Chemical and Process Engineering: New Frontiers, 2024, 45(1), e53
DOI: 10.24425/cpe.2023.147412

Enzymatic deacetylation of chitin treated with ionic liquids

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Article info:
Received: 15 July 2023
Revised: 20 September 2023
Accepted: 13 November 2023

Abstract
Despite its unique properties (biocompatibility and nontoxicity), chitin itself has limited application. Chitin is completely insoluble in most organic or inorganic solvents which can be beneficial when chitin is investigated as a support for chromatography or enzyme immobilization. These applications require the particles to have an extensive outer surface with a large number of reactive ligands. The increase in specific surface area of chitin particles can be performed by dissolution in ionic liquid and precipitation with water. To increase the number of reactive ligands (amine groups), deacetylation of the surface of chitin particles is necessary. The deacetylation process can be carried out by an enzymatic process with the enzyme, chitin deacetylase.
In our investigation, 21 ionic liquids were used for chitin particle structure modification followed by enzymatic deacetylation. Results proved the positive effect of modifications with ionic liquid on enzymatic deacetylation of the chitin surface with chitin deacetylase. For 12 samples the deacetylation increased the number of active ligands in comparison to natural chitin. The best results were observed for [Bmim][Br], [Emim][Cl] and [MPpip][Ac]. That could be correlated with an increase in outer surface area by increasing porosity of particles or by structural changes in chitin particles.

Keywords
chitin, chitin deacetylase, ionic liquids, enzymatic deacetylation

1. INTRODUCTION

Chitin is one of the most abundant natural biopolymers, present in the skeleton of crustaceans, insects and fungi. It is built of N-acetylglucosamine (GlcNAc) units with a small number of glucosamine (GlcN) units in a polymer chain, Fig. 1.

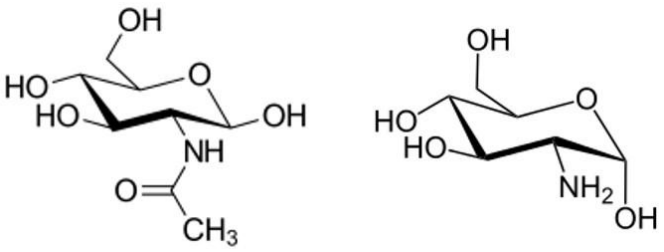


Figure 1. Structure of N-acetylglucosamine unit (left) and glucosamine unit (right).

Despite its unique properties (biocompatibility and nontoxicity), chitin itself has limited application due to its high crystallinity. Chitin is completely insoluble in most organic or inorganic solvents. However, thanks to this resistance to dissolution, chitin seems to be a good support for, e.g. chromatography or enzyme immobilization. These applications require the particles to have an extensive outer surface with a large number of reactive ligands. Unfortunately, chitin particles form structures with low porosity, so also with a low specific area. The structure of chitin particles can be turned into preferred directions (pore structure, corrugation structure) by dissolution in ionic liquid (IL) and precipitation with water. The influence of ionic liquids on chitin was reported for the first time in 2006 (Hanley et al, 2006). Since that time, it has been shown that several ionic liquids (ILs) can dissolve chitin (Jaworska et al., 2012; Kadokawa, 2019; Mohan et al., 2022; Shamshina and Berton, 2020). ILs are organic salts containing positively or negatively charged ions existing freely and stably in liquid form at temperatures below 100 °C (room temperature ionic liquid, RTIL). The charge of the ion is mainly due to electron imbalance (too many or too few) or to dislocations of charge at the surface of an ion. Usually, it is built with a large nitrogen-containing cation (e.g., imidazolium) or phosphorus (e.g., phosphonium), whereas anion can be organic (e.g., acetic, lactic) or inorganic (e.g., Cl^-, Br^-) and is much smaller.

The dissolution of chitin in ionic liquids is caused by different interactions between ILs and chitin, but after precipitation with water, often changes in chitin structure have been observed: an increase in porosity (Jaworska and Górak, 2016), increase in surface corrugation (Jaworska and Górak, 2018), changes in particle shape or size (Jaworska and Górak, 2018; Jaworska et al., 2018). These modifications significantly increase the size of the outer surface of chitin particles.

The low number of reactive groups at the surface of chitin particles is another objective for the industrial application of this polymer. The amine groups in glucosamine units (see Fig. 1)

are thought to be responsible for the biopolymer's reactivity. To increase the number of amine groups, deacetylation of the surface of chitin particles is necessary. The deacetylation process changes the unreactive N-acetylglucosamine units into reactive glucosamine units, and acetic acid is liberated. The deacetylation process can be carried out by chemical modification with strong alkali solutions (NaOH, KOH) or by an enzymatic process. Enzymatic deacetylation of N-glucosamine units can be carried out by the enzyme, chitin deacetylase (Arnold et al., 2020; Harmsen et al., 2019; Jaworska, 2012; Kaczmarek et al., 2019; Yang et al., 2022). Chitin deacetylase is the only enzyme that is able to hydrolyze the linkage between the acetyl and amine groups in the units of *N*-acetylglucosamine (GlcNAc) of chitin, transforming them into the glucosamine (GlcN) units. Contrary to the chemical process, enzymatic deacetylation avoids polymer degradation as it modifies only the GlcNAc units and does not make any changes in the length of the polymer chain. It is carried out only at available N-acetylglucosamine groups, which (in the case of chitin) are located only at the surface of particles (due to the firm crystal structure the enzyme is not able to penetrate inside a particle). The enzymatic deacetylation of chitin depends on chitin structure as it has been presented by Jaworska and Roberts (2016).

The changes in chitin particles made with ionic liquid can influence changes in the conformation of the polymer chains at the surface of the chitin particles so that it can influence the deacetylation of the surface GlcNAc units. Chitin treated with ionic liquids has been deacetylated chemically (Barber et al., 2014; Huet et al., 2021; Tolesa et al., 2019), but enzymatic deacetylation was rarely attempted (Ma et al., 2020).

The present paper aims to investigate the possibility of enzymatic deacetylation of chitin particles treated with ionic liquids. We expected that the number of deacetylated GlcNAc units at the surface of chitin particles would be correlated with the increase in porosity or corrugation of their surface or with the increase in surface development and changes in crystallography. In our investigation, 21 ionic liquids divided into two groups of ionic liquids have been used:

- ionic liquids with organic anion: 2-hydroxy ethyl ammonium formate ([EOHamm][Form]), 1-ethyl-3-methylimidasolium acetate ([Emim][Ac]), 1-methyl-1-propylpyridinium acetate ([MPpyrr][Ac]), 1-methyl-1-propylpiperidinium acetate ([MPpip][Ac]), choline acetate ([Chol][Ac]), 1-ethyl-3-methylimidasolium lactate ([Emim][Lact]) 1-methyl-1-propylpirolidinium DL-lactate ([MPpyrr][Lact]), 1-methyl-1-propylpiperidinium DL-lactate ([MPpip][Lact])
- Ionic liquids with inorganic anion: 1-allil-3-methylimidasolium chloride ([Amim][Cl]), 1-ethyl-3-methylimidasolium chloride ([Emim][Cl]), 1-buthyl, 3-methylimidasolium chloride ([Bmim][Cl]), 1-buthyl, 1-butyl,-2-3-dimethylimidasolium chloride ([Bdmim][Cl]), 1-hexyl-3-methylimidasolium chloride ([Hmim][Cl]), 1-octyl-3-methylimidasolium chloride ([Omim][Cl]), 1-allyl-3-methylimidasolium bromide ([Amim][Br]), 1-buthyl-3-methylimidasolium bromie ([Bmim][Br]), were kindly donated by Merck KgaA, 1-ethyl-3-methylimidasolium bromide ([Emim][Br]), 1-ethylpyridinium bromie ([Epyr][Br]), 1-ethyl-3-methylimidasolium iodine ([Emim][I]), 1-ethylpyridinium iodide ([Epyr][I], -(2-hydrohyethyl)pyridinium tetracyanoborate ([EOHpyr][TCB]).

Ionic liquids have been chosen based on our previous experiments on the interaction of ionic liquids with chitin (Jaworska and Górak, 2016; Jaworska and Górak, 2018; Jaworska et al., 2017; Jaworska et al., 2018) as well as on the interaction of the ionic liquid with chitin deacetylase (Aspras et al., 2016; Aspras et al., 2017).

2. MATERIALS AND METHODS

2.1. Chemicals

$\alpha-$Chitin, with the degree of acetylation of 95%, from shrimp was purchased from BioLog Heppe (Germany). Particles were ground, and the fraction of 25–50 μm was used for modification with ionic liquids.

The following ionic liquids were used for the modification of chitin:

- ionic liquids with chloride anion: 1-allil-3-methylimidasolium chloride ([Amim][Cl]), 1-ethyl-3-methylimidasolium chloride ([Emim][Cl]), 1-buthyl, 3-methylimidasolium chloride ([Bmim][Cl]), 1-buthyl, 1-butyl,-2-3-dimethylimidasolium chloride ([Bdmim][Cl]), 1-hexyl-3-methylimidasolium chloride ([Hmim][Cl]), 1-octyl-3-methylimidasolium chloride ([Omim][Cl]) were kindly donated by Merck KgaA,
- ionic liquids with bromide anion: 1-allyl-3-methylimidasolium bromide ([Amim][Br]), 1-buthyl-3-methylimidasolium bromie ([Bmim][Br]), were kindly donated by Merck KgaA, 1-ethyl-3-methylimidasolium bromide ([Emim][Br]), 1-ethylpyridinium bromie ([Epyr][Br]) were purchased from Iolitec,
- ionic liquids with iodine anion: 1-ethyl-3-methylimidasolium iodine ([Emim][I]), 1-ethylpyridinium iodide ([Epyr][I]) were purchased from Iolitec,
- ionic liquids with organic acid anion: 2-hydroxy ethyl ammonium formate ([EOHamm][Form]) were purchased from Iolitech, 1-ethyl-3-methylimidasolium acetate ([Emim][Ac]), 1-ethyl-3-methylimidasolium lactate ([Emim][Lact]) were purchased from Aldrich, 1-methyl-1-propylpyridinium acetate ([MPpyrr][Ac]), 1-methyl-1-propylpiperidinium acetate ([MPpip][Ac]), 1-methyl-1-propylpirolidinium DL-lactate ([MPpyrr][Lact]), 1-methyl-1-propylpiperidinium DL-lactate ([MPpip][Lact]) were prepared according to the method presented in [15]Jaworska et al. (2018),
- other ionic liquids: 1-(2-hydroxyethyl)pyridinium tetracyanoborate ([EOHpyr][TCB]) were kindly donated by Merck KgaA, choline acetate ([Chol][Ac]) were purchased from Iolitec.

All chemicals used in experiments were analytical grade.

2.2. Chitin deacetylase

The enzyme separated from *Absidia orchidis* NCAIM F 00642 was used in all experiments (the enzyme is not commercially available). The fungi were cultivated in a 5.0-L batch culture (26 °C, pH 5.5, YPG (yeast extract, peptone, glucose) nutrient medium, (Jaworska and Konieczna, 2001)) and separated from the nutrient medium by centrifugation (6000 rpm, 20 min, 4 °C). Next, the biomass was frozen and then slowly thawed and homogenized, and the crude cell extract was separated (centrifugation, 6000 rpm, 20 min, 4 °C) and salted out with ammonium sulphate (80% saturation) overnight at 4–6 °C. The solution was diafiltrated with HCl (pH 4.0) to remove ammonium (using a membrane module with a cut-off 10 kDa) and then concentrated by ultrafiltration (the same membrane module). This enzyme solution, adjusted to pH 4.0 (optimal pH) with HCl, was used in all experiments. The activity of the preparation was 6.5 U/mL, where 1 U is such amount of the preparation solution that forms 1 µmol of acetic acid during 10 min of reaction with chitosan (chitosan with acetylation degree (AD) = 22.8%) as a substrate, at 45 °C, pH 4.0.

2.3. Chitin treated with ionic liquid

Approx. 5 g of selected ionic liquid was weighed to a probe, and next 500 mg of α–chitin 25–50 µm) was added. Samples were incubated in an oil bath at 105 °C and mixed (200 rpm) for 48–72 h. After that time, water (5 mL) was added to a sample (mixing at vortex) to precipitate chitin. Chitin was washed at least 10 times with water, next at least five times with ethanol, and finally lyophilized.

2.4. Enzymatic deacetylation of chitin treated with ionic liquids

Approx. 120 mg of lyophilized modified chitin was weighed to a probe, and 3 mL of HCl (pH 4.0) solution was added. Samples were incubated at 30 °C and mixed (150 rpm) for 30 minutes, and the next enzyme solution (0.5 mL) was added to start the deacetylation reaction. The reaction was carried out in the same conditions (30 °C, 150 rpm) for 48 h to reach complete deacetylation of the chitin surface. After that time, the solution was sampled (2 mL) and mixed with 0.10 mL of 1.0 N NaOH to stop the reaction. Chitin was separated by centrifugation, and the released acetic acid amount in the clear supernatant solution was measured. The difference in liberated acetic acid in samples with and without enzyme was reported as a result of deacetylation process.

Additionally, an experiment with non-modified chitin was carried out as a comparison.

2.5. Analytical methods

The acetic acid concentration in the clear solution was analysed using the HPLC method: isocratic system (Varian ProStar 210) with HyperREZ XP Organic acid column (60 °C) and HyperREZ XO Carbohydrate H^+ Guard Column, water as eluent (0.5 mL/min), and refractometer detector (Varian ProStar 350). The quantification limit was evaluated at 10 nmol/mL with a standard deviation of 8% of the mean value. The method was validated for acetic acid determination in chitosan–HCl (pH 4.0) solutions.

The particle size distribution ($d_{10\%}$–$d_{90\%}$) and mean size of particles (d_{mean}) were evaluated using a Laser Diffraction Particle Size Analyzer LS 13- 320, Universal Liquid Module (Coulter–Beckman, USA), a mean value of 3 samples were taken. The standard deviation (SD) of d_{mean} was lower than 10%.

2.5.1. FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) measurements were taken using a Nicolet 6700 FT-IR spectrometer (Thermo Scientific, Waltham, MA, USA) equipped with photoacoustics MTEC model 300 accessory (Thermo Scientific, Waltham, MA, USA). Samples in the form of a powder were placed in the recesses of measurement cells without additional mechanical working. The following measurement parameters were used: resolution, 4 cm^{-1}; photoacoustic detector; number of scans, 256. For each sample, the spectra were collected over the range of 4000 to 500 cm^{-1}. Data collection and post-processing were performed using OMNIC software (v. 9.0, Thermo Electron Corp. USA). Each spectrum was analysed with the use of a linear baseline and pre-processed by means of Fourier smoothing.

2.5.2. Wide-angle X-ray diffraction (WAXD)

WAXD patterns were obtained on an URD 6 Seifert diffractometer (Rich. Seifert & Co. Röntgenwertk, Ahrensburg, Germany) employing the Bragg–Brentano reflection geometry method. For the measurements, copper radiation (CuKα, λ =1.54 Å) was used at 40 kV and 30 mA. The monochromatization of the radiation beam was achieved by a graphite monochromatizer placed across the monochromatized beam. A scintillation counter was used as a detector. The tests were carried out in the range of 2θ from 5° to 35° in steps of 0.01°, scanning speed of 0.1° per 15 s, ~ 25 °C. Selected WAXD diffractograms for different preparations are presented.

2.5.3. Electron microscope observation

A Nikon ELWD 03/OD75 Electron Microscope (Japan) was used for the SEM observations. Samples had been lyophilized and sprayed with gold before photographs were taken. Typical observations of at least 3–5 preparations have been presented.

3. RESULTS AND DISCUSSION

Ionic liquids can change the structure of chitin particles, which can influence the possibility of their enzymatic deacetylation. Ionic liquids also interact with the enzyme influencing its activity. Both effects are possible in the enzymatic deacetylation of

chitin particles treated with ionic liquids. First, we presented the changes in the structure of chitin particles after treatment with ionic liquids, next results of enzymatic deacetylation are presented, and finally the changes in chitin particles structure are discussed.

3.1. Structural modifications of chitin

The changes in chitin structure after treatment with ionic liquid strongly depend on the ionic liquid used as it was presented in our earlier paper (Jaworska and Górak, 2016; Jaworska and Górak, 2018; Jaworska et al., 2017; Jaworska et al., 2018). The ionic liquid can completely destroy the bonds stabilizing the particle structure, causing complete dissolution of chitin or can only weaken these bonds giving gelation of chitin particles. Depending on the ionic liquid, new structures, new particle size distribution or slight modification of the chitin surface could be observed after precipitation with water. The influence of ionic liquid on the changes of chitin particles is presented in Table 1 and Table 2.

Depending on the ionic liquid used for modification, changes in the structure of chitin particles, as well as changes in the size of chitin particles, were observed. Generally, slight changes in particle size and particle size distribution were observed. The only exception was observed for [Emim][Ac], [MPpyr][Ac], [MPpip][Ac] and [MPpip][Lact], where an increase in particle size was noticed, and for [EOHpyr][TCB] where a significant decrease in particle size was shown.

3.2. Enzymatic deacetylation of chitin

Chitin particles treated with ionic liquids have been enzymatically deacetylated with chitin deacetylase. The results of deacetylation were presented as the total amount of acetic acid liberated in the process, Fig. 2.

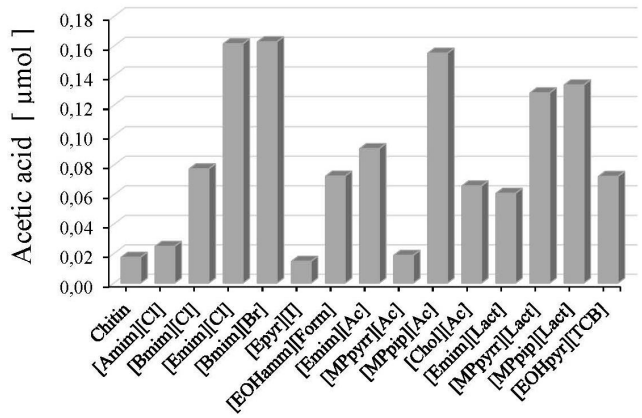


Figure 2. Total amounts of acetic acid liberated from chitin particles treated with ionic liquid after enzymatic deacetylation.

In all experiments, the same mass of chitin was used, so differences in the amount of acetic acid liberated during the deacetylation process resulted from the availability of N-acetylglucosamine units for the enzyme. An increase in availability could be a result of changes in particle structure or surface corrugation, or particle size distribution. The largest amounts of liberated acetic acid were obtained for chitin particles modified with [Bmim][Br], [Emim][Cl], [MPpip][Ac], [MPpip][Lact], [MPpip][Ac] for which 8–9 times more acetic acid was liberated that for natural chitin. These ionic liquids are responsible for significant changes in chitin structure (especially [MPpip][Ac], [MPpip][Lact], [MPpip][Ac]) or in chitin porosity ([Bmim][Br], [Emim][Cl]). Contrary to them, for [Bdmim][Cl], [Hmim][Cl], [Omim][Cl], [Amim][Br], [Emim][Br], [Epyr][Br], [Emim][I] enzymatic deacetylation was not observed as the amount of acetic acid liberated during the process was below the detection limit. This can suggest that acetyl groups present originally at the surface of chitin particles were no more available for the enzyme after modification due to structural changes in the particles. It is also possible that it was caused by the interaction of ionic liquid linked to the structure of chitin particles with the enzyme.

3.3. Wide-angle X-ray diffraction (WAXD)

Selected sets of WAXD diffraction patterns with Miller's indicators for chitins treated with various ionic liquids and enzymatic deacetylation are presented below. We have chosen particles with the largest amount of liberated acetic acid ([Bmim][Br] and [Emim][Cl], Fig. 3) and particles where no deacetylation was observed ([Emim][I] and [Emim][Br], Fig. 4).

Four characteristic peaks appear on the obtained diffraction patterns (Fig. 3a–b; Fig. 4a–b), which characterize the crystalline structure of chitin. The main crystal peaks were distinguished in the places of maximum radiation intensity diffracted on the lattice planes (h,k,l), which occur at the positions $2\theta = 8.9^\circ$ (020), 12.4° (021), 18.9° (110), 25.9° (013) for natural chitin.

As a result of the treatment of natural chitin with ionic liquids and after enzymatic treatment, for diffraction patterns obtained for [Bmim][Br] or [Emim][Cl], we do not observe significant changes in the position of the diffracted radiation maxima, which proves that the molecular structure is preserved in ordered areas.

However, after treatment of natural chitin with ionic liquids [Emim][I] and [Emim][Br], we observed clear shifts in the position of four characteristic maxima of crystal peaks, which after treatment with ionic liquid and enzymatic deacetylation occur at the positions of $2\theta = 9.4^\circ$ (020), 12.7° (021), 19.4° (110), 26.4° (013) for [Emim][Br]. The observed effect is the result of the compaction of the ordered structure after the treatment of chitin with both ionic liquids and enzymatic deacetylation. The confirmation of this phenomenon is the reduction of interplanar distances in the chitin lattice, which was calculated from Bragg's law.

Table 1. Typical changes in the structure of chitin particles.

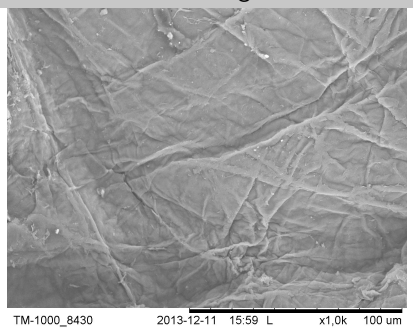
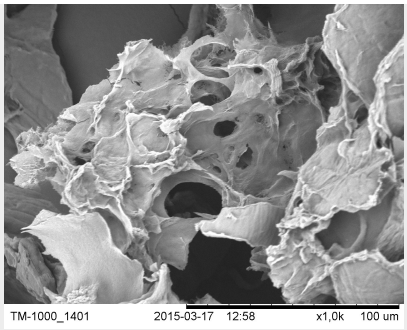
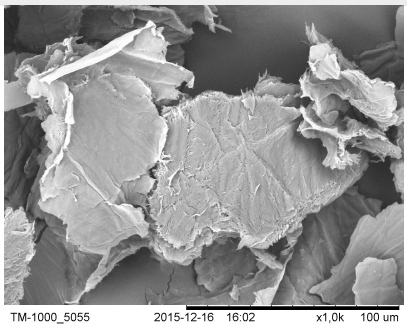
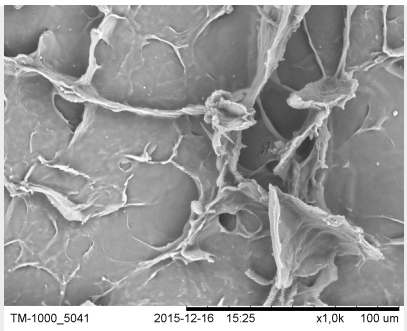
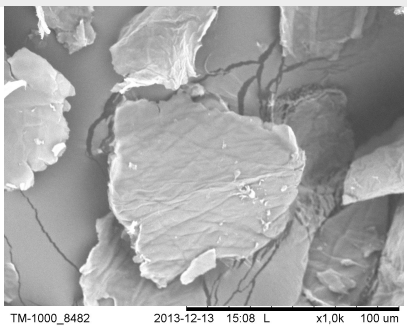
Chitin structure	Ionic liquid	SEM images
Chitin	—	
Changes in particle structure	[MPpyr][Ac] [MPpip][Ac] [Emim][Ac] [MPpip][Lact]	
Changes in porosity	[EOHam][Form], [Amim][Cl], [Emim][Cl], [Bdmim][Cl], [Omim][Cl] [Amim][Br], [Emim][Br] [Emim][I]	
Changes in surface corrugation	[EOHam][Form] [MPpyr][Ac], [MPpyr][Lact] [MPpip][Ac], [MPpip][Lact] [Chol][Ac] [Emim][Ac], [Emim][Lact] [Bmim][Cl], [Omim][Cl] [Bmim][Br],[Emim][Br] [Epyr][I], [Emim][I] [EOH pyr][TCB]	
No changes	[Hmim][Cl] [Epyr][Br]	

Table 2. Size of chitin particles after treatment with ionic liquids.

Ionic liquid	Mean particle size [μm]	Particle size distribution, d_{10-90} [μm]	References
Chitin	74	34–126	Jaworska et al., 2018
[Amim][Cl]	75	32–126	Jaworska and Górak, 2016
[Emim][Cl]	66	26–112	Jaworska et al., 2017
[Bmim][Cl]	62	23–106	Jaworska and Górak, 2016
[Bdmim][Cl]	72	30–117	Jaworska and Górak, 2016
[Hmim][Cl]	72	27–122	Jaworska and Górak, 2016
[Omim][Cl]	72	34–126	Jaworska and Górak, 2016
[Amim][Br]	71	26–116	pp
[Bmim][Br]	71	27–117	pp
[Emim][Br]	67	13–122	Jaworska et al., 2017
[Epyr][Br]	83	27–146	pp
[Epyr][I]	76	22–138	Jaworska et al., 2017
[Emim][I]	67	17–129	pp
[EOHam][Form]	63	17–119	pp
[Chol][Ac]	64	18–104	pp
[Emim][Ac]	753	120–1527	Jaworska et al., 2018
[MPpyr][Ac]	662	62–1581	Jaworska et al., 2018
[MPpip][Ac]	406	41–1053	Jaworska et al., 2018
[Emim][Lact]	76	32–143	Jaworska et al., 2018
[MPpyr][Lact]	82	24–148	Jaworska et al., 2018
[MPpip][Lact]	328	31–1310	Jaworska et al., 2018
[EOHpyr][TCB]	48	5–106	Jaworska and Górak, 2018

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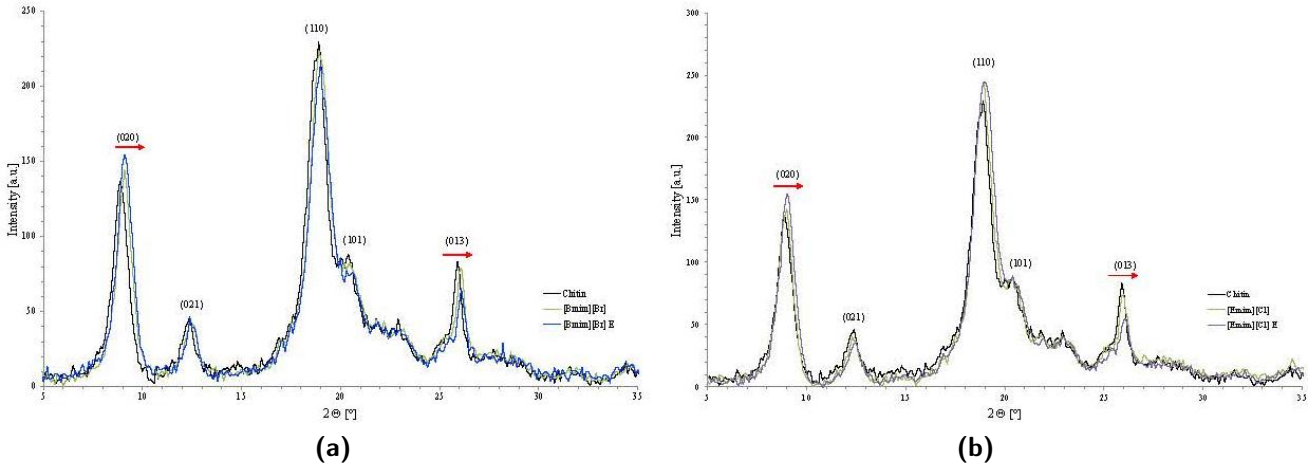
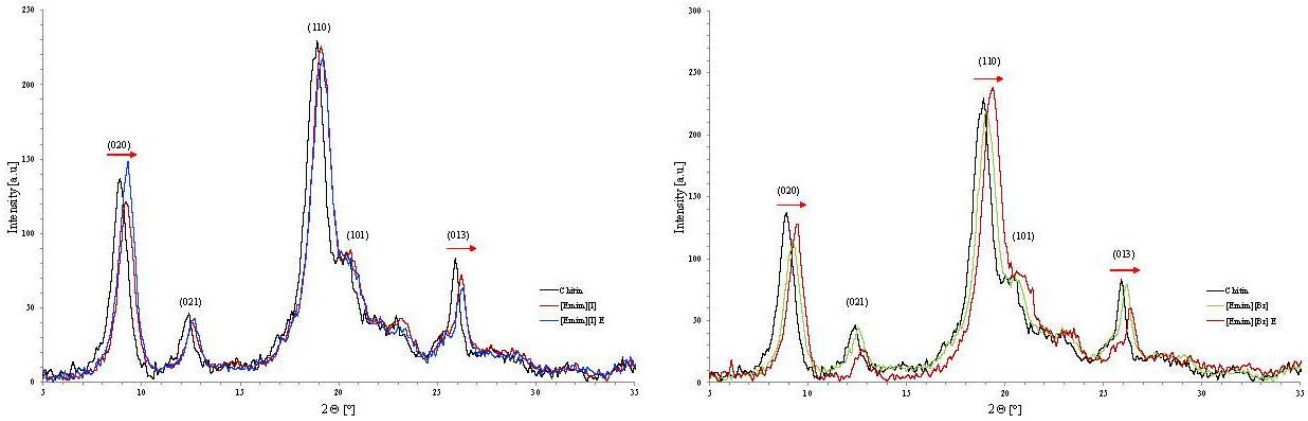


(a) **(b)**

Figure 3. Wide-angle X-ray diffraction (WAXD) of natural chitin, chitin after ionic liquid treatment and after enzymatic deacetylation (E); a) [Bmim][Br] b) [Emim][Cl].



(a) **(b)**

Figure 4. Wide-angle X-ray diffraction (WAXD) of natural chitin, chitin after ionic liquid treatment and after enzymatic deacetylation (E); a) [Emim][I] b) [Emim][Br].

The interplanar distances d_{hkl} for individual families of lattice planes were calculated using Bragg's law:

$$2d_{hkl} = n \cdot \lambda \tag{1}$$

where: n is a natural number, the positions of peaks related to a given family of planes were calculated, λ – is the wavelength of Cu–K radiation ($\lambda = 1.54$ Å), θ – is half the Bragg angle corresponding to the crystalline peak (rad).

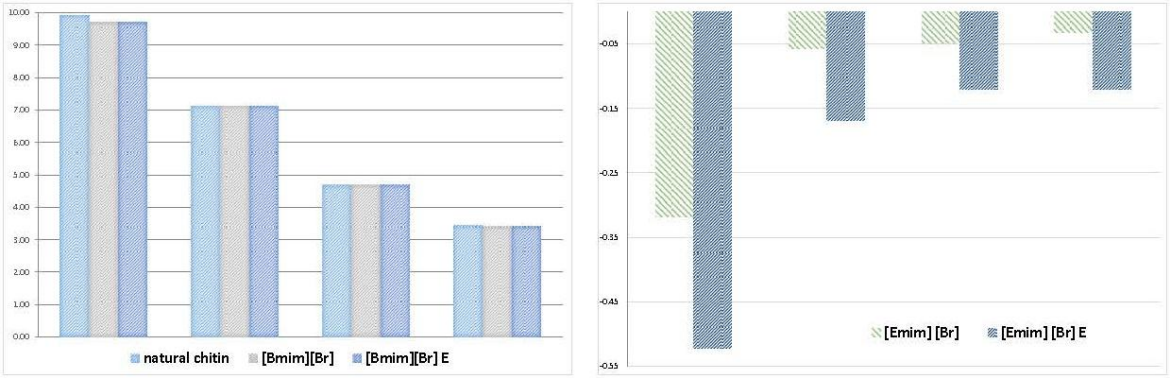
Exemplary results are presented graphically for selected chitin samples after treatment with ionic liquid and enzymatic deacetylation, Fig. 5.

After the treatment of chitin with the ionic liquid [Bmim][Br] and after enzymatic deacetylation (E), there are no changes in the interplanar distances. However, in the case of chitin treated with the ionic liquid [Emim][Br] and after enzymatic deacetylation (E), the interplanar distances in the molecular structure of chitin decrease. This compaction of the particle structure could be a reason of the low or even no availability of GlcNAc units in chitin chains, which could be enzymatically deacetylated.

3.4. FTIR spectra of chitin

The changes in chitin chemical structure caused by modification with ionic liquids and by enzymatic deacetylation were analyzed based on FTIR spectra. We have chosen particles with the largest amount of liberated acetic acid [Bmim][Br] and [Emim][Cl], and particles where no deacetylation was observed [Emim][I] and [Emim][Br], Figs. 6–9.

Figures 6 and 7 show the spectra for natural chitin samples and after treatment with ionic liquids [Bmim][Br], [Emim][Cl] and enzymatic deacetylation (E). For the obtained spectra, no differences in the chemical structure of chitin are observed, also for the spectra of chitin after treatment, no new bands appear, and no disappearance of the existing bands is observed. Slight changes can be observed only in the case of the Amide II band at the maximum position of 1556 cm^{-1}, in the first stage of processing, narrowing of the band top and then its widening. This effect may be the result of the compaction of the chitin molecular structure and its subsequent loosening to its original state. It is probably related to the influence of ionic liquids on intermolecular interactions between chitin chains.



(a) **(b)**

Figure 5. Interplanar distances (d_{hkl}) calculated from XRD patterns: a) [Bmim][Br] b) difference of the obtained values compared to native chitin for [Emim][Br].

Presented spectra proved that ionic liquids were not linked to chitin structure as no additional bonds were detected. In such a case the activation effect of [Bmim][Br] (Aspras et al., 2017) can not be the explanation of a significant increase in deacetylated units. Additionally, no changes in bands correlated with glucosamine units were observed. That could be explained by a relatively small increase in the amount of GlcNAc units in comparison with the total number of these units in chitin particles. The changes in acetylation degree could be below the sensitivity of the detection method.

Figures 8 and 9 show the spectra for natural chitin samples and after treatment with ionic liquids [Emim][I], [Emim][Br] and enzymatic deacetylation (E). In the case of these samples,

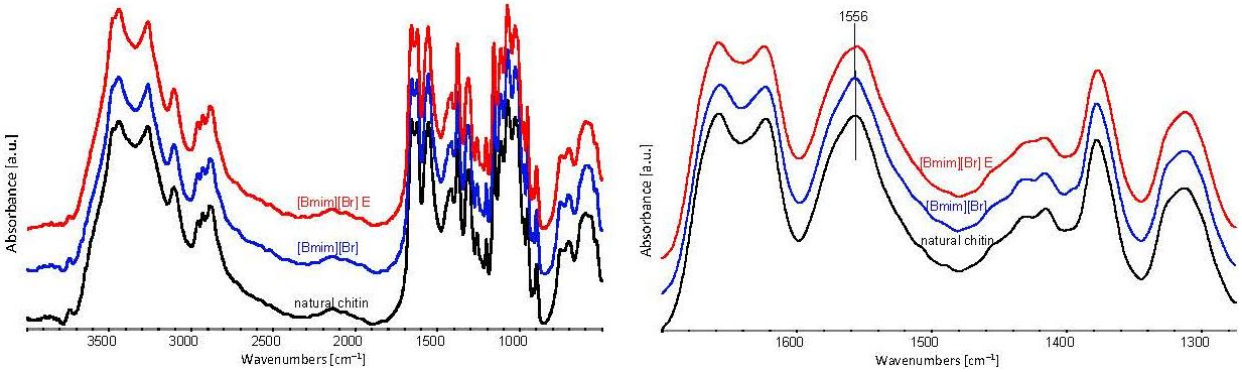


Figure 6. FTIR spectra of natural chitin, chitin after treatment with [Bmim][Br] and after enzymatic deacetylation (E); a, b) spectra in a different range of wavenumbers.

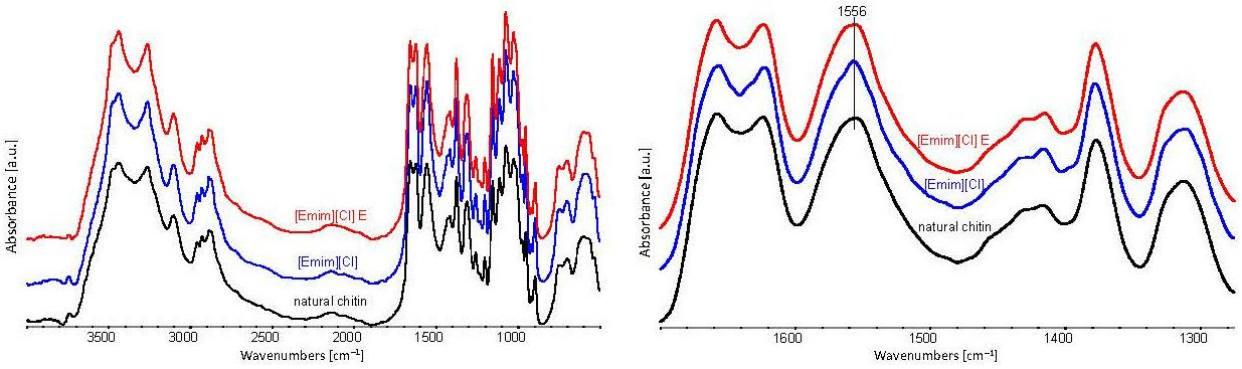


Figure 7. FTIR spectra of natural chitin, chitin after treatment with [Emim][Cl] and after enzymatic deacetylation (E); a, b) spectra in a different range of wavenumbers.

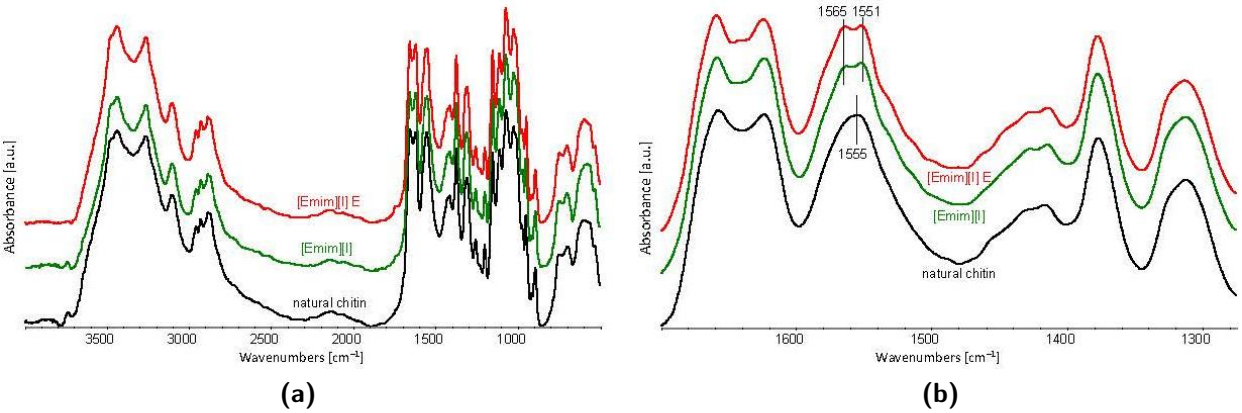


Figure 8. FTIR spectra of natural chitin, chitin after treatment with [Emim][I] and after enzymatic deacetylation (E); a, b) spectra in a different range of wavenumbers.

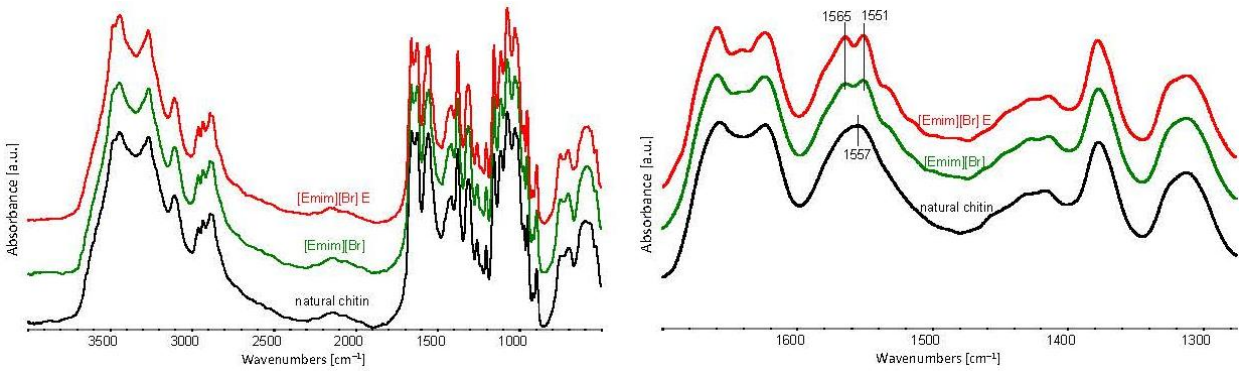


Figure 9. FTIR spectra of natural chitin, chitin after treatment with [Emim][Br] and after enzymatic deacetylation (E); a, b) spectra in a different range of wavenumbers.

significant differences in the molecular structure of chitin are observed in the spectra. New bands appear as a result of the formation of new molecular structures. In the case of the Amide II band at the maximum position of 1557 cm^{-1}, we observed its separation into two bands at the maximum position of 1565 cm^{-1} and 1551 cm^{-1}. This effect may be the result of compaction of the ordered crystalline structure and loosening of the less ordered or amorphous structure. It is probably related to the characteristic influence of these ionic liquids on intermolecular interactions between adjacent chitin chains.

4. DISCUSSION AND CONCLUSIONS

Although chitin is one of the most widespread biopolymers, its industrial application is rather small. It can be changed by modification of the structure of chitin particles by ionic liquids and by enzymatic deacetylation. It has been shown that ionic liquids can change the surface of chitin particles, increasing its porosity and corrugation as well as changing particle size distribution giving large agglomerates or decreasing particle size. Additionally, modified chitin can be enzymatically deacetylated increasing the number of active amine ligands present at the surface of chitin particles.

The presented investigations proved the positive effect of modifications with ionic liquid on enzymatic deacetylation of the chitin surface with chitin deacetylase. For 12 samples, the deacetylation gave larger amounts of liberated acetic acids than it was observed for natural chitin. The best results were observed for [Bmim][Br], [Emim][Cl] and [MPpip][Ac]. That could be correlated with an increase in outer surface area by increasing porosity of particles or by structural changes in chitin particles. For these particles, no changes in crystallinity were observed. Contrary to these best results, for 7 samples no deacetylation was noticed. For these particles also changes in porosity were detected, but, more importantly, changes in crystallinity were also noticed. After modification with ionic liquids, the arrangement of chitin chains was more compact, which could influence lower availability of GlcNAc units. After precipitation with water, probably stronger interchain interactions took place, which caused more compact structure that was more difficult to deacetylate.

As a conclusion, we can state out that chitin particles modified with ionic liquids, which can significantly increase the development of the outer surface without changes in the degree of crystallinity or its lowering, are most suitable for enzymatic deacetylation of chitin.

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