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One-pot chitin pulping using recyclable superbase-based protic ionic liquid

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ABSTRACT

The application of ionic liquids and deep eutectic solvents offers a promising opportunity for a more environmentally friendly and straightforward chitin purification process from crustacean shells. Nonetheless, the insufficient recyclability of these ionic solvents poses a challenge to the long-term sustainability of such extraction methods. Thus, there is a strong imperative to focus on employing easily recyclable ionic liquids for chitin isolation, enhancing the overall sustainability of the process.

In this investigation, a direct chitin purification procedure that utilized pulping liquors consisting of the superbase-based protic ionic liquid 1,5-diazabicyclo[4.3.0]non-5-enium acetate and its precursor, acetic acid, was developed. It was demonstrated that these pulping liquors were capable of simultaneously deproteinate and demineralize shrimp shells to generate chitins with higher purity, degree of *N*-acetylation and crystallinity than commercially obtained chitin. More significantly, the pulping liquors can be recycled to their pure form in high quantity by simple distillation under reduced pressure, allowing the reuse of these mixtures, which give chitin of nearly identical purity.

1. Introduction

As a result of the detrimental impacts of climate change, the excessive reliance on fossil resources for fuel and material production has emerged as a pressing global issue. Biomass stands out as the essential sustainable alternative for carbon-based materials, making its conversion into fuel and materials a pivotal aspect of contemporary technological advancements. Chitin is one of the most abundant biomolecules in nature. It is mainly found in the exoskeletons of crustaceans and insects and the internal shell of mollusks. Chitin and its derivatives, chitosan and glucosamine are already being used in the food industry, and their uses in several packaging, medicinal and biotechnological applications have been demonstrated (Rinaudo, 2006). Overall, the chitin and chitosan markets are expanding quickly and are expected to achieve revenues of USD 3 billion (Shamshina et al., 2019) and USD 5 billion (Negi et al., 2021), respectively, by 2027.

The conventional method for chitin extraction consists of two main steps: 1) demineralization and 2) deproteination, which removes the other two main constituents of crustacean and mollusk shells: calcium carbonate and protein. These steps can also be conducted in reverse order (No & Meyers, 1995). Demineralization is generally performed by mixing the shell waste with dilute hydrochloric acid, which neutralizes and converts calcium carbonate into water-soluble calcium chloride (No & Meyers, 1995). Deproteination typically involves treating the waste with a dilute alkaline solution (e.g. aqueous sodium hydroxide solution) at elevated temperatures (65–100 °C), which dissolves the protein present (No & Meyers, 1995). This established method is versatile and can be used for different crustacean and mollusk shell waste. However, it requires a multi-step process in which non-renewable chemicals are used to generate unwanted demineralization and deproteination waste, leading to issues in water treatment. Moreover, using strongly acidic and basic solutions at high temperatures can damage the structural integrity of chitin (Berezina, 2016). Therefore, a milder and more straightforward process to isolate chitin from crustacean and mollusk shell waste is desirable. In recent years, the use of novel solvents such as ionic liquids (ILs) and deep eutectic solvents (DESs) for sustainable purification of chitin has become an active area of research and development.

ILs are pure ionic compounds with low melting points (e.g. <100 °C). One of their many potential applications is biomass processing due to their ability to dissolve several biopolymers, which are generally insoluble in other solvents. Direct extraction of chitin from crustacean waste by using imidazolium-based ILs was widely reported (Shamshina,

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2019). However, only a few ILs have shown real potential, as chitin has low solubility in most ILs, and the extraction or dissolution process typically requires a long heating time (over 12 h) (Shamshina, 2019).

In contrast, the dissolution of proteins in ILs is more accessible with respect to the heating temperature and time. For example, it was demonstrated that feather keratin, a fibrous protein, could be dissolved in the IL *N*,*N*-dimethylethanolammonium formate with relatively short heating time (100 °C, 7 h) (Idris et al., 2014). The solubility of keratin in this IL was high (25 % by weight). The same IL was also found to dissolve zein, a plant protein (Choi & Kwon, 2011). An alternative approach to purifying chitin that focused on the dissolution of protein and neutralization of calcium carbonate using an IL-type pulping liquor was reported by Shamshina et al. (2016). In their study, the utilized pulping solvent [NH_3OH][OAc] was generated by mixing hydroxylamine (NH_2OH) and acetic acid (AcOH) in a 1:1 ratio, in which the starting materials are weak base ($pK_b = 8.1$; $pK_aH = 6.0$) (Kirby et al., 2006) and weak acid ($pK_a = 4.8$) respectively. This weak acid and base combination would generate a substance that can be described as a pseudo-protic IL (Wylie et al., 2023; Yoshizawa et al., 2003). The authors envisaged that the basic functionality of [NH_3OH][OAc] could remove the protein, and the acidic one could remove the calcium carbonate of shrimp shells simultaneously (Shamshina et al., 2016). The major obstacle preventing IL-based chitin isolation processes from commercialization is that these solvents are expensive. Also, recycling these ILs is not straightforward, especially in separating the dissolved protein and calcium contents from the ILs.

Using a similar approach, DESs consisting of choline or betaine have also been investigated for chitin pulping recently (Khajavian et al., 2022). These DESs are often comprised of carboxylic acids which act as both hydrogen bond donors and Brønsted acids that neutralize the calcium carbonate of the crustacean shells. These DESs were able to isolate chitin in high purity. For example, Zhu et al. (2017) utilized DESs consisting of choline chloride–malonic acid mixtures for the isolation of chitin from lobster shells. However, like in the case of ILs, the recycling of the solvents is not straightforward. Wang et al. (2022) demonstrated the possibility of separating the dissolved substances, i.e., protein and calcium compounds, from DESs by precipitation using an anti-solvent (ethanol). Yet, this type of separation could not fully isolate the dissolved substances.

We propose an alternative approach to isolate chitin from crustacean shells, which possesses the advantages of previously reported ionic solvent-based pulping processes but with enhanced recyclability. In the proposed method, superbase-based distillable ionic liquid (DIL) 1,5-diazabicyclo[4.3.0]non-5-enium acetate ([DBNH][OAc]) (Fig. 1), was used to dissolve the protein content of the shrimp shells selectively. Such DIL can be conveniently synthesized by the neutralization of selected organic base (i.e., DBN) and simple carboxylic acids (i.e., AcOH) (Fig. 2) and has demonstrated high recyclability to either pure ionic liquid or their starting compounds (Ostonen et al., 2016; Parviainen et al., 2015).

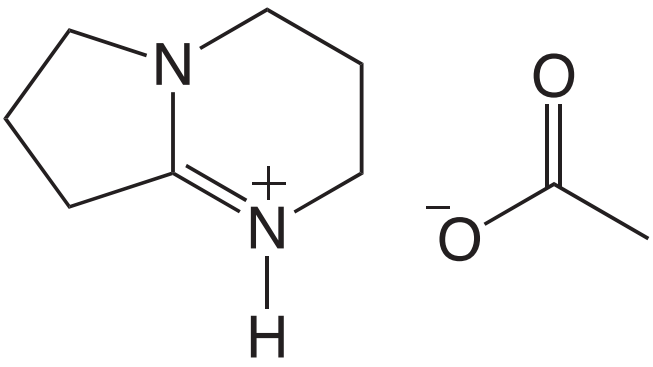

Fig. 1. Distillable ionic liquid 1,5-diazabicyclo[4.3.0]non-5-enium acetate ([DBNH][OAc]).

Moreover, from the sustainability point of view, this IL was demonstrated to have relatively low toxicity and was safe for large-scale usage (Ruokonen et al., 2016).

Since DBN is a superbase ($pK_b = 0.90$), the 1:1 mixture of DBN and AcOH is expected to produce negligible free acids. Thus, in our proposed method, AcOH is added in excess to neutralize the calcium carbonate in the shrimp shells in the same pot (Fig. 2). The product of the neutralization reaction, i.e., calcium acetate, is highly soluble in water. Hence, any residual calcium acetate can be eliminated by washing the chitin samples with water after they are filtered from the [DBNH][OAc]-AcOH mixtures. Utilizing AcOH, as opposed to alternative acids, for the neutralization of calcium carbonate enhances the efficiency of the pulping liquor recycling process. This is primarily attributed to AcOH's integration into the ionic liquid structure. Additionally, its relatively low boiling point allows for the recycling of excess AcOH through distillation.

Using [DBNH][OAc]-AcOH mixtures as pulping liquors would result in chitins with high quality. Moreover, the liquors can be effectively purified by distillation, allowing the recycling of the DIL and the process.

2. Materials and methods

Two types of shrimp shells (feedstocks A and B) were provided as food waste from a local seafood restaurant (Tsui Wan Seafood Restaurant) in Hong Kong, China. Feedstock A was exoskeletons of shrimp cooked in boiling water. Feedstock B was exoskeletons from another batch of uncooked shrimp. The feedstocks were washed with tap water, dried in an oven at 60 °C until the absence of weight loss and then ground in a household pulverizer to obtain a powder of different sizes through sieves. The shrimp shell powder used in this investigation has dimensions smaller than 125 μm. A commercial sample of α-chitin from shrimp shells by the chemical isolation was purchased from Sigma-Aldrich Co. (product of USA). 1,5-Diazabicyclo[4.3.0]-5-nonene (DBN) was purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). HCl was purchased from AnalaR NORMAPUR (made in France). Glacial acetic acid (AcOH) was purchased from RCI Labscan Ltd. Sodium hydroxide was purchased from Sigma-Aldrich Co. (a product of Sweden). All chemicals were of analytical grade and were used without further purification.

2.1. Preparation of pulping liquors ([DBNH][OAc]-AcOH mixtures)

The pulping liquors were prepared by mixing different ratios of DBN and AcOH using a modified procedure outlined in Druel et al. (2018). Specifically, 3.72 mL (65 mmol) of acetic acid was slowly added to 6.18 mL (50 mmol) of 1,5-diazabicyclo(4.3.0)-5-nonene (DBN) in a round-bottom flask under a nitrogen atmosphere, within an ice bath with vigorous stirring. Stirring was maintained for at least 30 min after adding all the AcOH to ensure complete reaction. The resulting pulping liquors had a molar ratio of 1.3 of AcOH to DBN, and they appeared as transparent fluid at room temperature. These liquors were stored under a nitrogen atmosphere. Pulping liquors with various AcOH to DBN ratios can be prepared using the same method.

2.2. Pulping of chitin

The shrimp shell powder and [DBNH][OAc]-AcOH mixtures were combined in specific mass ratios (5 wt% or 10 wt%) and stirred in an oil bath with magnetic stirring for 2–12 h at 100–120 °C (as detailed in Table 1) under a nitrogen atmosphere. After the mixing process, the mixture was allowed to cool, and then the pulping liquors were separated using a centrifuge (operating at 60,000 rpm for 10 min) to obtain undissolved material. This material was subsequently washed with distilled water using a centrifuge (at 30,000 rpm for 5 min) five times to eliminate any residual liquor. Finally, the samples were washed and dried at 60 °C in an oven overnight, resulting in the production of the

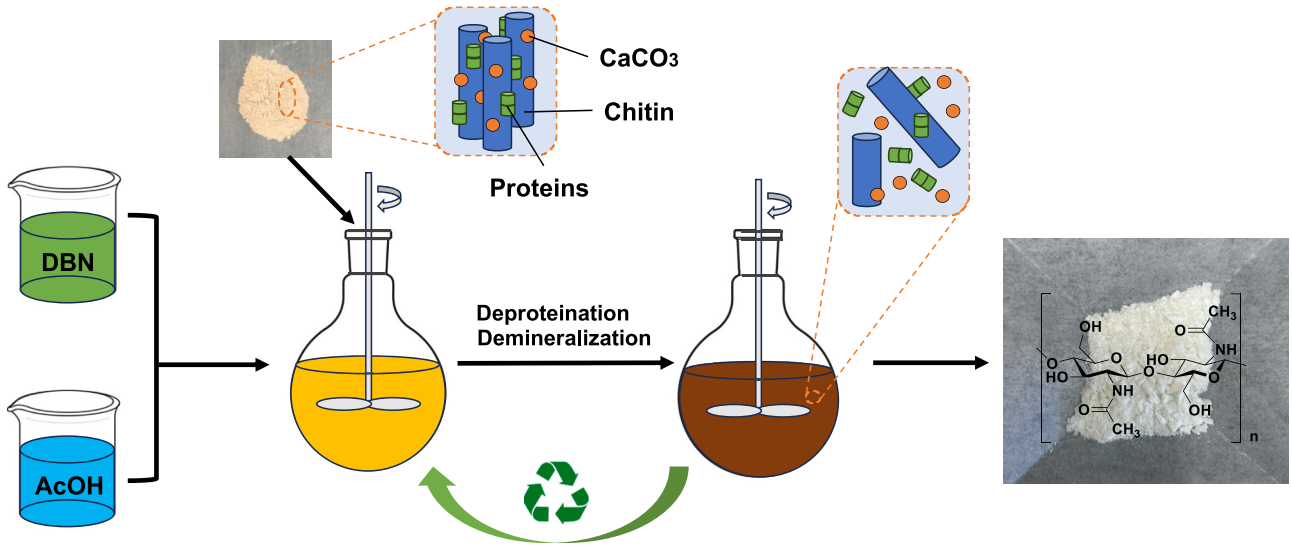

Fig. 2. Proposed method of chitin purification of this study.

Table 1
Chitin yield and purity of samples obtained by pulping using [DBNH][OAc]-AcOH mixtures. PC refers to practical grade chitin. A: cooked shrimp shells with the size of <125 μm as raw materials; B: raw shrimp shells with the size of <125 μm as raw materials.

Sample	Ratio of AcOH: DBN	Feedstock	Load (%)	T (°C)	Time (h)	Yield (%)	Chitin Content (%)
0	1.00	A	10	100	2	35	55
1	1.60	A	10	100	2	26	66
2	1.40	A	10	100	2	26	77
3	1.30	A	10	100	2	27	70
4	1.60	B	10	100	2	35	83
5	1.60	A	10	100	3	29	66
6	1.40	A	10	100	3	24	78
7	1.40	B	10	100	3	35	85
8	1.40	A	10	100	6	21	78
9	1.40	B	10	100	6	35	85
10	1.40	A	5	100	2	22	78
11	1.40	A	5	110	2	22	86
12	1.40	B	5	110	2	38	86
13	1.40	A	5	120	2	21	84
14	1.40	B	5	120	2	34	85
15	1.40	A	5	120	6	22	85
16	1.40	B	5	120	6	32	90
17	1.40	A	5	120	12	23	85
18	1.40	B	5	120	12	30	91
PC							86

desired product (Fig. 2).

2.3. Determination of chitin yield

The yield of the chitin sample was calculated using Eq. (1):

$$\text{Yield}\% = \frac{n_1 \times 100}{n_0} \quad (1)$$

n_1 is the dried weight of the chitin sample, and n_0 is the dried weight of the dried shrimp shell powder.

2.4. Determination of chitin content

The chitin contents were determined using the modified version of the reported method (Black & Schwartz, 1950; King et al., 2017), which involves sequential decalcification and deproteination steps. For decalcification, 6 mL of 1 M HCl was added to 0.25 g of sample in a 20 mL vial (loosely capped) along with a stir bar, and the suspension was stirred in a 90 °C oil bath for 1 h. The solid was centrifuged, and the supernatant was discarded. The precipitate was repeatedly washed with additional DI water (~20 mL), centrifuged, and the supernatant removed until it reached a pH of 7.

For deproteination, 0.25 g of the samples was treated with 6 mL of NaOH solution (1.25 M) at 90 °C for 1 h. After filtering the solid and washing it with DI water to neutralize the pH, the samples were dried overnight at 60 °C and weighed to determine the chitin content.

2.5. Thermogravimetric analysis (TGA)

Thermogravimetric (TG) and derivative thermogravimetric (DTG) analyses of feedstocks and isolated chitin samples were carried out using a Perkin Elmer Pyris 6 TGA Thermogravimetric Analyzer at a temperature ramping of 10 °C/min from 50 to 800 °C under nitrogen atmosphere.

2.6. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the samples (in the forms of KBr disk and film) were recorded with Spectrum 100 Optica FT-IR spectrometer over the frequency range of 4000–400 cm^{-1} at a resolution of 8 cm^{-1}.

The degree of *N*-acetylation (DA) was estimated using Eq. (2), as reported by Domszy and Roberts (1985):

$$\text{DA}\% = \frac{\frac{A_n 1655}{A_n 3450}}{1.33} \times 100 \quad (2)$$

A1655 and A3450 are the value of absorbance at the 1655 cm^{-1} and 3450 cm^{-1} of the FTIR spectra using the baseline method, respectively.

2.7. X-ray diffraction (XRD)

Powder X-ray Diffraction of samples was conducted by using X-ray Diffractometer (Rigaku SmartLab 9 kW — Advance) with Cu Kα radiation. The scan speed was 10° min^{-1}. The index of crystallinity (CrI) was estimated via Eq. (3) according to Focher et al. (1990):

$$\text{CrI} = \frac{I_{110} - I_{am}}{I_{110}} \times 100\% \quad (3)$$

where I_{100} represents the height of peak corresponding to (110) facet and I_{am} is the height correlating to amorphous diffraction at $2\theta = 16°$.

Subtraction of background was carried out before calculation (Focher et al., 1990).

2.8. Average molecular weight measurement

The average molecular weights of chitin samples were estimated by viscosity measurements. The viscosity measurements were performed by using Ubbelohde capillary viscometer (Φ = 0.4–0.5 mm) at 25 °C. The sample solution of concentration of 0.05 g dL^{-1} in 5 % LiCl/DMAc were prepared. The intrinsic viscosity [η] can be calculated by testing five concentration gradients for each sample solution by directly diluting the initial sample solution in viscometer. The viscosity-average molecular weight (Mw) was calculated based on Mark–Houwink–Sakurada equation (Eq. (4)):

$$[\eta] = KMw^{\alpha} \quad (4)$$

where α = 0.95 and K = 7.6 × 10^{-5} dL g^{-1} (Martin & Gérard, 2002).

2.9. Recycling of pulping liquor

The used pulping liquor (i.e., [DBNH][OAc]-AcOH mixture) isolated from chitin in Section 2.2 was distilled under reduced pressure (1 kPa) at the heating temperature of 150 °C to separate it from the dissolved materials. The distillate was weighed and analyzed by ^{1}H NMR (see Section 2.10) to verify its purity and AcOH:DBN ratio. After the introduction of additional AcOH to the distillate to refill the acid that was neutralized by the calcium carbonate, this pulping liquor was used to purify chitin again under identical pulping conditions.

2.10. Proton nuclear magnetic resonance (NMR) spectroscopy

The fresh and recycled pulping liquor [DBNH][OAc]-AcOH mixture was dissolved in dimethyl sulfoxide-d_6. The solution was then analyzed using a Bruker Ultrashield™ 400 MHz NMR spectrometer. ^{1}H NMR spectra were acquired under a 30° flip angle, a scan number of 16, and a recycle delay (D1) of 10 s.

3. Results and discussion

3.1. Chitin pulping and contents

The pulping liquors for this investigation were synthesized by mixing DBN and AcOH in various ratios. Since DBN is a superbase that would substantially deprotonate AcOH, excess AcOH was introduced to ensure sufficient free acid is present to neutralize and remove the calcium carbonate. These mixtures were envisioned to be solutions of AcOH in DIL [DBNH][OAc]; for instance, an initial mixing ratio of 1.60 between AcOH and DBN would give a solution of [DBNH][OAc]-AcOH with 1:0.60 molar ratio.

The chitin pulping process was optimized to give high chitin content (purity) product by adjusting the AcOH/DBN ratio, processing time, heating temperature, and concentration of feedstocks A and B (Table 1). It was demonstrated that an over-excess of AcOH could weaken the performance of deproteination. For pulping of feedstock A, the chitin content of sample 1 (66 %), which was isolated using AcOH/DBN ratio of 1.60, was considerably lower than that isolated using the ratio of 1.40, i.e., sample 2 (77 %) (Table 1). Furthermore, the liquor mixture with AcOH/DBN ratio of 1.40 was also able to isolate chitin of higher purity from shrimp shell B (85 % as opposed to 83 % using 1.60 acid/base ratio). These observations were likely because an over-excess of AcOH would dilute the "IL portion" (i.e., [DBNH][OAc]) of the pulping liquor. On the other hand, pulping liquors consisting of AcOH/DBN ratios of 1.00 (55 %, sample 0) and 1.30 (70 %, sample 3) also gave lower chitin content than that of 1.40 under identical conditions (77 %, sample 2) due to inadequate decalcification performance. Thus, a DBN/AcOH ratio of around 1:1.4 provided a fine, optimized balance for efficiently performing demineralization and deproteination.

In most cases, the increase in heating time did not show apparent effects on the chitin content. However, an increase in heating temperature (to 110–120 °C) provided products of higher chitin contents, with 84–91 % obtained for samples 11–18. To put these figures into context, the chitin content in practical grade chitin (PC) obtained from a major chemical vendor (Sigma Aldrich) was reported to be 79 % by Shamshina et al. (2016). The current study estimated the chitin content of PC to be 86 %, using the Black and Schwartz (1950) method. Thus, it was demonstrated that the purity of the chitin samples 11–18 is similar to or better than the industry standard PC.

The chitins' yield and purity were found to be largely feedstock-dependent: across parallel conditions (samples 1, 4, 6–18), shrimp shell B (30–38 % yield, 83–91 % purity) tended to give better numbers than those of A (21–29 % yield, 66–86 % purity) (Table 1).

The molecular weights of PC and the purest chitin sample, obtained as sample 18, were also estimated using viscosity measurements (see 2.8). Their values were found to be similar, with PC weighing 650 kDa and sample 18 weighing 677 kDa.

3.2. Thermogravimetric analysis (TGA)

TGA was first carried out on feedstocks A and B, which revealed three main decomposition steps (Fig. 3). The first step occurred around 50–150 °C and was likely due to water evaporation and protein decomposition. The second step, at about 300 °C, was attributed to the degradation of chitin saccharide backbones, including dehydration of polysaccharide rings and polymerization and decomposition of acetylated and deacetylated units. The final step, which occurred at around 600 °C, involved the thermal decomposition of calcium carbonate to calcium oxide. The thermograms of feedstocks A and B demonstrated that substantial calcium substances (about 20 %) were present after TGA.

TGA was then carried out on the chitin samples and PC. As demonstrated in Fig. 3, the thermograms of the samples with the highest chitin contents, 17 and 18, are like the ones of PC. The first decomposition step (50–150 °C) associated with water evaporation and protein decomposition was hardly noticeable. This demonstrated that the deproteination of the process of this study was effective. The purified samples then underwent decomposition of the chitin itself. Unlike feedstocks A and B, the thermograms of chitins reached around 0 % weight at nearly 650 °C. This, together with the lack of residual materials at the end of TGA, indicated that the decalcification was effective. Conversely, decalcification did not occur noticeably without excess AcOH (sample 0). The

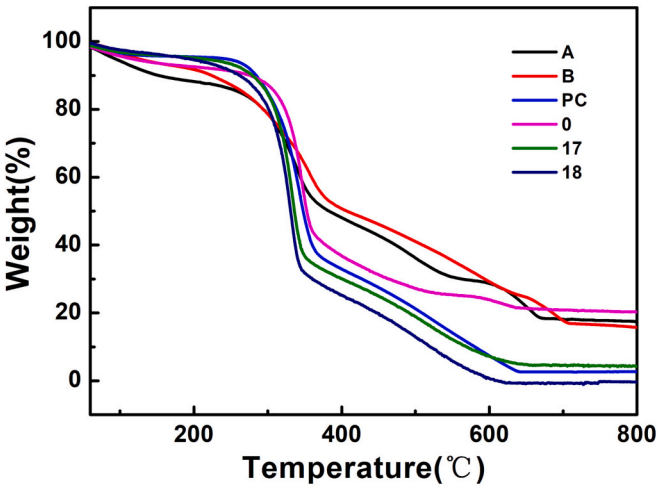

Fig. 3. TGA thermograms of the sample **A, B, PC, 0, 17** and **18**.

maximum degradation temperatures (DTG_{max}) of the isolated chitin were around 327–346 °C (Table 2), which were within the basic range of chitin thermal degradation (300–460 °C) reported by Stawski et al. (2008), and generally slightly lower than that of PC (342 °C). There was no noticeable correlation between the experimental conditions and DTG_{max} obtained (Table 2). It should be noted that the thermal properties of chitin were found to be feedstock-dependent previously (Stawski et al., 2008).

3.3. Fourier-transform infrared spectroscopy (FTIR)

IR spectroscopy was used to analyze the shrimp shells and chitin samples, as shown in Table 3 and Fig. 4. As observed, the IR spectra of the isolated chitin samples are very similar to the one of PC, notably in the fingerprint region (600–1400 cm^{-1}). The bands at 3467 and 3261 cm^{-1} were due to the symmetric stretching vibration of NH_2 and OH groups, respectively. The absorption band at around 2880 cm^{-1} (C—H stretching) was also observed in both PC and the samples obtained in this study. The amide I band split at 1662 cm^{-1} and 1624 cm^{-1}, which was attributed to the existence of intermolecular (–CO··NH–) and the intramolecular hydrogen bond (–CO··$HOCH_2$–). Moreover, the spectra displayed the amide II band at 1554 cm^{-1} and amide III at 1316 cm^{-1}. In the spectra of shrimp shells A and B, much of the absorptions associated with chitin overlapped with those of calcium carbonate (~1425 cm^{-1}) and protein (e.g., 1460–1680 cm^{-1}) in the shrimp shells and could only be observed after extraction with the [DBNH][OAc]-AcOH mixtures. The DA values of the pulped chitin were also estimated by IR with the Domszy and Roberts (1985) method, which used the amide I band (absorbance at 1655 cm^{-1}) as a measure of the acetyl group content and the hydroxyl band (absorbance at 3450 cm^{-1}) as the internal standard. As demonstrated in Table 2, the DA% of all chitin samples isolated in this process were generally higher than the one of PC (80 %). These findings demonstrated that this process sufficiently retained chitin's acetyl group during its purification. On the other hand, clear relationships between DA and DTG_{max} (Table 2) of the chitin samples were not observed.

3.4. X-ray diffraction (XRD)

The XRD patterns of the samples are illustrated in Fig. 5. The shrimp shells A and B share a similar XRD pattern with two major peaks (2θ = 9.1° and 19.2°) associated with α-chitin and one peak (2θ = 29.3°) ascribed to $CaCO_3$, which is consistent with the structure of shrimp shell in the literature (Tolesa et al., 2019; Zhao et al., 2019). After purification, the peaks of $CaCO_3$ were eliminated or became negligible. In addition, compared with the two feedstocks, the major peaks intensify, and additional peaks appear in the diffractograms of samples 17 and 18. These peaks at 9.1°, 12.5°, 19.2°, 20.7°, 23.3° and 26.2° can be assigned to the (020), (021), (110), (120), (130) and (013) facets of α-chitin, respectively (Yuan et al., 2020). The intensified diffraction peaks of α-chitin indicate that the concentration of α-chitin is increased after the removal of $CaCO_3$. The estimated value of the index of crystallinity (CrI) of feedstock A was 89.3 %. After purification of this feedstock, the CrI increased to 96.1 % (sample 17). Similarly, the CrI of feedstock B (88.5 %) increased to 95.4 % (sample 18). These findings demonstrated that the purification process enhanced the crystallinity of the chitin. The crystallinity of the purified chitins 17 and 18 was also higher than that of PC (94.1 %).

Table 2
Degrees of *N*-acetylation (DA) and maximum degradation temperatures (DTG_{max}) of chitin samples. PC refers to practical grade chitin. A: cooked shrimp shells with the size of <125 μm as raw materials; B: raw shrimp shells with the size of <125 μm as raw materials.

Sample	Ratio of AcOH: DBN	Feedstock	Load (%)	T (°C)	Time (h)	DA (%)	DTG_{max} (°C)
11	1.40	A	5	110	2	89	330
12	1.40	B	5	110	2	80	346
13	1.40	A	5	120	2	97	338
14	1.40	B	5	120	2	96	333
15	1.40	A	5	120	6	89	327
16	1.40	B	5	120	6	92	332
17	1.40	A	5	120	12	84	335
18	1.40	B	5	120	12	83	335
PC						80	342

Table 3
Assignments of FTIR spectra (cm^{-1}) of chitin samples.

Functional group and vibration modes	Sample 17	Sample 18	PC
O—H stretching	3441	3443	3442
N—H stretching	3267–3104	3268–3105	3273–3102
CH_3 symmetric stretch and CH_3 asymmetric stretch	2882	2885	2890
C—O secondary amide stretch	1658	1655	1658
C—O secondary amide stretch	1626	1624	1629
N—H bend, CN– stretch	1552	1559	1556
CH_2 bend and CH_2 deformation	1424	1416	1417
CH bend and CH_3 deformation	1382	1378	1380
CH_2 wagging	1318	1318	1315
Asymmetric bridge oxygen stretching	1157	1156	1156
C–OC– asymmetric stretch	1022	1020	1025
CH_3 wagging	951	954	952
CH ring stretching	892	896	898

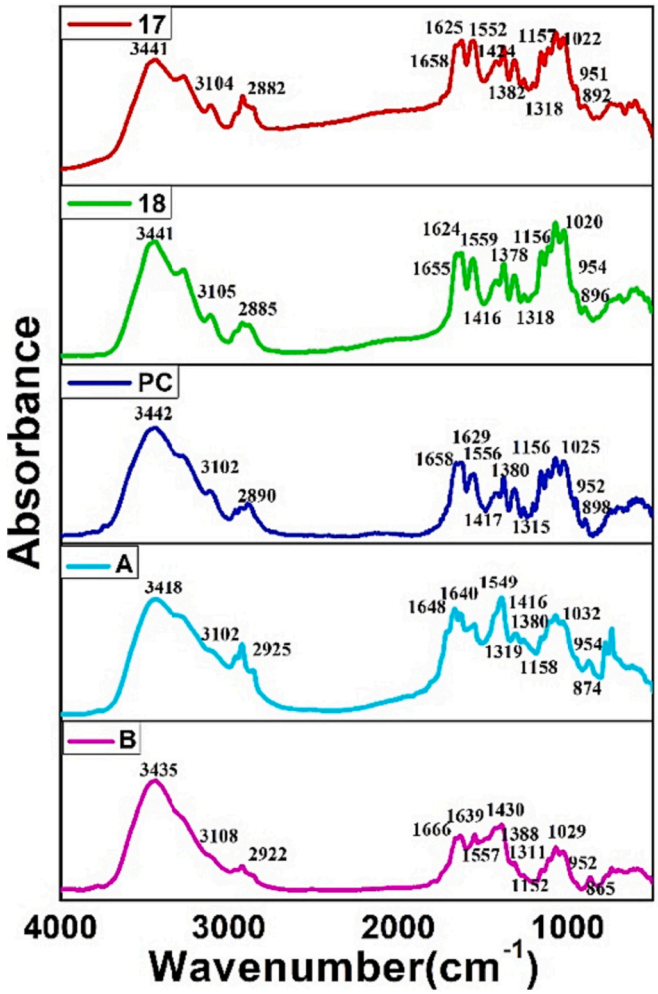

Fig. 4. FTIR spectra of the chitin samples **17, 18, PC** and shrimp shells **A** and **B**.

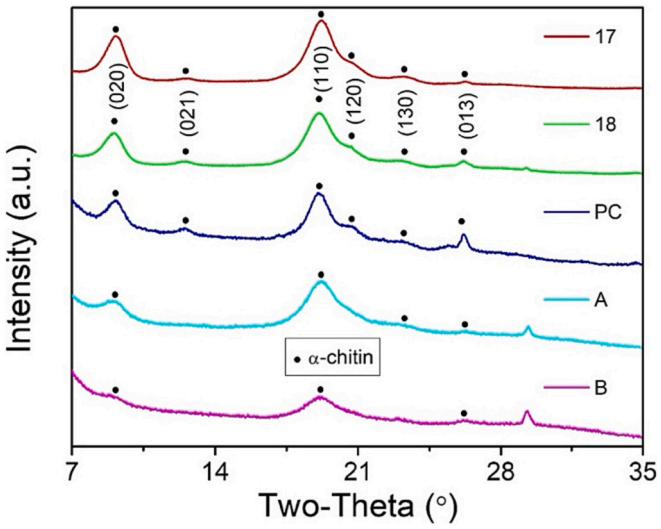

Fig. 5. XRD patterns of shrimp shells and chitin samples. Labelled peaks refer to those associated with chitin.

3.5. Recycling of pulping liquor ([DBNH][OAc]-AcOH mixture)

To demonstrate that the dissolved protein and calcium acetate can be separated from the pulping liquor and thereby the [DBNH][OAc]-AcOH mixture can be recycled after the chitin pulping process, the filtrate of sample 13, which contained the liquor and the dissolved materials, was distilled under reduced pressure. 92 wt% of the used pulping liquor was recycled as a colourless liquid mixture after distillation. A small amount of the liquor might be lost during work-up (e.g. centrifugation) and in the distillation apparatus. Using semi-quantitative ^{1}H NMR, the purity of the recycled pulping liquor was analyzed (Fig. 6). The only significant signals found in the ^{1}H NMR spectrum of the recycled [DBNH][OAc]-AcOH mixture were associated with the hydrogen atoms attached to the carbon chain of $[DBNH]^{+}$ (1.8, 2.0, 2.8, 3.3, 3.4 and 3.6 ppm) and the $[OAc]^{-}$/AcOH (1.7 ppm), which indicated the recycled pulping liquor has excellent purity. The ^{1}H NMR spectra of both the recycled and fresh liquors exhibit similarity, as illustrated in Fig. 6. The signals displayed in these spectra correspond to similar chemical shifts, also indicating the absence of impurities in the samples. The ratio of AcOH:DBN of the distillate was also estimated by integration, which was found to be 1.3. AcOH was then added to the distillate to bring the ratio back to 1.4 for reuse in pulping.

Identical conditions of pulping as sample 13 (5 % loading, 120 °C, 2 h heating) were used to purify the chitin from feedstock A using the recycled pulping liquor. The yield and purity of chitin obtained from the 2nd cycle were 24 % and 84 %, respectively, which were very similar to the ones obtained in the 1st cycle (21 % and 84 %, respectively). These results demonstrated that this chitin pulping process is highly recyclable with consistently good yield and quality chitinous products obtained using the old pulping liquor.

4. Conclusions

In this study, we have demonstrated a straightforward ionic liquid-based procedure to isolate chitin, using pulping liquors consisting of different ratios of DIL [DBNH][OAc] and AcOH, which were used to deproteinate and demineralize the shrimp shells, simultaneously. The pulping liquor could be synthesized conveniently by acid-base neutralization of two widely available chemicals, DBN and AcOH, at different ratios. This chitin isolation method distinguishes itself from other IL or DES-based procedures by providing a route to recycle the DIL in their original forms in excellent recovery by distillation. Moreover, this method retains the advantages of more recent studies, namely that it is carried out in a one-pot fashion without the need for an antisolvent to isolate the chitin.

The chitin samples isolated using this method possess qualities comparable to or better than commercial-grade chitin, as exhibited by their high purity, degree of *N*-acetylation and crystallinity. It was demonstrated that the type of feedstock, acid-base ratio, and pulping conditions played a crucial role in affecting the various properties of the isolated chitin. The optimized procedure unveiled in this report has high adaptability as good quality chitins were shown to be obtained from multiple feedstocks. While this pulping process offers several advantages over the conventional method, it is important to note that the use of distillation for pulping liquor recycling can be energy intensive. Therefore, conducting a comprehensive study to compare the energy efficiency and cost-effectiveness of the two methods would be highly desirable.

CRediT authorship contribution statement

Qingqing Tao: Data curation, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Felipe Nunes Henriquez:** Investigation, Validation, Visualization, Writing – review & editing. **Kang Ding:** Investigation, Methodology, Visualization, Writing – original draft. **Wai Lun Man:** Resources, Writing – review & editing. **Matthew Y. Lui:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

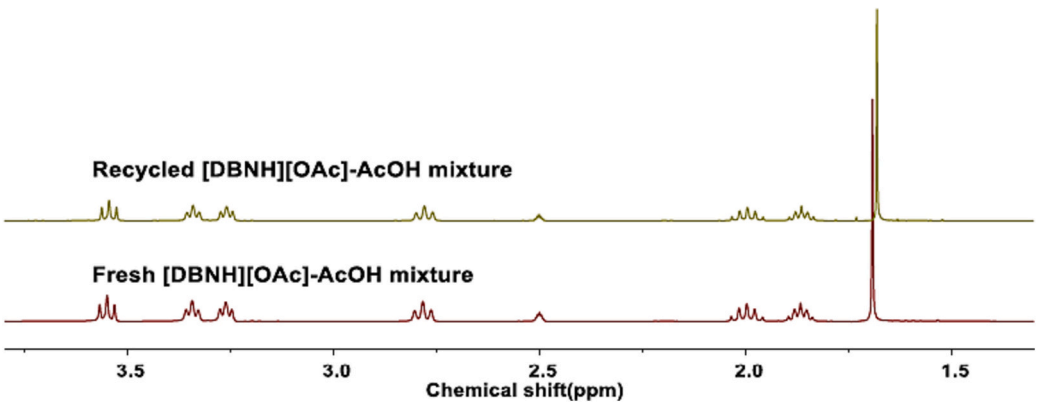

Fig. 6. ^{1}H NMR spectra of fresh and recycled pulping liquors.

the work reported in this paper.

Data availability

Data will be made available on request.

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