan was significantly increased by covalent modification.

1. Introduction

Chitosan is a highly basic polysaccharide with one amino group and two hydroxyl groups, and it exhibits special properties such as low solubility and high viscosity [23]. Chitosan is mainly derived from crab and shrimp shells, and it is the most bountiful natural polysaccharide after cellulose [26]. As an abundant renewable resource, chitosan has fruitful applications in industrial areas, but poor solubility has limited its further applications. Since the active primary amino group and hydroxyl group make chitosan suitable for side group attachment under mild reaction conditions, chemical modification such as grafting of polyphenols may be an effective approach to improve functional properties of chitosan [3,15,26].

Polyphenols are plant secondary metabolites, and they represent the largest class of natural antioxidants [21]. Besides, polyphenols have various positive bioactivities such as modulation of gut ecology, hence polyphenols have recently drew a lot of scholarly attention [6,37]. Different polyphenols may have distinct structures, and they can react dissimilarly with chitosan and form chitosan–polyphenol complexes with different physicochemical properties. Systematic investigation of different chitosan–polyphenol complexes may give new insight into the influence of polyphenol skeleton on the reactivity of chitosan–polyphenol covalent complexation. Since chlorogenic acid (CA), gallic acid (GA) and cinnamic acid (CAA) are commonly found in plant materials such as fruits, and they are polyphenols with biological properties such as antimicrobial and anticancer effects [11,19,31]. Thus, CA, GA and CAA were chosen as polyphenol models and investigated in this study.

During the past few years covalent complexes prepared by polyphenols have received a lot of scholarly attention, since they can combine advantageous properties of polyphenols and other biopolymers [8]. Previous studies reveal that grafting of ferulic acid onto chitosan can provide chitosan with improved thickener potential and antioxidant properties [3]. In addition, our previous study shows that chitosan–(−)-epigallocatechin-3-gallate covalent complexes prepared by hydroxyl-free radical grafting are water-soluble at neutral pH, which may be due to grafting of hydrophilic hydroxyl groups [18]. Chitosan–polyphenol covalent complexes may exhibit different physicochemical properties from chitosan and polyphenols alone, and they may positively affect human health [8]. However, chitosan–polyphenol covalent complexes are mainly prepared via enzymatic grafting and

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free radical grafting now [1,3,16,18]. These modification procedures first convert polyphenols into corresponding quinones, and the quinones then react with nucleophilic groups to yield either Schiff-bases or Michael-type adducts. These oxidative reactions lead to colored chitosan derivatives, and oxidation of polyphenols may be detrimental to their antioxidant properties due to loss of hydroxyl groups [2]. The colored covalent complexes with weakened antioxidant power may not be desirable in food products. Thus, other methods have been developed to produce chitosan–polyphenol covalent complexes, and carbodiimide-mediated coupling reaction has recently attracted a lot of attention [34]. In this study carbodiimide-mediated coupling method was utilized to synthesize chitosan–polyphenol covalent complexes. Since chitosan–polyphenol covalent complexes may exhibit different structural and functional properties from chitosan–polyphenol physical complexes, a systematic research on the comparison of covalent and physical complexes prepared by chitosan and polyphenols may gain a better understanding of how the covalent and physical complexations affect structural and functional properties of chitosan and promote applications of the complexes in food industry.

Accordingly, this research aimed to first covalently modify chitosan with polyphenols, and then characterize structural and functional properties of chitosan–polyphenol covalent and physical complexes.

2. Materials and methods

2.1. Materials

Low-molecular-weight chitosan (50–190 kDa, with deacetylation degree of 75–85%) was purchased from Sigma–Aldrich (USA). Chlorogenic acid (CA) (>98%, purity) and gallic acid (GA) (>98%, purity) were purchased from Beijing BSZH science company. Cinnamic acid (>95%, purity) was obtained from Beijing Chemical Works. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and *N*-hydroxysuccinimide (NHS) were purchased from Xiya Reagent Company (Chengdu, China). Folin–Ciocalteu reagent, 2,2′-azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 6-hydroxyl-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox) were obtained from Sigma–Aldrich (Sigma Chemical Co., St. Louis, MO). All other chemicals were of analytical grade, unless otherwise stated.

2.2. Preparation of chitosan–polyphenol complexes

2.2.1. Synthesis of chitosan–polyphenol covalent complexes

Chitosan (0.25 g) was slowly dispersed in 50 mL distilled water and stirred overnight at 20 °C to obtain uniform chitosan dispersion. Briefly, 1.5 mmol polyphenol (CA, GA or CAA) and 3 mmol EDC·HCl were dissolved in 50 mL ethanol at 4 °C, and 3 mmol NHS was further added to the solution. The mixture solution was stirred at 4 °C for 1 h, and the solution was then gradually added to chitosan dispersion under continuous stirring (150 rpm). The pH of the resultant solution was carefully adjusted to 6.5 using NaOH solution (0.1N). The solution obtained was stirred (150 rpm) at 4 °C for 1 h. Then the reaction was further carried out at 20 °C for 24 h. Thereafter, the samples were dialyzed for 72 h against distilled water at 4 °C to remove the free polyphenols (molecular weight cutoff 3500 Da) and then finally freeze-dried to acquire chitosan–polyphenol covalent complexes. Control chitosan was prepared according to the above procedure but without the addition of EDC·HCl, NHS and polyphenols. Because it was observed that control chitosan and native chitosan (chitosan without any treatment) were identical in terms of all structural and functional properties shown in our experiments, the data regarding native chitosan were omitted so as to make this paper more clear.

2.2.2. Preparation of chitosan–CA physical complex

The total phenolic content of chitosan–CA covalent complex was determined according to the Folin–Ciocalteu method [10] using CA as a standard. The total phenol content was expressed as mg CA equivalents/g dry complex. If 1 g of chitosan–CA covalent complex consisted of M_1 g of chitosan and M_2 g of CA, then M_2 g of CA dissolved in ethanol was gradually added to aqueous dispersion containing M_1 g of chitosan. The pH of the resultant solution was carefully adjusted to 6.5. The mixture solution was stirred (150 rpm) at 4 °C for 2 h and then at 20 °C for 24 h. The final product was lyophilized to acquire chitosan–CA physical complex without dialysis (SI.6).

2.3. Structural characterization of chitosan–CA complexes

2.3.1. Fourier transform infrared (FTIR) spectroscopy

The infrared spectra were recorded with the potassium bromide (KBr) pellet method in a Spectrum 100 FT-IR spectrophotometer (PerkinElmer, UK). The sample (2 mg) was dried and well blended with 198 mg of desiccated KBr. Potassium bromide was used as a reference in this measurement. The spectra of powder samples recorded between 1000 and 3000 cm^{-1} with accumulation of 16 scans and a resolution of 4 cm^{-1}.

2.3.2. UV–vis spectroscopy

In order to better separate UV–vis spectra of different samples and compare shifts of spectra, different concentrations of samples were applied. Chitosan, CA, chitosan–CA physical complex and covalent complex were dissolved in 0.5% (v/v) acetic acid with magnetic stirring to obtain solutions at proper concentrations. UV–vis absorption spectra were recorded using a Shimadzu UV-1800 spectrophotometer (Tokyo, Japan) in the wavelength range of 210–500 nm.

2.3.3. X-ray diffraction (XRD) measurement

Chitosan and chitosan–CA complexes were carefully milled to small fine particles. The powder samples were placed on quartz sample holders. XRD measurements were carried out on D8 Advance X-ray diffractometer (Bruker, Germany). XRD patterns were recorded over a 2θ range from 10° to 80° at room temperature using Cu Kα radiation [39].

2.3.4. Morphological analysis

Field-emission scanning electron microscopy (FESEM) was applied to observe the morphology of the surface of samples in powder form. Chitosan and chitosan–CA complexes were mounted on a metal stub and sputter-coated with gold. Morphological analysis was performed on S-4700 FESEM (Hitachi Ltd., Japan) at an accelerating voltage of 20 kV.

2.4. Functional properties of chitosan–CA complexes

2.4.1. ABTS radical scavenging assay

The activity of scavenging $ABTS^{\bullet+}$ was evaluated as described by Siddhuraju [27], and the antioxidant capacity was expressed as nmol Trolox equivalents (TE)/mg sample using Trolox as a standard.

2.4.2. DPPH radical scavenging assay

The activity of scavenging $DPPH^{\bullet}$ was performed according to previous studies [12], and the antioxidant capacity was expressed as nmol Trolox equivalents (TE)/mg sample using Trolox as a standard.