Carbohydrate Polymers 177 (2017) 217–223

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

One-pot production of chitin with high purity from lobster shells using choline chloride–malonic acid deep eutectic solvent

Ping Zhu, Zhongji Gu, Shu Hong, Hailan Lian*

College of Materials Science and Technology, Nanjing Forestry University, Nanjing, 210037, China

ARTICLE INFO

Chemical compounds studied in this article:
Choline chloride (PubChem CID: 6209)
Thiourea (PubChem CID: 2723790)
Urea (PubChem CID: 1176)
Glycerol (PubChem CID: 753)
Malonic acid (PubChem CID: 867)
Hydrochloric acid (PubChem CID: 313)
Sodium hydroxide (PubChem CID: 14798)
Hydrogen peroxide (PubChem CID: 784)
Calcium carbonate (PubChem CID: 10112)

Keywords:
Chitin
Deep eutectic solvents
Lobster shells
Physicochemical properties

ABSTRACT

For the first time in this study, chitin was solely extracted from lobster shells through a fast, easy and eco-friendly method using deep eutectic solvents (DESs), consisting of mixtures of choline chloride–thiourea (CCT), choline chloride–urea (CCU), choline chloride–glycerol (CCG) and choline chloride–malonic acid (CCMA). The physiochemical properties of the isolated chitins were compared with those of the chemically prepared one and commercial one from shrimp shells. Results showed that CCT, CCU and CCG DESs had no important effect on the elimination of proteins and minerals, while chitin obtained by CCMA DES showed a high purity. The yield (20.63 ± 3.30%) of chitin isolated by CCMA DES was higher than that (16.53 ± 2.35%) of the chemically prepared chitin. The chitin obtained by CCMA DES could be divided into two parts with different crystallinity (67.2% and 80.6%), which also had different thermal stability. Chitin from lobster shells showed porous structure, which is expected to be used for adsorption materials and tissue engineering.

1. Introduction

Chitin is the second largest natural polysaccharide biopolymer after cellulose, with the annual output of ca. 10^{10}–10^{12} Tons (Roberts, 1992). It is an insoluble linear polymer consisting of β-(1, 4) linked N-acetyl-D-glucosamine units and widely exists in crustacean shells, insect exoskeleton and the cell walls of many fungi and some algae (Lehane, 1997; Martin, 1998; Muzzarelli, 1999; Simpson et al., 1994). Chitin and its derivatives, such as chitosan (obtained by the deacetylation of chitin), display outstanding biocompatibility, biodegradability, null toxicity, thermal and chemical stability. Due to these properties, these polymers have many application areas including food, medicine, agriculture, textiles and other related fields (Hirano, 1996).

The lobster processing industry produces a large number of by-products such as lobster shells, which can account for 50–70% of raw materials (Roberts, 1992). These by-products are currently used to produce low-value aquatic feed and bio-fertilizers, or directly discarded resulting in serious environmental concerns and disposal problems that require additional costs. Therefore, it is crucial to turn these wastes into useful products for the sustainability of the lobster industry. As an important chitin source, lobster shells have been considered to be the potential material for commercial production of chitin (Nguyen, Zhang, Barber, Su & He, 2016). Lobster shells contain not only chitin, but also high content of minerals, proteins and a small amount of pigments. The production of chitin is essentially the removal of other substances.

According to the current research, the extraction of chitin is classified into two different procedures, the chemical isolation and the biological isolation of chitin. But both of these procedures involve the same steps: demineralization and deproteinization (Shimahara & Takiguchi, 1988). Traditionally, the chemical method employs strong acid and alkali to remove minerals and proteins, respectively. However, this method will produce a large number of corrosive acid-base wastewater and may bring about serious environmental problems. To overcome the defect, many studies have been performed in relation to the utilization of biological methods including enzymatic reactions and microbial fermentation (Sawssen, Olfa, Islem, Kemel & Moncef, 2015; Younes, Ghorbel-Bellaaj, Nasri, Chaabouni, Rinaudo & Nasri, 2012; Younes, Hajji, Frachet, Rinaudo, Jellouli & Nasri, 2014). With these methods, the above problem of environmental pollution is avoided. Moreover, the molecular weight and crystallinity of the obtained chitin are higher than those of the chemically prepared chitin (Pacheco et al., 2011). However, it is insufficient

* Corresponding author.
E-mail address: lianhailan@njfu.edu.cn (H. Lian).

http://dx.doi.org/10.1016/j.carbpol.2017.09.001
Received 17 June 2017; Received in revised form 31 August 2017; Accepted 1 September 2017

to remove minerals and proteins from raw materials in these biological processes (Setoguchi, Kato, Yamamoto & Kadokawa, 2012). Besides, longer fermentation cycles and expensive enzymes prevent the promotion of these methods.

Recently, ionic liquids (ILs), as a more sustainable alternative to volatile organic solvents, are used for the direct extraction of chitin from raw materials (Barber, Griggs, Bonner & Rogers, 2013 ;Setoguchi et al., 2012; Qin, Lu, Sun & Rogers, 2010). However, certain ILs show some drawbacks, such as high cost and toxicity, which are not suitable for biological applications (Sharma, Mukesh, Mondal & Prasad, 2013). Therefore, a more economic, efficient and eco-friendly technique would be desirable for the production of chitin.

Deep eutectic solvents (DESs), as a greener alternative to conventional ILs, are fluids obtained by the adequate mixture of the hydrogen bond acceptor (HBA) and donor (HBD) which are capable of self-association through H-bonding interactions to form eutectics with lower melting points in comparison to each individual component (Abbott, Boothby, Capper, Davies & Rasheed, 2004; Abbott, Capper, Davies, Munro, Rasheed & Tambyrajah, 2001). Compared with traditional ILs, DESs possess more advantages such as null toxicity, low cost, ease of syntheses, biodegradability, negligible volatility, etc. For these properties, DESs have various applications including organic synthesis, dissolution media, extraction processes and materials chemistry (Zhang, De, Royer & Jérôme, 2012). It has been reported that DESs could be used as extraction and dissolution media of some biopolymers including cellulose, lignin and starch (Francisco, Bruinhorst & Kroon, 2012). Furthermore, the dissolution of chitin has also been reported (Mukesh, Mondal, Sharma & Prasad, 2014; Sharma et al., 2013). To the best of our knowledge, no attempts have been made so far to isolate chitin solely from crustacean shells using DESs. In this present study, DESs consisting of mixtures of choline chloride–thiourea (CCT 1:1), choline chloride–urea (CCU 1:2), choline chloride–glycerol (CCG 1:2) and choline chloride–malonic acid (CCMA 1:2) were used to extract chitin from lobster shells. The physicochemical characteristics of the obtained materials were studied by comparing with the chemically prepared chitin and commercial chitin from shrimp shells.

2. Materials and methods

2.1. Materials

Lobster shells were collected from Nanjing, Jiangsu, China. The cooked lobster shells were washed with tap water, dried in the DHG-9240A heating and drying oven at 100 °C and then grinded in a pulverizer to obtain powder (particle size < 0.15 mm). A commercial sample of α-chitin from shrimp shells by the chemical isolation was purchased from Aladdin Industrial Corporation. Choline chloride, thiourea, urea, glycerol and malonic acid were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). HCl, NaOH, H_2O_2 and $CaCO_3$ were purchased from Nanjing Chemical Reagent Co., Ltd. All chemicals were of analytical grade and were used without further purification.

2.2. Synthesis of DESs

Both the hydrogen bond acceptor (HBA) and donor (HBD) molecules were mixed and heated in a three-neck flask under the magnetic stirring until a homogeneous, colorless solution was formed. The DESs were shown in Table 1. The properties of DESs, such as freezing point, viscosity and conductivity, have a great impact on the application of DESs (Abbott, Boothby, Capper, Davies & Rasheed, 2004). In this study, hydrogen bond donor (HBD) types and molar ratio were determined by the freezing point and viscosity. The low freezing point and viscosity favor the separation of chitin from DESs during the chitin extraction process. Reaction temperature was determined by the short synthesis time of DESs. Afterwards, the solution was cooled to room temperature and stored in a desiccator to reduce moisture absorption.

2.3. Chitin extraction

A typical process for the extraction of chitin from lobster shells was carried out as follows. The milled lobster shells (10.00 g) and DESs were mixed and stirred at different temperature for a certain time as shown in Table 2. After the mixture had been subjected to centrifuge into supernatant and precipitate, the supernatant and precipitate were respectively centrifuged with distilled water at 4000 rpm for several times (5 min each time) until neutral pH was achieved shown in Fig. 1. Two resulted solid samples were obtained and then decolorized by 10% (w/v) H_2O_2 at 80 °C. Finally, the purified samples (sample S and sample P) were dried in an oven at 100 °C to constant weight. The yield of chitin was determined according to the weight difference of the raw material and extracted chitin. DESs exhibit similar physicochemical properties to ILs. It was reported that chitin in raw materials could be dissolved in ILs and obtained from the supernatant during centrifugation (Setoguchi et al., 2012; Qin et al., 2010). Reference to the extraction of chitin by ILs, the proportion of the lobster shells and DESs, temperature and time of the chitin extraction process were determined by the high yield of chitin from the supernatant (sample S).

In comparison with the samples obtained by DESs, the chemical procedure for the isolation of chitin from lobster shells was conducted as follows (Al Sagheer, Alsughayer, Muslim & Elsabee, 2009 ;Bharty, 2015; Marei, El-Samie, Salah, Saad & Elwahy, 2016). Demineralization was performed by treating the powders (10.00 g) with 6% (w/v) HCl solution at room temperature for 2.5 h with a solution to solid ratio 25 mL/g. The resulted solid fraction was centrifuged with distilled water to neutrality. Deproteinization was carried out using alkaline treatment with 10% (w/v) NaOH solution at 90 °C for 3 h. The resulted chitin was then centrifuged with distilled water until neutral pH was achieved. Finally, it was decolorized by 10% (w/v) H_2O_2 at 80 °C. The purified chitin (sample AA) was dried in an oven at 100 °C.

2.4. Chemical composition analysis of the isolated chitins

The ash content was determined gravimetrically by incinerating the sample (1–2 g) till a constant weight in a muffle furnace at 600 °C (Mohanasrinivasan et al., 2014). The moisture content was determined by drying the sample in a vacuum oven at 105 °C until the weight was constant (Marei et al., 2016). The protein content remaining in the sample was analyzed with the absorbance spectrum, according to a method reported by Nicholas J. Kruger. (Kruger, 1994). Prior to the analysis, the solid sample was treated with 5% (w/v) NaOH in order to collect the supernatant (Setoguchi et al., 2012). The appearance was determined by the senses. Chinese standard (GB/T 29941-2013) was used for reference and evaluation.

2.5. Fourier tran sform infrared spectroscopy (FTIR)

The FTIR spectra of the samples (in the forms of KBr disk and film) were recorded with a Nicolet 360 spectrometer over the frequency range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}.

2.6. X-ray powder diffractometry (XRD)

X-ray diffraction data were collected with CuKα radiation (λ = 1.5406) at 40 kV and 30 mA using a Rigaku SmartLab9 diffractometer. The 2θ angle was scanned between 5° and 55°. The crystalline index (CrI; %) was calculated according to Eq. (1).

$$CrI_{110} = \left[\frac{I_{110} - I_{am}}{I_{110}}\right] \times 100 \quad (1)$$

Where I_{110} is the maximum intensity at $2\theta \cong 20^{\circ}$ and I_{am} is the