SUPERMOLECULAR STRUCTURE OF CHITIN AND ITS DERIVATIVES IN FTIR SPECTROSCOPY STUDIES

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1. Introduction

It was found out that during butyrylation of the krill chitin followed by estrification of the obtained dibutyrylchitin, characteristic changes of the supermolecular structure of the materials take place. Chemical reactions and structural changes accompanying the transformation: krill chitin - dibutyrylchitin - regenerated chitin were investigated by means of the spectroscopic methods (FTIR, FTNIR, FT Raman) as well as by the measurements of wide-angle X-rays diffraction (WAXS). In the obtained IR spectra specific changes of Amide I band were noticed. The resolution of Amide I band was ascribed to the influence of hydrogen bonds on the oscillation frequency of C=O groups. The observation was the basis for the new model of a supermolecular structure of chitin in which a hydrogen bond is formed in every second C=O group [1].

2. Measurements and equipment.

2.1. Parameters of measuring system

2.1.1. X-ray diffraction measurements

The measurements of wide-angle X-ray diffraction (WAXS) for all the examined samples were carried out in the reflection mode at a room temperature using a Seifert URD-6 diffractometer with a scintillation counter connected to a computer. Ni-filtered Cu Kα radiation was applied. The accelerating voltage value was 40 kV and the plate current intensity - 30 mA. The diffraction scans were collected between 2Θ values from 2° to 60° with a step of 0.1°.

2.1.2. Measurements in the range of medium IR 4000 - 400 cm^{-1}

All the measurements were carried out using FTIR spectrometer of MAGNA 860 type, a product of NICOLET.

The fibres were cut into 1 - 3 mm pieces and next they were blended with powdered sodium chloride (spectr. grade). The samples were then pressed under 200 MPa to obtain a tablet. The tablet was placed in a measuring chamber of the spectrometer equipped with a mirror beam collimator (focused 16×). As a reference, a tablet made of NaCl (without fibres) was used.

The following measuring parameters were applied:
range – 4000 - 400 cm^{-1}, resolution – 4 cm^{-1}, number of scans – 128,
source of radiation – IR, detector – DTGS, beamsplitter – KBr.

2.1.3. Measurements in the range of near IR (FTNIR) 10600 - 5600 cm^{-1}
The measurements were carried out for the fibres which were not mechanically processed. The fibres were placed in a measuring tube in such a manner as to ensure the homogeneous exposition to radiation of the whole sample.

The following measuring parameters were used:
range – 1600 - 5600 cm^{-1}, resolution – 8 cm^{-1}, number of scans – 512,
source – halogene lamp, detector – InGaAs, beamsplitter – CaF_2.

2.1.4. Measurements in the range of medium FT Raman
All the measurements were carried out using FTIR spectrometer of MAGNA 860 type to be equipped with FT Raman module, a product of NICOLET.

The following measuring parameters were applied:
range – 4000 - 100 cm^{-1}, resolution – 8 cm^{-1}, number of scans – 50 000,
source of radiation – NdYag 1064 nm, energy source – 0.6 W,
detector – InGaAs, beamsplitter – KBr.

3. Results and discussion

The WAXS measurements of the krill chitin proved that its supermolecular structure is ordered and the value of its crystallinity degree is high. As a result of the butyrylation process leading to obtaining dibutyrylchitin, a deep transformation of the polymer, material structure takes place. In Figure 1 a set of diffractograms of the krill chitin and dibutyrylchitin has been presented.

By comparing WAXS diffractograms of chitin and dibutyrylchitin it can be seen that the butyrylation process causes the disappearance of diffraction reflexes in the ordered area accompanied by the broadening of the remaining reflexes. The calculations of the supermolecular structure parameters contain in Table 1. The conclusion of this parameters is the obtained dibutyrylchitin is characterized by significantly lower crystallinity degree as well as by the smaller size of the crystalline regions, which results from a small structural ordering of the obtained polymer.

The alkaline treatment of dibutyrylchitin (5% KOH and at 20 °C - series A, at 50 °C - series B, at 70 °C - series C and at 90 °C - series D) to obtain the regenerated chitin brings about a reverse chemical process in which the supermolecular structure of chitin is gradually be-

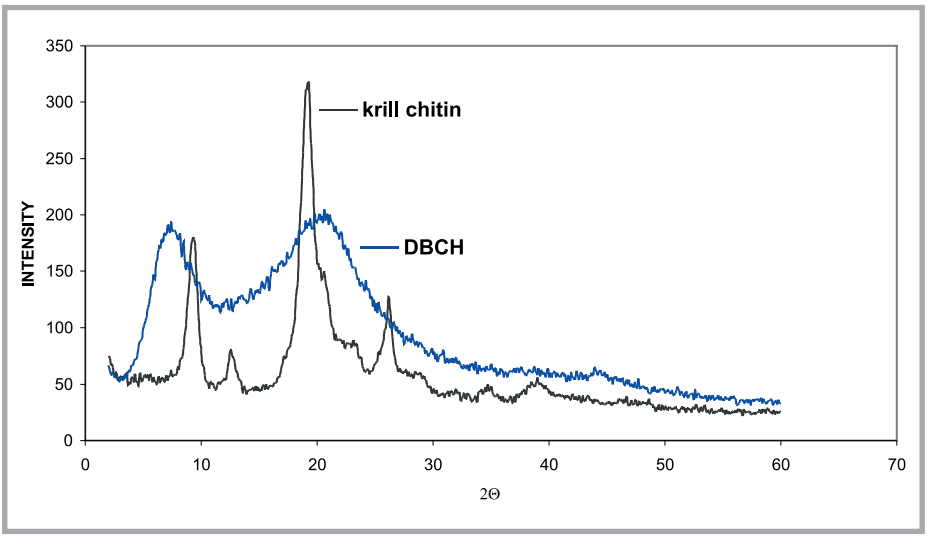

Figure 1. *WAXS diffraction patterns for krill chitin and DBCH fibres.*

Table 1. *Results of WAXS examination for DBCH, krill chitin, and products of DBCH alkaline hydrolysis in 5 % KOH at 20 °C.*

Sample	Treatment time	Degree of crystallinity	Crystallite dimensions				Interplanar distance			
			$D_{(010)}$	$D_{(220)}$	$D_{(100)}$	$D_{(110)}$	$d_{(010)}$	$d_{(220)}$	$d_{(100)}$	$d_{(110)}$
	min	%	nm	nm	nm	nm	nm	nm	nm	nm
DBCH	-	32	2.0	-	0.91	-	1.2	-	0.43	-
A1	10	37	2.2	-	1.4	-	1.2	-	0.43	-
A2	20	37	2.1	-	1.4	-	1.17	-	0.43	-
A3	30	44	1.9	3.2	2.7	-	1.08	0.66	0.46	-
A4	60	47	4.1	5.9	4.7	5.2	0.98	0.69	0.46	0.34
A5	120	62	4.2	7.5	6.8	4.2	0.99	0.70	0.46	0.34
A6	240	72	4.7	5.8	5.2	4.8	0.98	0.69	0.46	0.34
A7	480	74	4.5	4.9	4.6	4.0	0.98	0.69	0.46	0.34
A8	960	77	4.7	4.3	4.6	4.9	0.99	0.69	0.46	0.34
krill chitin	-	78	6.9	7.8	5.2	6.3	0.96	0.69	0.46	0.34

ing regained and thus the configuration of the polymer macromolecules becomes similar to the crystalline network of the krill chitin (Table 1) [1]. Random, gradual debutyrylation and formation of hydrogen bonds probably stabilizes the crystalline network defects and brings about the broadening of the crystalline reflexes as seen in the diffractograms of the regenerated chitin (Figure 2). The krill chitin, dibutyrylchitin and products of its deestrification were also examined by means of the different techniques of the FTIR method, i.e. the

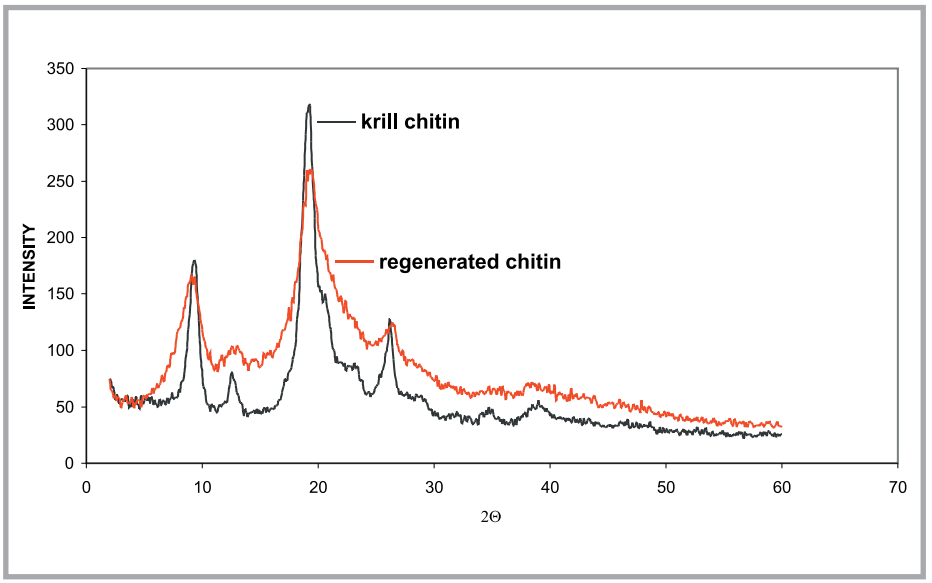

Figure 2. *WAXS diffraction patterns for krill chitin and regenerated chitin obtained using KOH.*

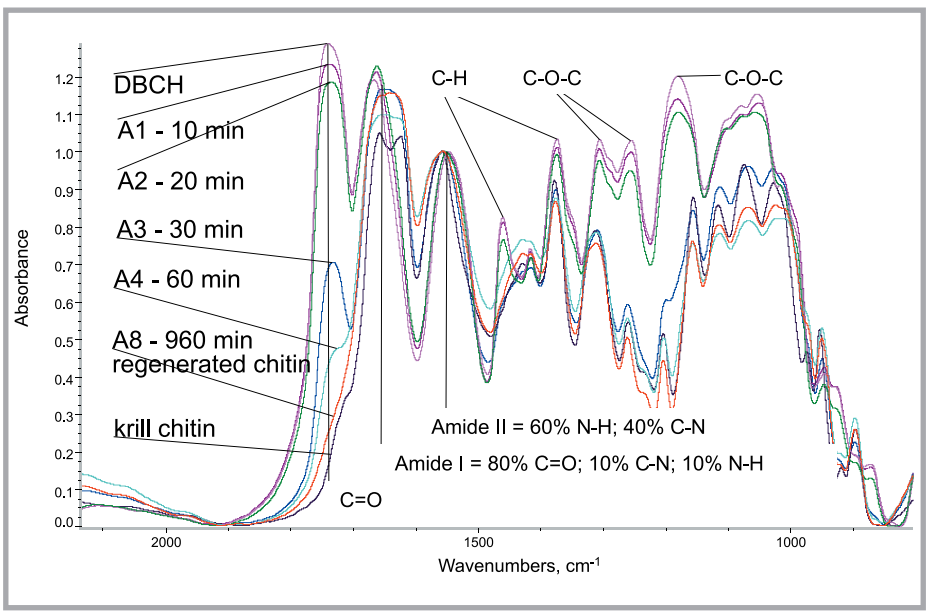

Figure 3. *FTIR spectra ranging from 2150 to 850 cm^{-1} for DBCH fibres, krill chitin and products of alkaline hydrolysis of DBCH at 20 °C; A1 – 10 min, A2 – 20 min, A3 – 30 min, A4 – 60 min, A8 - 960 min.*

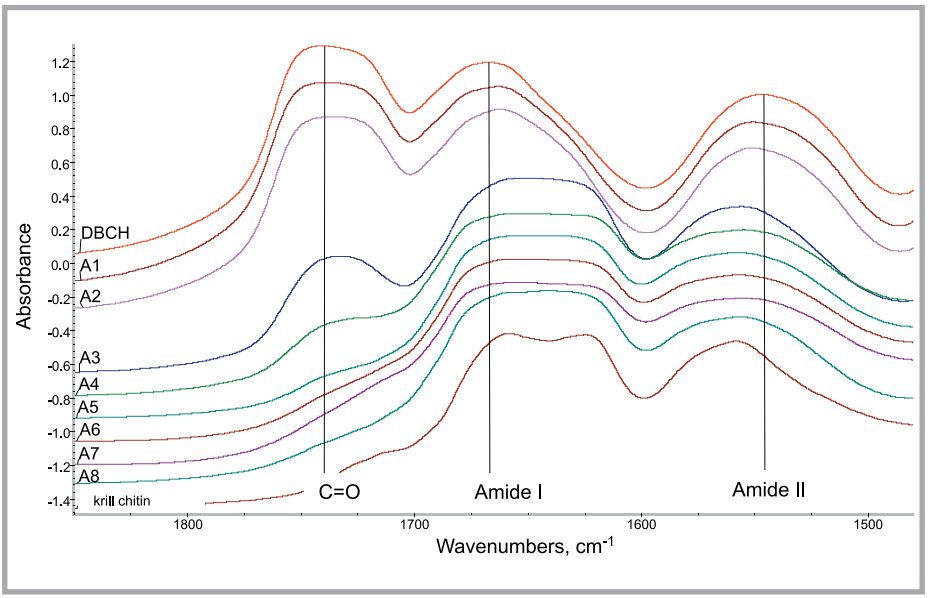

Figure 4. *FTIR spectra ranging from 1850 - 1480 cm^{-1} for DBCH fibres, krill chitin and products of alkaline hydrolysis of DBCH at 20 °C (A1 – 10 min, A2 – 20 min, A3 – 30 min, A4 – 60 min, A5 – 120 min, A6 – 240 min, A7 – 480 min, A8 - 960 min).*

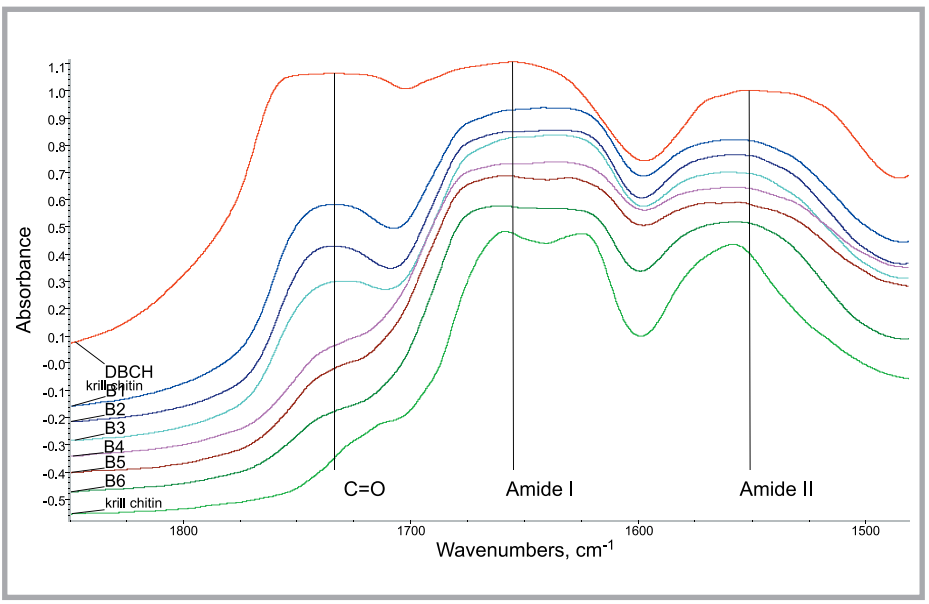

Figure 5. *FTIR spectra ranging from 1850-14800 cm^{+1} for DBCH fibres, krill chitin and products of alkaline hydrolysis of DBCH at 50 °C (B1 – 10 min, B2 – 20 min, B3 – 30 min, B4 – 60 min, 120 min, B6 – 180 min).*

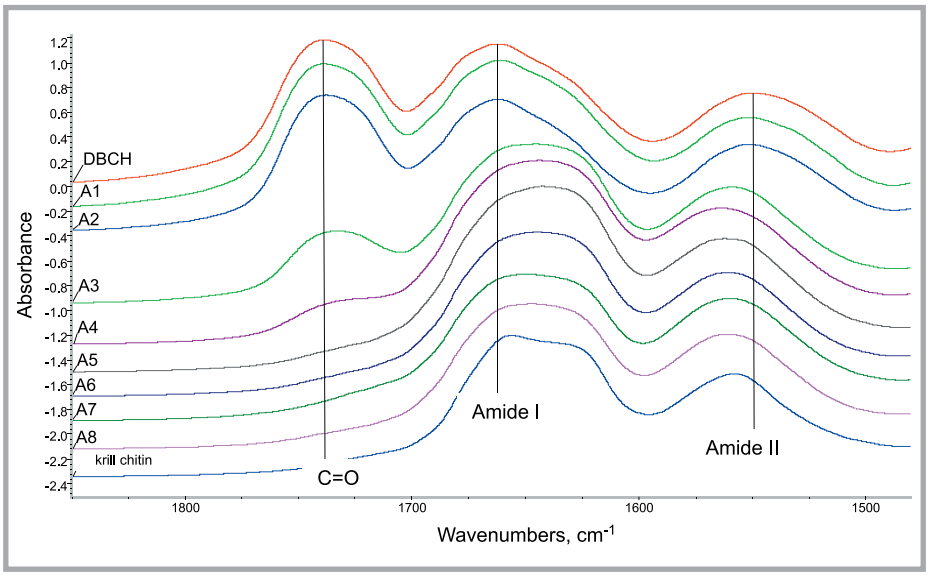

Figure 6. *FTIR spectra ranging from 1850 cm^{-1} to 1480 cm^{-1}for samples pastilles with NaCl including the samples of krill chitin and DBCH fibres - untreated and after alkaline treatment in 5 % KOH solution at 20 °C. DBCH - untreated fibres; A-1 after 10 min of treatment, A-2 - 20 min., A-3 - 30 min., A-4 - 60 min., A-5 - 120 min., A-6 - 240 min., A-7 - 480 min., A-8 - 960 min.*

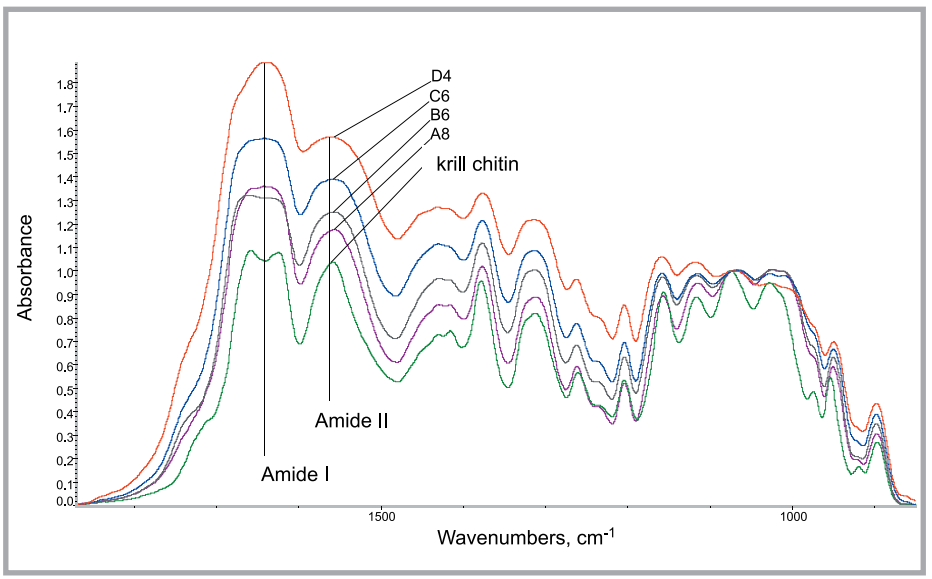

Figure 7. *FTIR spectra ranging from 1850 cm^{-1} to 850 cm^{-1} for pressed samples including krill chitin and DBCH fibres treated with 5 % KOH. A-8 - after 960 min. at 20 °C, B-6 - after 180 min. at 50 °C, C-6 - after 180 min. at 70 °C, D-4 after 60 min. at 90 °C. Spectra normalized in relation to C-O-C band at 1075 cm^{-1} .*

pelleting of fibres with NaCl under pressure, powdering with NaCl and using ATR . The obtained spectra revealed differences in the band shape of Amide I group. In Figures 3 - 5 FTIR spectra from the middle range of IR (2150 - 850 cm^{-1}) for the deesterified DBCH fibres (in 5 % KOH aq. solution at 20 °C and 50 °C) pelletted with NaCl have been shown. In all the experiments carried out (at 20 °C - series A, at 50 °C- series B, at 70 °C - series C and at 90 °C - series D) changes of carbonyl C=O band from Amide I (Figure 7). In the course of the alkaline treatment, the amount of the butyric substituents decreases, and thus the distances between macromolecules become smaller. Due to this phenomenon the C=O oscillator of an amide group enters the region of the hydrogen bond interactions. The maximum of Amide I absorption band then shifts towards lower energies, e.g. to 1662 cm^{-1} for A2 sample (Figure 6) in which there still are numerous butyric substituents. Next, the gradual statistic formation of hydrogen bonds between C=O and O-H oscillators of the adjacent macromolecules takes place. As a result, the defects of the crystalline network in the fibre material become stabilized, which affects the supermolecular structure and tenacity of the fibres.

The diversification of the intermolecular interactions brings about broadening of Amide I band from 1662 cm^{-1} to about 1624 cm^{-1} . Powdering of chitin with NaCl leads to the liberation of C=O oscillators from the hydrogen bonds and to the increase of the band intensity at 1662 cm^{-1} (Figure 6).

The ATR examination results in obtaining the information coming from the polymer layer with the thickness close to the length of the absorbed wave. Also FTIR spectra prepared

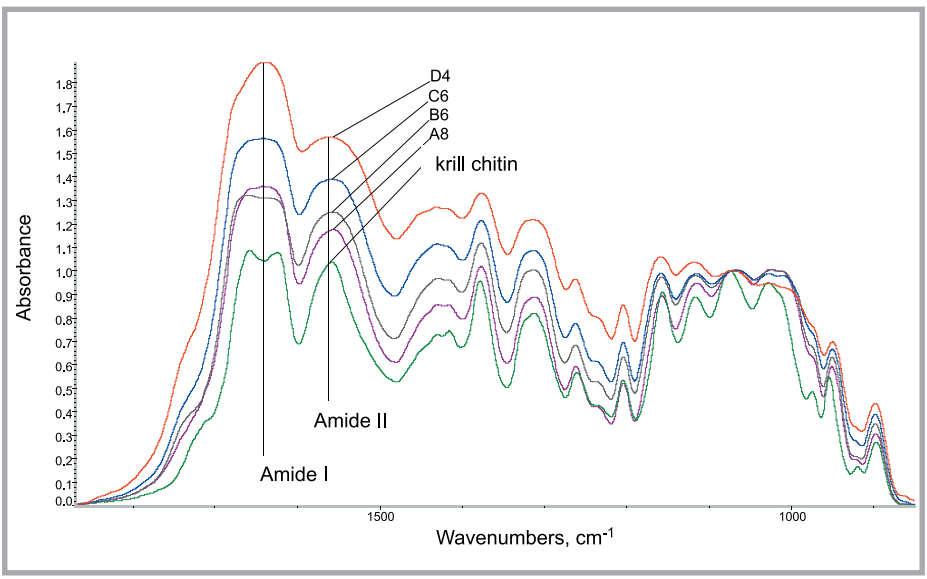

Figure 8. *FTIR-ATR spectra ranging from 3700 cm^{-1} to 500 cm^{-1} including krill chitin and DBCH fibres. DBCH - untreated fibres, A-1 - after 10 min. of treatment in 5 % KOH at 20 °C . A-2 - after 20 min., A-3 - 30 min, A-4 - 60 min., A-5 - 120 min., A-6 - 240 min., A-7 - 480 min., A-8 - 960 min.*

using ATR for both the krill and regenerated chitin show a symmetric band resolution of C=O oscillator from Amide I group (Figure 8).

In Figure 9 FTNIR spectra from the range 9000 - 6000 cm^{-1} have been presented. They mainly come from II and III overtones of the basic oscillations. The FTNIR technique can only be applied for the samples of respectively large masses (ca 0.1 g). As in the spectra no highly splited up bands can be found, the explicit interpretation of the chemical changes occurring during the process is difficult. However, a distinct resolution of the carbonyl band for the krill chitin can be observed.

Raman spectroscopy is another instrumental technique applied in this paper. In this method the radiation scattered on the surface of the examined material excited to the emmision by a laser beam is analyzed. Raman emmision is probably generated in a deeper layer of the material in comparison with the radiation examined by means of the ATR technique. In the Raman technique, however, more intensive bands can be derived from symmetric oscillators. Amide I band is created by a strongly polarized C=O oscillator. As the band free of hydrogen interactions, it can be considered as a polar oscillator (C^{+}=O^{-}), whereas in a hydrogen bond - as an oscillator of a higher symmetry (>C=O H-O-). Such an interpretation results from the fact that Raman emmision is conditioned by making the oscillators vibrate. Very often vibrations cause a significant rise of a sample temperature, due to which the sample may even get burnt. A high temperature of the material may also lead to the breaking of hydrogen bonds and the shift of the maximum of Amide I emmision towards higher energy values, characteristic for a free C=O group (Figure 10).

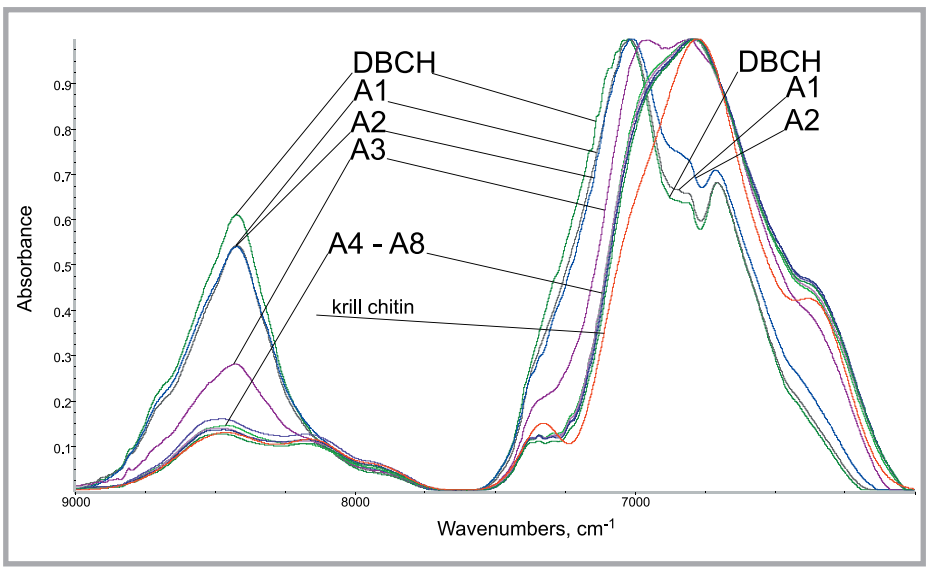

Figure 9. *FT NIR spectra ranging from 9000 cm^{-1} to 6000 cm^{-1} including krill chitin and DBCH fibres treated with 5 % KOH at 20 °C. DBCH - untreated fibres, A-1 - after 10 min of treatment, A-2 - 20 min., A-3 - 30 min., A-4 - 60 min., A-5 - 120 min., A-6 - 240 min., A-7 - 480 min., A-8 - 960 min.*

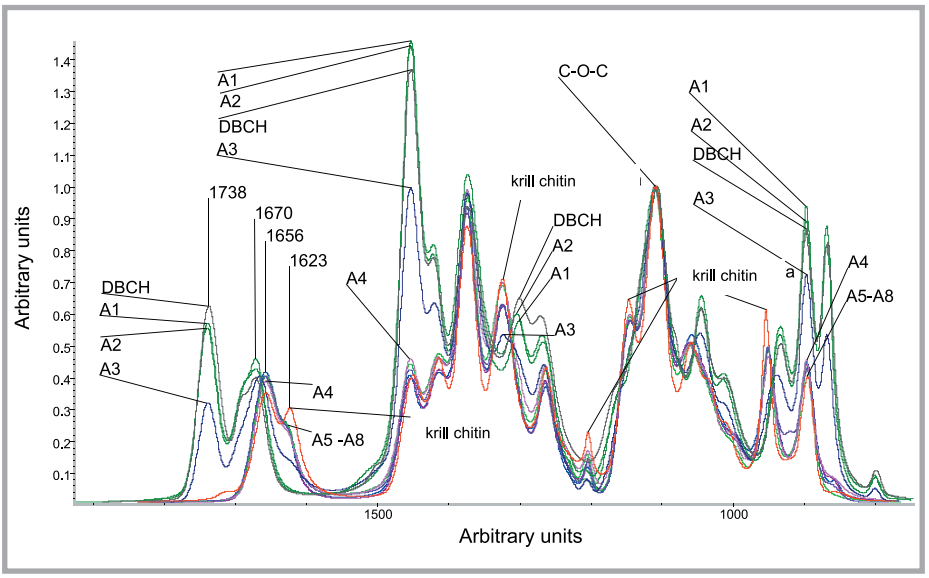

Figure 10. *FT Raman spectra of DBCH fibres, krill chitin and of alkaline hydrolysis products of DBCH at 20 °C (samples: A1 – 10 min, A2 – 20 min, A3 – 30 min, A4 – 60 min, A5 – 120 min, A6 – 240 min, A7 – 480 min, A8 - 960 min).*

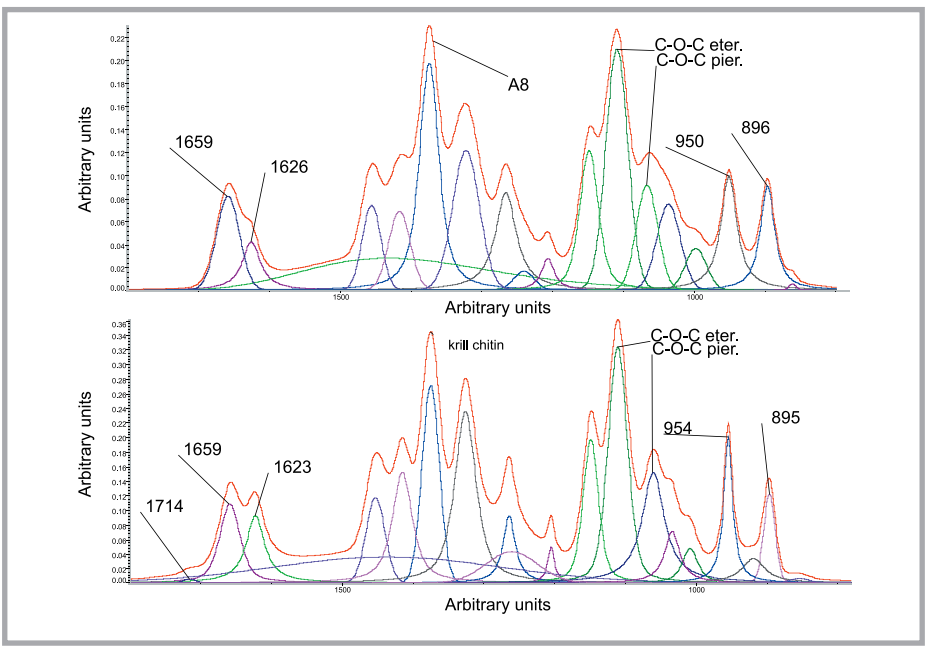

Figure 11. *Raman spectra shift distribution ranging from 1800 – 780 cm^{-1} for krill chitin and fibres from regenerated chitin (GRAMS software).*

Figure 11 presents the distributions of Raman spectra prepared by means of GRAMS software for the krill and regenerated chitin.

As it is known, chitin is a polymer of highly ordered structure. It can take the form of three polymorphic modifications called α–, β- and γ-chitin, respectively. These modifications are characterized by a different orientation of their particular molecular chains in the crystalline regions. The investigation of the supermolecular structure of chitin by means of X-ray diffraction was first carried out by Gonell [2]. Hermans [3] elaborated a model of the chitin structure similar to that of the other polysaccharides (cellulose), while Carlstrom proposed a conformation model of a chitin molecular chain showed in Figure 12 [4]. Figure 13 shows two projections of α-chitin structure presented by Blackwell and Minke [5, 8, 10]. Atom configuration in a molecular chain of β-chitin was demonstrated by Dweltz [6, 7, 9] (Figure 14).

The effect of a symmetric resolution of C=O band observed in the krill chitin spectra obtained by means of different techniques results from its crystallographic network and chitin conformation. It can be suggested that intermolecular hydrogen bonds are formed in an every second amide group with OH group from sixth carbon atom of an adjacent macromolecule. This leads to the conclusion that Amide I band is symmetrically splited

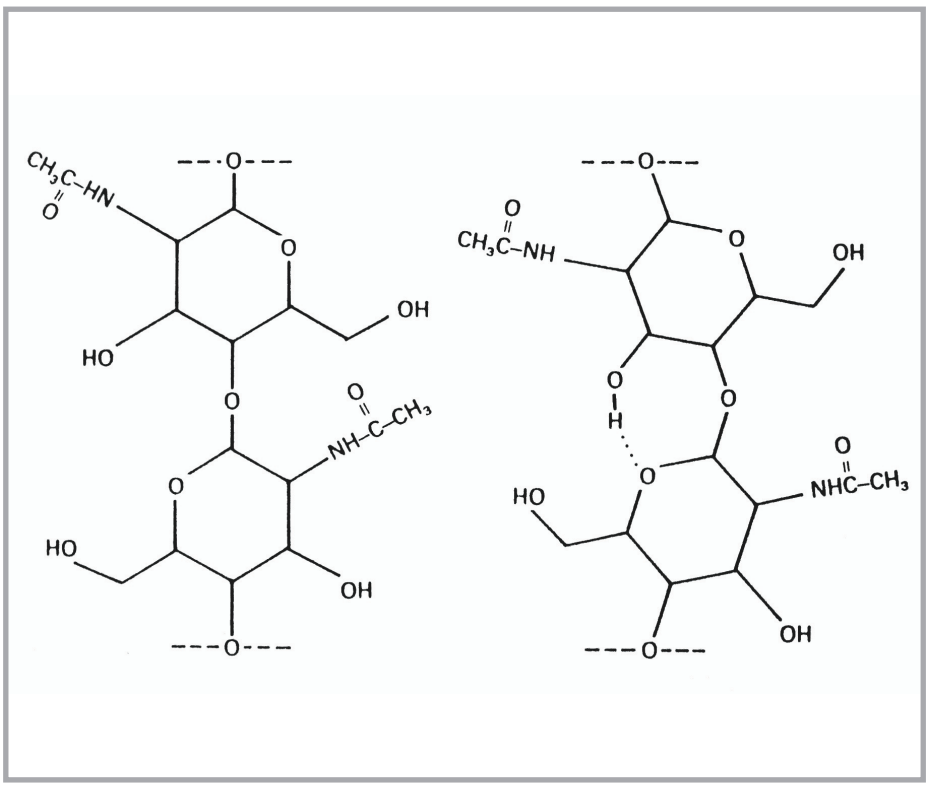

Figure 12. *Chain conformation for chitin according to Carlstrom [4].*

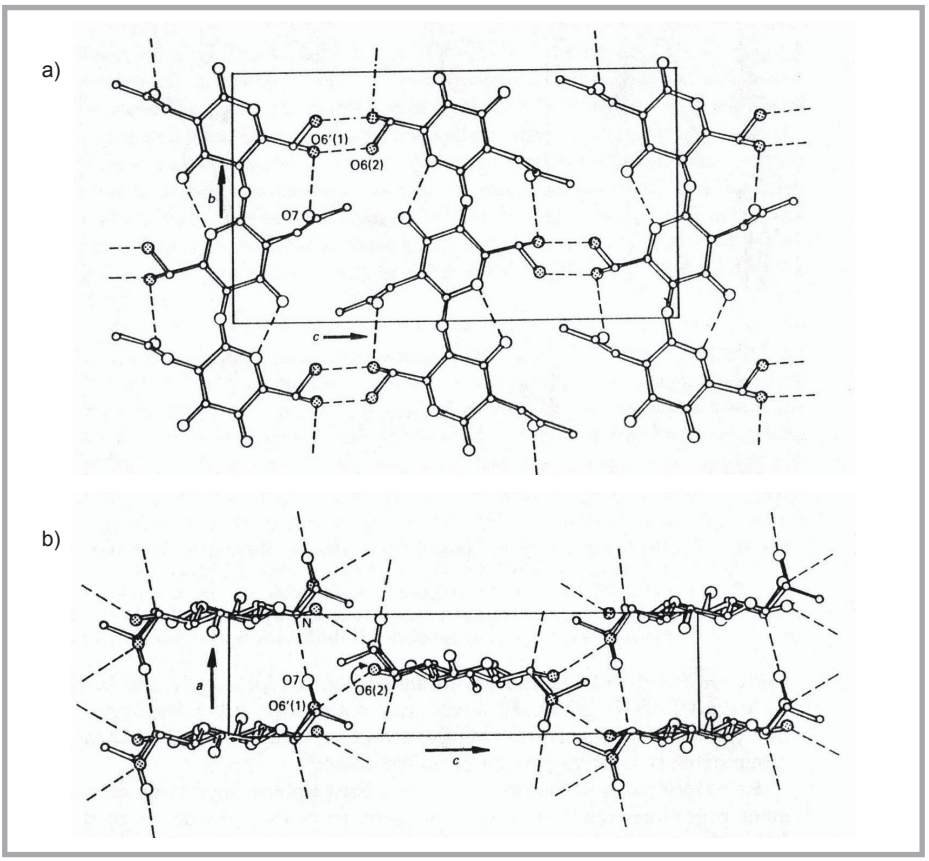

Figure 13. *Structure of α-chitin proposed by Blackwell et al. [5, 8, 10]; a) projection of C(3$_1$') O-H··· O(5$_1$) bonds combined with C(6$_1$')O-H··· O(6$_1$'), b) projection of C(2$_1$)N-H··· O=C (7$_3$) bonds combined with C(6$_2$)O-H··· O(6$_1$'.*

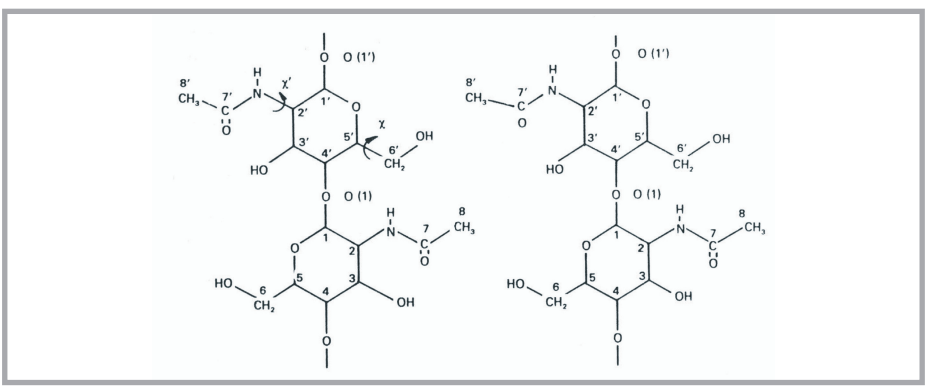

Figure 14. *Atom configuraton in β-chitin elaborated by Dweltz et al [6, 7, 9].*

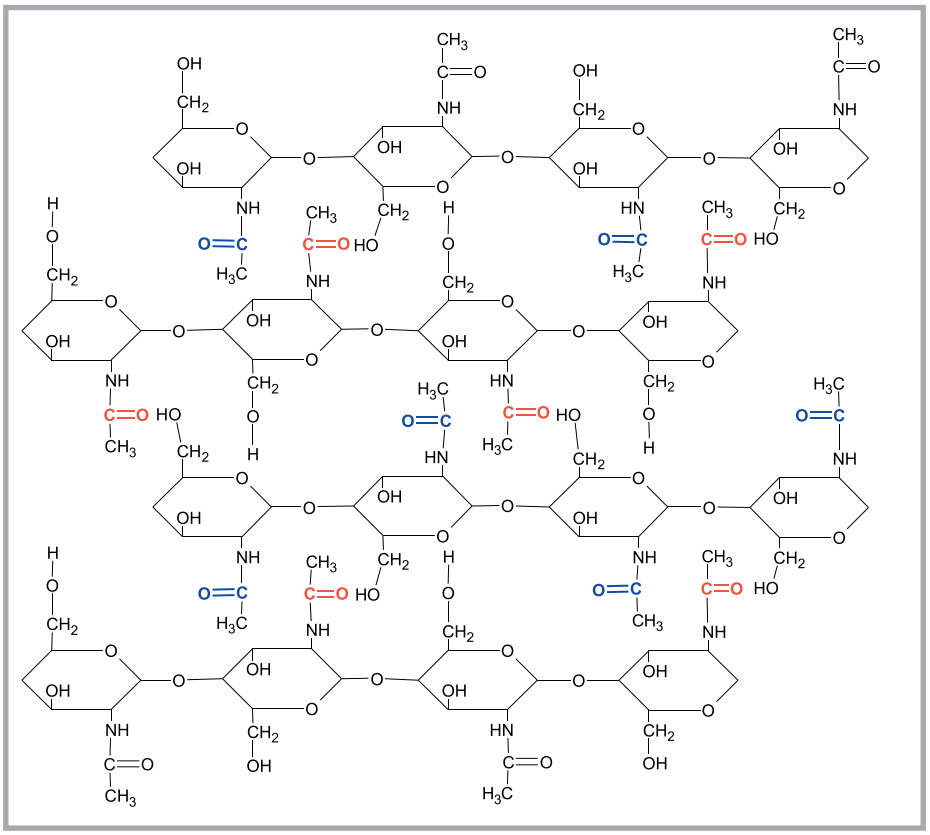

Figure 15. *Scheme of krill chitin supermolecular structure considering symmetric resolution of carbonyl band from amide group. Half of them takes part in hydrogen bonds with OH group of contiguous macromolecule, the remaining are free.*

up into two C=O oscillations, either from any group bound with OH group of the adjacent macromolecule or from free oscillations. It has been illustrated by the proposed scheme of the supermolecular structure of the krill chitin (Figure 15, 16.a - 16.c).

The modelled structure (Figure 16.a - 16.c) was generated first in a form of a four pyranose-ring oligomer. The obtained molecule was then multiplied twice to give an initial structure. Intra- and inter-molecular hydrogen bonds were added according to information derived from IR spectra and literature. Such a conformation was then preliminary optimized using the molecular mechanics MM3 force-field.

In order to find a final minimum semi-empirical calculations (a PM5 level of parametrization with a MOZYME algorithm for large molecules [12]) were done. All calculations have been performed with the modelling package CAChe Work System Pro v. 6.1.12.33 for Windows [13].

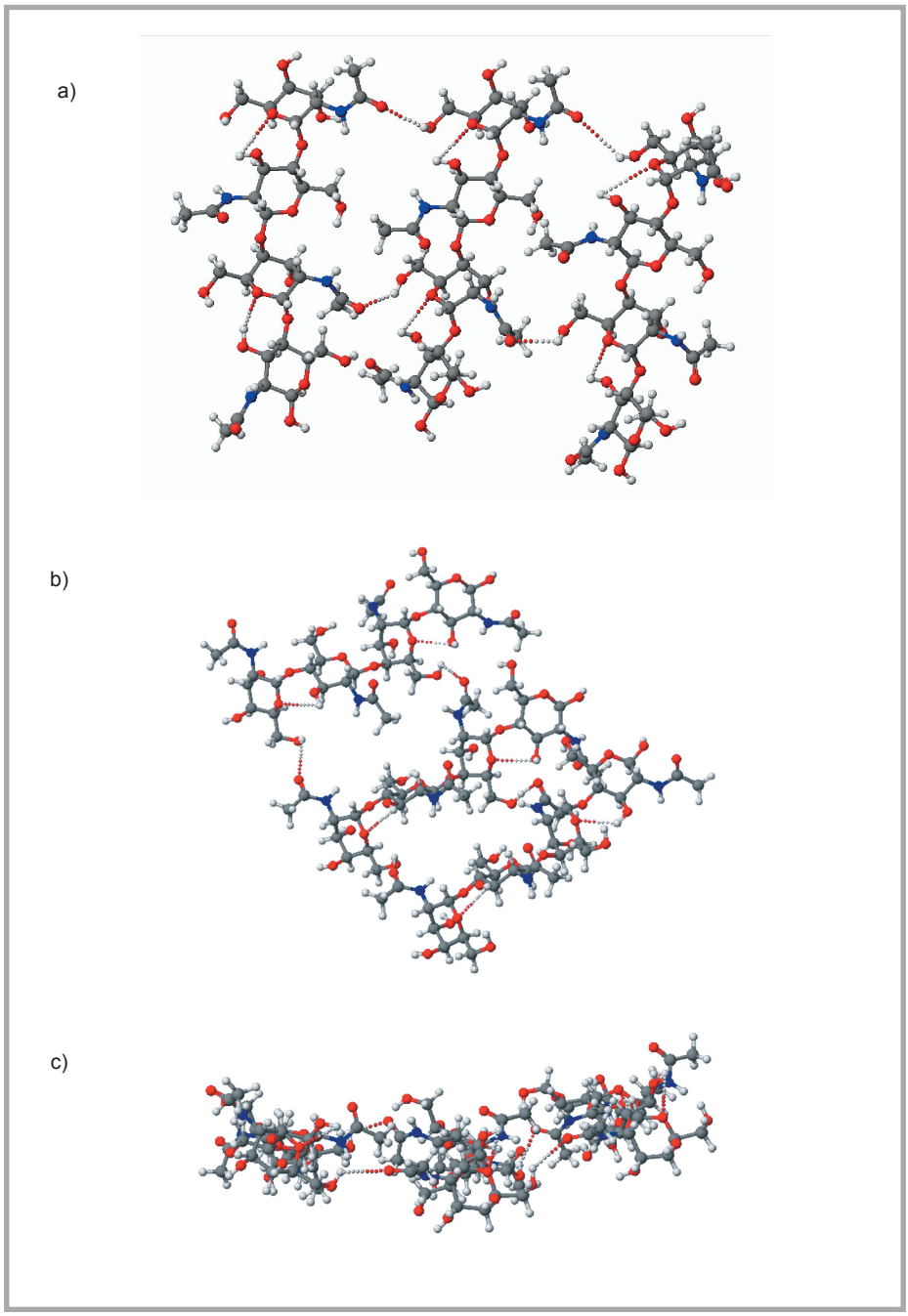

Figure 16 (a,b,c). *The modelled structure was generated first in a form of a four pyranose-ring oligomer.*

The calculated heat of formation of the studied oligomer was –4160.24 kcal/mol. Intermolecular hydrogen bonds between carbonyl oxygen atoms of acetamide groups and C6 CH_2-OH hydrogen atoms have energy range 4.40-5.27 kcal/mol. Their distance are between 2.50 and 3.12 Å. The energy of intramolecular hydrogen bonds is very small and they could be neglected. Carbonyl oxygen atoms that are not involved in hydrogen bonds are directed out of the oligomer plane.

4. Conclusions

1. Spectroscopic examinations carried out using different techniques revealed the characteristic changes of Amide I band shape that occurred during the transformation process: krill chitin - dibutyrylchitin - regenerated chitin.
2. It was found out that Amide I band resolution results from the influence of hydrogen bonds on C=O group oscillation frequency. This assumption was the basis for elaborating a new model of the molecular structure of chitin. In the model hydrogen bond is formed by an every second C=O group.

5. References

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