Study on the degradation of chitosan by pulsed electric fields treatment

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ABSTRACT

Chitosan solution was processed by applying pulsed electric fields (PEF) with different strengths up to 25 kV cm^{-1}. Changes of the physicochemical properties of chitosan, such as molecular weight and crystallinity degree, were measured by analyses of scanning electron microscopy (SEM), viscosity molecular weight (M_v), X-ray diffractometry (XRD), FTIR and UV spectra. The results showed that after being treated at 25 kV cm^{-1}, the chitosan granules were significantly deformed with many pits and cracks appeared on the surface. M_v was decreased with the increasing electric field strength, for example, the M_v was decreased from 2.81×10^5 Da (initial chitosan) to 1.54×10^5 Da after the PEF treatment at 25 kV cm^{-1}. Meanwhile, the crystalline region of the treated sample was significantly damaged from XRD patterns. All results showed that the PEF technique is a possible method to obtain low molecular-weight chitosan.

Industrial Relevance: Chitosan solution was treated by using pulsed electric fields (PEF) with different strengths up to $25 \text{ kV}\cdot\text{cm}^{-1}$. Changes of the physicochemical properties of chitosan were measured by analyses of scanning electron microscopy (SEM), viscosity molecular weight (M_v), X-ray diffractometry (XRD), FTIR and UV spectra. The results showed that the PEF technique is a promising method for scale-up industrial manufacture of low-molecular-weight chitosan.

1. Introduction

Chitosan, a linear copolymer consisting of β-1,4-linked 2-amino-2-deoxy-D-glucopyranose and 2-acetamido-2-deoxy-D-glucopyranose units, is a natural polymer generally obtained by extensive deacetylation of chitin isolated from crustacean shells. Owing to its biocompatibility, biodegradability, and biological activities, chitosan as a functional biopolymer has gotten wide acceptance in diverse fields, such as food, pharmaceutics, biomedicine, pharmacology, biotechnology, biomaterials, and cosmetics (Sugano, Fujikawa, Hiratsuji, Nakashima, & Hasagaea, 1980; Sashiwa, Saimoto, Shigemasa, Ogawa, & Tokura, 1990; Shahidi, Arachchi, & Jeon, 1999; Suh & Matthew, 2000; George & Abraham, 2006; Khor & Lim, 2003; Rinaudo, 2006; No, Meyers, Prinyawiwatkul, & Xu, 2007).

However, chitosan obtained from the deacetylation of chitin has a high molecular weight (over 10^5 Da) and low solubility in aqueous solvent, which limits its applications. With the progresses of deeply studying of chitosan, it has been found that comparing with higher molecular weight chitosans, chitosans with lower molecular weight have significantly different biological activities, such as antifungal activity, antibacterial activity, and antitumor activity (Tarsi, Corbin, Pruzzo, & Muzzarelli, 1998; Qin et al., 2004; Liu, Bao, Du, Zhou, & Kennedy, 2006; Qin et al., 2006; Chien, Sheu, & Lin, 2007). For instance, the inhibition rate against *Escherichia coli* (*E. coli*) of chitosan was increased from 81% to 99% with the decrease of molecular weight from 15.4×10^5 Da to 1.5×10^5 Da after ultrasonic wave treatment (Liu et al., 2006). Thus the development of a novel technology for reduction of molecular weight of high molecular weight chitosan to low molecular weight chitosan, without modification of its chemical structure, is of great interest (Yue, He, Yao, & Wei, 2009).

The methods for preparing low molecular weight chitosan can be chemical, enzymatic or physical technologies. The chemical treatment such as hydrogen peroxide degradation and acid hydrolysis is a very common and fast method for producing series of chitosan oligomers. However, it has the main disadvantage of causing environmental pollution (Liu et al., 2006). As for the enzymatic method, such as the application of chitosanase, proteases, and cellulases (Jeon, Park, & Kim, 2001; Qin et al., 2004; Li et al., 2005), it seems to be generally preferable to chemical reactions because the reaction is under more gentle conditions and the molecular weight distribution of the product is more controllable. However, the expensive cost of the specific enzymes, such as chitinase, limits its wide application on industrial scale. The third method of manufacturing low molecular weight chitosan or chito-oligomers is called physical methods, such as sonication, irradiation, microwave or other physical methods. Its outstanding advantages are environmentally friendly, high efficiency, as well as lower reaction temperature thus resulting in little side-effects. (Muzzarelli & Rocchetti, 1985; Chen, Chang, & Shyur, 1997; Lim, Khor, & Koo, 1998; Choi, Ahn, Lee, Byun, & Park, 2002; Czechowska-Biskup,

Rokita, Lotfy, Ulanski, & Janusz, 2005; Liu, Du, & Kennedy, 2007; Feng, Du, Li, Hu, & Kennedy, 2008).

The technology of pulsed electric fields, a non-thermal food processing method, has been widely used not only for non-thermal pasteurization, but also for enhancing chemical reactions as well as large molecules modifying (Jeyamkondan, Jayas, & Holley, 1999; Han, Zeng, Zhang, & Yu, 2009; Han, Zeng, Yu, & Chen, 2009). Numerous studies have demonstrated that the prominent advantages of PEF treatment are low temperature, continuous processing, short treatment time, uniform treatment as well as energy saving (Raso & Heinz, 2006). Concerning the exhibition of PEF on starch modification, as evidenced in our previous papers (Han, Zeng, Zhang et al., 2009; Han, Zeng, Yu et al., 2009), we inferred that PEF treatment should have positive effect on degradation of large molecular chitosan. The aim of this paper was to investigate the effect of PEF treatment on degradation of large molecular chitosan for the purpose of developing a novel green-processing method for low molecular chitosan.

2. Experimental part

2.1. Materials

Chitosan (degree of deacetylation = 95%) was purchased from Aoxing Biology Company (Zhejiang, China). Acetic acid, acetone, and sodium–hydroxide were obtained from Hui Shi Biochemical Regant Co., Ltd. (Shanghai, China). All reagents used in this experiment were of analytical grade.

2.2. PEF system

Chitosan solution was treated in a bench-scale, continuous PEF system (SCUT PEF Team, the South China University of Technology, China), showed in the paper (Han, Zeng, Zhang et al., 2009). Throughout the study, a square-wave pulse was generated with the pulse duration (τ) of 20 μs, pulse frequency (f) of 1000 Hz and pulse number of 12. The main advantage of the square-wave pulse is to deliver electrical energy at the maximum voltage during most of the pulse duration (Raso & Heinz, 2006). The treatment chamber is consisted of two parallel copper plate electrodes and a tubular insulator body made of Teflon. By placing a perforated Teflon parallel to the planar electrode in the volume between the electrodes, it is able to enhance the electric field considerably in the openings of the Teflon. The gap between electrodes is 3.00 mm and the diameter of the cylindrical treatment zone is 3.00 mm. The chitosan solution was pumped (Watson Marlow 323E/D Pump, USA) to the treatment chamber to receive the PEF treatment. The flow rate of the suspension was calculated and controlled by a rotameter (Model FM-01, Ningbo Jiutian Meter Company, China). A digital oscilloscope (Tektronix TDS220, Beaverton, OR) monitored the voltage and current of PEF treatments. The type K thermocouples were inserted into the inlet and outlet of the chamber to measure the sample temperature. After the PEF treatment, the PEF-treated samples were cooled down spontaneously at the room temperature. The untreated sample, pumped through the circuit without applying PEF treatment, was used as reference. Before and after every treatment, the PEF system was cleaned and disinfected with 75% (v/v) ethanol solution and rinsed with sterile distilled water.

2.3. PEF treatments

Chitosan (20.0 g) was dissolved in 10% (v/v) acetic acid solution (2000 mL) and the electric conductivity of the chitosan solution was 198.5 μS cm^{-1}. Afterwards, the chitosan solution was pumped through the treatment chamber to receive the PEF treatment at 15 kV cm^{-1}, 20 kV cm^{-1}, and 25 kV cm^{-1}, respectively. Throughout the experiments, the flow rate of chitosan suspension was set at

100 mL min^{-1}, and the sample temperature was controlled below 50 °C to avoid overheating. After PEF treatments, some solution was taken for UV test and the left was adjusted to pH 7–8 by the addition of 2 M NaOH. The product was then precipitated by the addition of acetone, and a white precipitate was obtained, filtered off, and dried at 50 °C overnight. Dry chitosan samples were obtained for following studies.

2.4. Scanning electron microscopy (SEM)

The morphology of chitosan samples were examined with an E-SEM XL30 microscope (FEI Company, Eidhoven, the Netherlands). Samples were placed on a specimen holder with the help of double-sided scotch tape and sputter-coated with gold (5 min, 2 mbar). Finally, each sample was transferred to a microscope where it was observed at an acceleration voltage of 20 kV and a vacuum of 9.75×10^{-5} torr.

2.5. Viscosity average molecular weight

Viscosity molecular weight of chitosan was measured in triplicates by dilute solution capillary viscometry at 30 ± 0.05 °C using a Cannon Ubbelohde four bulb shear dilution viscometer. Filtered chitosan solutions (0.05–0.2% in 0.2 M CH$_3$COOH/0.1 M CH$_3$COONa) were equilibrated to 30 °C. The intrinsic viscosity was determined by linear regression of the graph of reduced viscosity against concentration ($R^2 > 0.96$).

The viscosity average M_v was calculated from the intrinsic viscosity [η] using the Mark–Houwink equation as following:

$$[\eta] = kM_v^{\alpha} \tag{1}$$

The value of k and α is 6.589×10^{-3} and 0.88, respectively (Wang, Bo, Li, & Qin, 1991).

2.6. X-ray diffraction

The X-ray diffraction (XRD) pattern was obtained using a D/max 2200X-ray diffractometer (Tokyo, Japan), with a conventional copper target X-ray tube set at 30 kV and 30 mA. The X-ray source was Cu Ka radiation. Data were collected from 2θ of 5° to 40°(θ being the angle of diffraction) with a step width of 0.02°, step time of 0.3 s, scanning speed of 4°/min, divergence slit width of 0.20 mm, scatter slit width of 0.60 mm, and receiving slit width of 0.20 mm at room temperature. All samples were dried at 40 °C till constant moisture in a vacuum oven, then about 50.00 mg samples were added into the slide for packing prior to X-ray scanning.

2.7. FTIR

The FTIR spectra of the non-PEF and PEF-treated chitosan were recorded in powder form in KBr discs in the range of 4000–400 cm^{-1} with a Vector 33 FTIR spectrometer (Bruker, Germany).

2.8. UV

UV–vis absorption spectra were obtained using a UV–vis recording spectrophotometer (UV-2501PC, China) in the range of 200–800 nm.

3. Results and discussion

3.1. SEM

SEM micrographs of non-PEF-treated and various PEF-treated chitosan granules were shown in Fig. 1. It can be seen from Fig. 1A that the initial chitosan exhibited a nonporous, smooth membranous
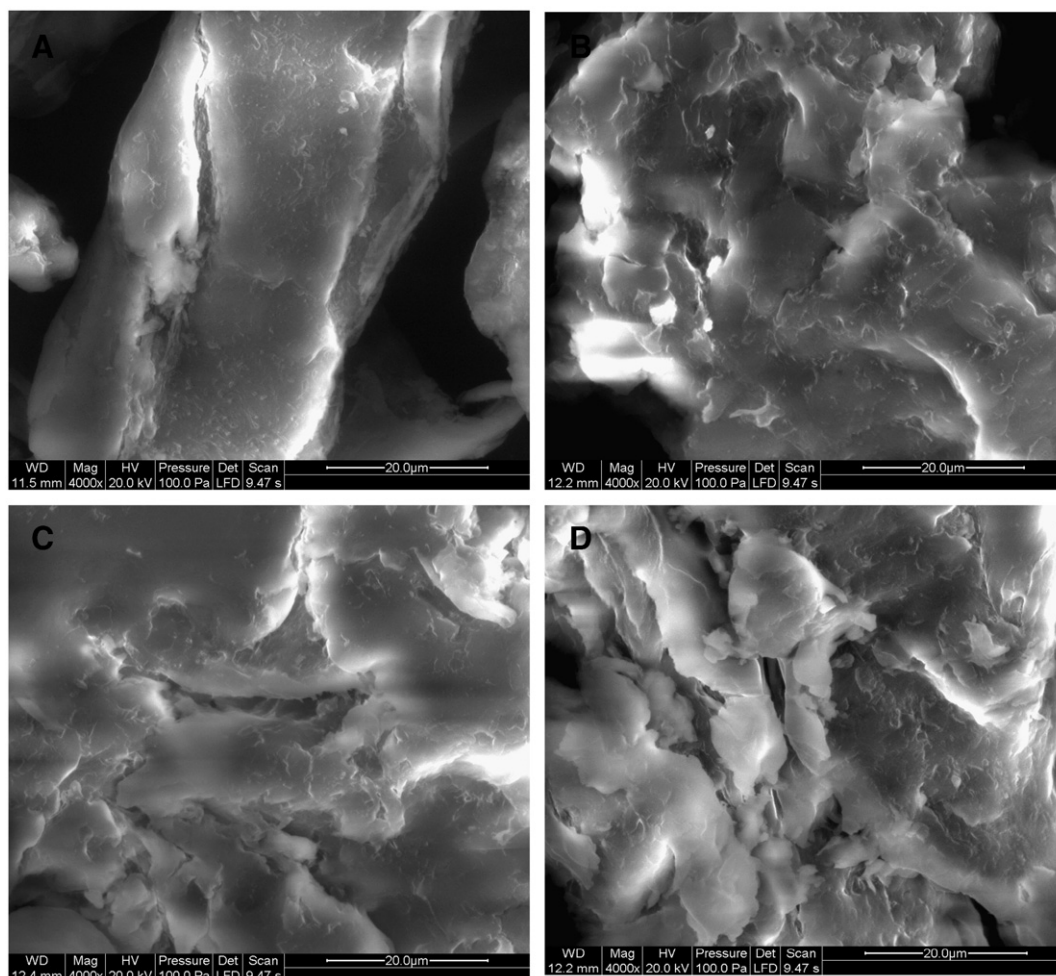
Fig. 1. SEM of chitosan samples treated at different electric field strengths: (A) 0; (B) 15 kV cm^{-1}; (C) 20 kV cm^{-1}; and (D) 25 kV cm^{-1}.

phases consisting of dome shaped orifices and microfibrils, which was similar with the morphology of chitosan showed elsewhere (Singh et al., 2009). After being subjected the PEF treatment at 15 kV cm^{-1}, some coarseness or pits had been generated on the surface of chitosan (Fig. 1B). Moreover, after being treated at more severe condition (20 kV cm^{-1}, Fig. 1C), some cracks were emerged, indicating that the granular structure had been changed by the PEF treatment. And on the most severe condition of PEF treatment at 25 kV cm^{-1}, it was demonstrated that the granule was significantly deformed (Fig. 1D).

3.2. Viscosity average molecular weight (M_v)

The viscosity average molecular weight (M_v) of the non-PEF treated and PEF-treated chitosan are listed in Table 1.

M_v of PEF-treated chitosan was decreased with the increasing electric field strength. Compared with the initial chitosan, after being treated at 15 kV cm^{-1}, 20 kV cm^{-1}, and 25 kV cm^{-1}, the decreased degree of M_v was 19.57%, 35.23%, and 45.19%, respectively. This may

be mainly due to the hydroxyl radicals accelerated by PEF, which can oxidize a broad range of organic compounds (Wang, Huang, & Wang, 2005). The hydroxyl radicals generated in the experimental systems might attack the β-D-(1→4) glycosidic bonds of chitosan and subsequently break the glycosidic linkages (Raso & Heinz, 2006). As a result, the viscosity average molecular weight of PEF-treated chitosan was decreased.

3.3. XRD patterns

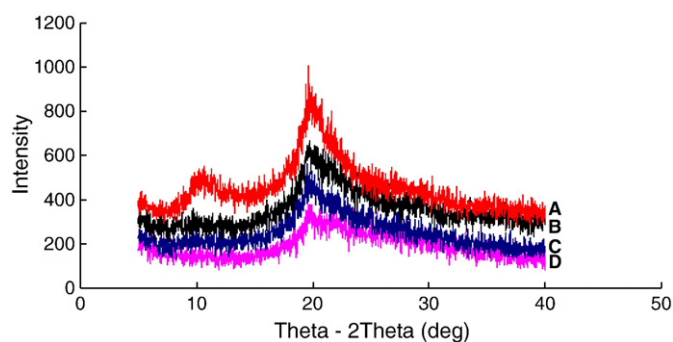X-ray diffraction (XRD) patterns of initial and various treated chitosan samples were shown in Fig. 2.

Table 1
Viscosity average molecular weight of chitosan after different strengths.

Treatment (kV cm^{-1})	M_v ($\times 10^5$ Da)
0	2.81 ± 0.04
15	2.26 ± 0.03
20	1.82 ± 0.01
25	1.54 ± 0.02

All values represent the means ± standard deviation; $n = 3$.

Fig. 2. GPC of chitosan treated at different electric field strengths: (A) 0; (B) 15 kV cm^{-1}; (C) 20 kV cm^{-1}; and (D) 25 kV cm^{-1}.

The XRD pattern of initial chitosan, with main peaks at about 10.4° and 20.2°(2θ), is coincided with the pattern of the 'L-2 polymorph' of chitosan reported previously (Saito & Tabeta, 1987). As for the PEF-treated samples, their XRD patterns exhibited differently. At relative lower intensity (15 kV cm^{-1}), the diffraction pattern of treated sample was changed and the height of the characteristic peaks at 10.4° and 20.2°(2θ) was decreased. When the electric field strength was further increased to 20 and 25 kV cm^{-1}, it was obviously shown that the crystalline region of the treated sample was significantly damage. As a result, the peak at 10.4°(2θ) was almost disappeared. Meanwhile, the peak at 20.2°(2θ) was nearly disappeared especially after being treated at 25 kV cm^{-1}.

The reason for this change was that, after the high intensity PEF treatment, the chitosan granules had been damaged as shown from the SEM micrographs, which meant that the crystalline region was destroyed. As a result, the intensity of the characteristic peaks was decreased.

3.4. FTIR spectra

The FTIR spectra of the degraded chitosan as well as initial chitosan are shown in Fig. 3.

From Fig. 3, it can be found that the characteristic absorption bands of initial chitosan are appeared at 1643 cm^{-1} (Amide I), 1599 cm^{-1} (–NH$_2$ bending) and 1325 cm^{-1} (Amide III). The absorption bands at 1154 cm^{-1} (asymmetric stretching of the C–O–C bridge), 1086 and 1032 cm^{-1} (skeletal vibration involving the C–O stretching) are characteristics of its saccharine structure.

Most of the characteristic adsorption peaks of chitosan were appeared in the FTIR spectra of PEF-treated sample. However, the following differences among them can be observed. The relative absorption intensity of –NH$_2$ band bending at 1599 cm^{-1} was enhanced with the increase of applied electric field strength, on the contrary, the relative absorption intensity of C–H band bending and C–H band stretching at 1382 cm^{-1} was weakened with the increase of electric field strength, which indicated that the intermolecular and intramolecular hydrogen bonds of chitosan were weakened and its crystallinity was reduced after PEF treatments.

3.5. UV spectroscopy

The UV absorption spectra of the initial chitosan and degraded chitosan samples are shown in Fig. 4. For all the samples, as can be seen, the peaks at 245 and 290 nm are appeared, which are ascribed to carbonyl and carboxyl groups. However, the relative absorption intensity of the peaks in these positions were weakened with the increase of electric field strength, which would imply that carbonyl
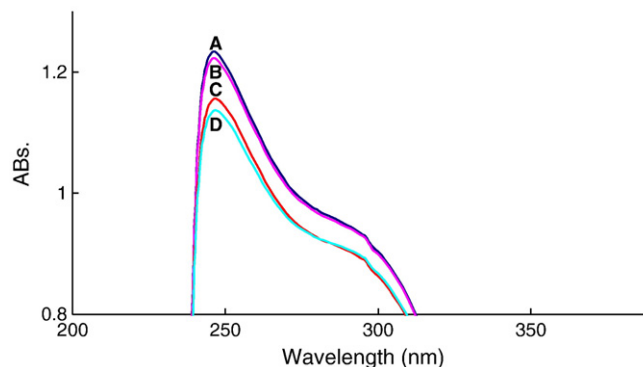

Fig. 4. FTIR spectrum of chitosan treated at different electric field strengths: (A) 0; (B) 15 kV cm^{-1}; (C) 20 kV cm^{-1}; and (D) 25 kV cm^{-1}.

and carboxyl groups in chitosan were partly destroyed by the PEF treatment.

4. Conclusions

In the present study, the effects of PEF treatment (up to 25 kV cm^{-1}) on the physicochemical properties of chitosan were investigated. The results from SEM images showed that the granule was significantly deformed with many pits and cracks. M_v was decreased with the increasing electric field strength. Meanwhile, it was observed that the crystalline region of the treated sample at 25 kV cm^{-1} was significantly damaged, which implies the degradation of high molecular weight chitosan. In conclusion, all results of this research reveal that PEF treatment has significant effect on chitosan molecular structure, which may result in changes of its physchemical and biochemical properties and deserve to be further researched.

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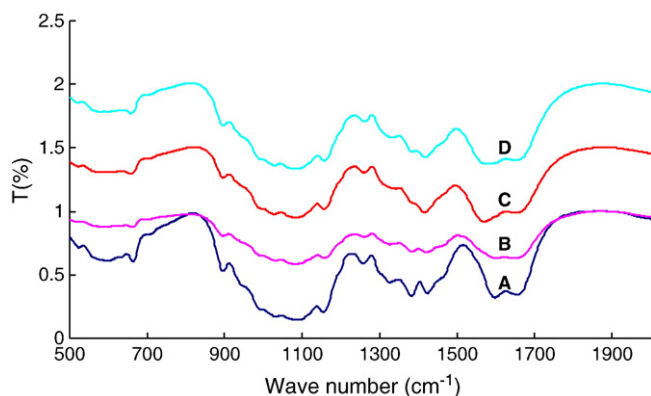
Fig. 3. XRD patterns of chitosan treated at different electric field strengths: (A) 0; (B) 15 kV cm^{-1}; (C) 20 kV cm^{-1}; and (D) 25 kV cm^{-1}.

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