COMPARATIVE STUDY OF CELLULOSE AND ITS NITROGENATED DERIVATIVES

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Abstract

In this paper structural characteristics and properties of cellulose and its nitrogenated derivatives – chitin and chitosan, have been studied. The obtained results showed that substitution of hydroxyl group in C2 of anhydroglucose unit of cellulose on nitrogen-containing groups leads to transformation of crystalline unit cell, increasing of interplanar distances and cell volume, and reduction of packing density of crystallites. Since cellulose contains lesser amounts of atoms C and H, the combustion of this polysaccharide requires lower oxygen amount than of nitrogenated derivatives; as a result, the exothermic value of combustion enthalpy increases when going from cellulose to chitosan and chitin. Study of hydrophilic properties revealed that wetting enthalpy and sorption of water vapor depend on the degree of amorphicity (Y) of the biopolymers. At the same Y-value the hydrophilic properties of cellulose and chitosan containing hydroxyl or amino groups were higher than of chitin containing hydrophobic acetyl groups. In the sequence: cellulose-chitin-chitosan, an increase in hydrolyzability of the biopolymer samples was observed. Furthermore, three methods for determination of degree of crystallinity and amorphicity such as X-ray, calorimetry and sorption, were discussed.

Keywords: Cellulose, Chitosan, Chitin, Structure, Crystallinity, Amorphicity, Thermodynamic properties, Hydrophility, Hydrolyzability

1. Introduction

Cellulose and its nitrogenated derivatives - chitin and chitosan, are the most abundant organic substances on Earth [1-3]. Cellulose is present in plant cell wall in a form of nanofibrils [3, 4], while nanofibrillated chitin is found in tissues of some marine animals, insects and fungi [5, 6]. Chitin performs a skeleton function in lower eukaryotes similar to that of cellulose in plants. Chitosan with various degree of deacetylation, usually from 70 to about 100%, is extracted from chitin by means of alkaline deacetylation process [6, 7]. Cellulose and its nitrogenated derivatives are linear semicrystalline biopolymers. Macromolecules of cellulose consist of repeat anhydroglucose units (AGU), whereas macromolecules of nitrogenated derivatives are built from partially substituted AGU and linked with each other by1,4-beta-glucosidic bonds. In chitin the hydroxyl group at C2 in AGU is substituted on acetylamino group, while in chitosan on amino group.

As is known, natural plant cellulose has a monoclinic crystalline unit cell called CI_β [4]. Chitin in the nature can be in three allomorph crystalline forms: alpha, beta and gamma [8-10]. The most abundant orthorhombic alpha-form is present in chitin samples isolated from sea animals (crabs, shrimps, lobsters, krill, etc.), insects, fungi and some microorganisms. The monoclinic beta-allomorph is found in the chitin of squid pens, tube-worms and some others sources. Rare gamma form of chitin with poorly studied crystalline structure can be isolated from some yeast [11]. The beta and gamma forms are instable and after some treatments these forms of chitin are transformed irreversibly into more stable alpha allomorph of chitin. Chitosan of alpha type extracted from orthorhombic alpha-chitin has also an orthorhombic crystalline form, but other parameters of crystalline unit cell [8].

Numerous scientific papers have been devoted to description of structure, properties and applications of individual types of biopolymers, such as cellulose, chitin and chitosan. Unfortunately, these papers do not clarify how the substitution of hydroxyl groups at C2 of AGU

affects the structure and properties of biopolymers. The study of this issue was a main purpose of the current paper.

2. Experimental

2.1. Materials

Various cellulose samples were used: cotton cellulose (CC) of Hercules, kraft pulp (KP) of Weyerhaeuser and microcrystalline cellulose (MCC) Avicel PH-101 of FMC BioPolymer were used. Purified powders of alpha-chitin of crab shells (degree of acetylation DA= 94%) and alpha- chitosan (DA=15%) were acquired from Sigma-Aldrich. Beta-chitin (DA = 91%) was isolated from squid pens using the procedure [6].

2.2. Methods

The investigations were carried out by methods of X-ray diffraction [12, 13], calorimetry [14, 15] and sorption [16]. Interplanar distances (d) in crystallites were calculated from X-ray diffraction peaks using Bragg's equation:

$$d = 0.5\ \lambda/\sin\theta \tag{1}$$

Degree of crystallinity (X) and degree of amorphicity (Y) of the samples were calculated, as follows [12, 13]:

$$X = \int I_{cr}\,d\theta / \int I_o\,d\theta \tag{2}$$

$$Y = 1 - X \tag{3}$$

where I_o is total intensity of the corrected X-ray diffractogram after subtraction of incoherent background; I_{cr} is intensity of the crystalline scattering.

Degree of acetylation (DA) of nitrogenated polysaccharides was calculated from results of elemental analysis [6]:

$$DA,\ \% = (58.34\ C/N) - 300 \tag{4}$$

where C and N is percentage of carbon and nitrogen in the analyzed sample of chitin or chitosan.

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Van der Waals volume (w) of hydroxyl, amino and acetylamino groups was determined by method of additive increments [17]. Specific gravity of crystallites (ρ_c) was calculated from parameters of crystalline unit cells of cellulose and nitrogenated derivatives.

For monoclinic unit cell: $\rho c = nM/(N\ a\ b\ c\ \sin\gamma)$ (5)

For orthorhombic unit cell: $\rho c = nM/(N\ a\ b\ c)$ (6)

where n is number of links in crystalline unit cell; M is molecular weight of one link; N is Avogadro number.

The inverse value, ρ_c^{-1} is a specific volume of crystallites, V_c.

Hydrolyzability was studied by gravimetric method after treatment of the samples with boiling 3M HCl for 1 h followed by washing to neutral pH and drying.

3. Results and discussion

X-ray diffractogram of cellulose Iβ (e.g. MCC) shows three characteristic peaks at 2θ angle of 14.6-14.8, 16.3-16.5 and 22.5-22.7^{o} (Fig.1). Replacement of one secondary hydroxyl group at C2 on amino group in α-chitosan and acetylamino group in α- or β-chitin leads to a shift of the diffraction peaks to smaller angles (Fig. 1, 2). Thus, as a result of substitution the increase of interplanar distances typical for crystalline lattice of CIβ is observed (Table 1).

Table 1. Typical interplanar distances (d, nm) in crystalline cell of the biopolymers

Cellulose Iβ	α-Chitosan	α-Chitin	β-Chitin
0.392-0.394	0.42-0.43	0.46-0.47	0.43-0.45
0.54-0.55	0.80-0.81	0.70-0.71	0.68-0.69
0.60-0.61	0.84-0.85	0.94-0.95	0.91-0.92

Based on parameters of crystalline unit cells of the biopolymers [4, 8-10, 18-20], values of specific volume (Vc) and specific gravity of crystallites (ρc) were calculated.

Table 2. Structural characteristics of crystallites of various biopolymers

Characteristics	Cellulose Iβ	α-Chitosan	α-Chitin	β-Chitin
a, nm	0.79	0.81	0.47	0.48
b, nm	0.82	0.84	1.89	0.93
c, nm	1.034	1.034	1.032	1.038
γ^{o}	97	90	90	97.5

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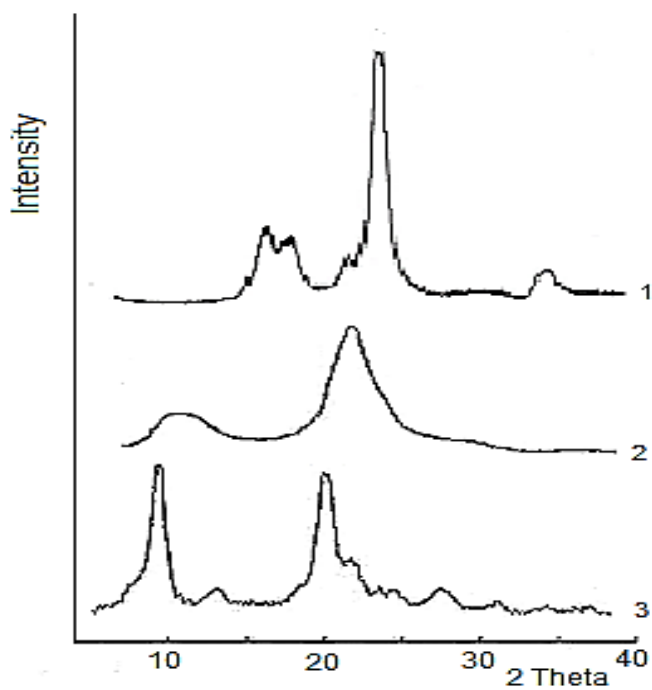| Characteristics	Cellulose Iβ	α-Chitosan	α-Chitin	β-Chitin
V_c, cm³/g	0.617	0.658	0.680	0.680
ρ_c, g/cm³	1.62	1.52	1.47	1.47

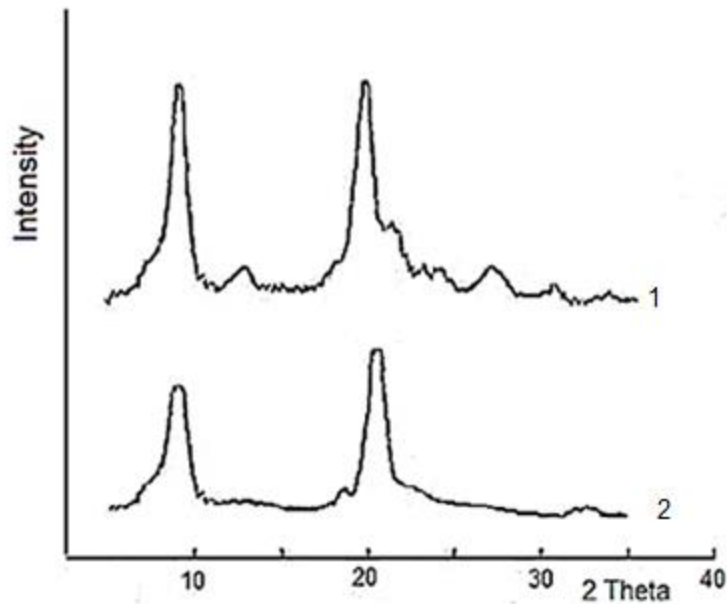**Figure 1**. X-ray diffractograms of samples of cellulose Iβ (1), α-chitosan (2) and α-chitin (3)

Figure 2. X-ray diffractograms of α-chitin (1) and β-chitin (2) samples

The calculations showed (Table 2) that in the sequence: cellulose - chitosan - chitin, an increase of volume and reduction in packing density of crystallites is observed due to the substitution of very small hydroxyl group (w = 0.010 nm^3) in cellulose on amino group (w = 0.012 nm^3) in chitosan and large acetylamino group (w = 0.052 nm^3) in chitin, which is accompanied by distortion of the system of hydrogen bonds. This result is confirmed by the increase of interplanar distances in crystallites of nitrogenated polysaccharides compared to crystallites of CIβ (Table 1).

Introduction of nitrogen-containing groups in AGU leads to appreciable change in crystalline structure of CIβ. As shown in Fig. 3, inclusion of amino group causes transformation of monoclinic crystalline unit cell of CIβ into orthorhombic unit cell of α-chitosan (α-CS) with decrease of γ angle from 97 to 90° and increase in **a** and **b** parameters.
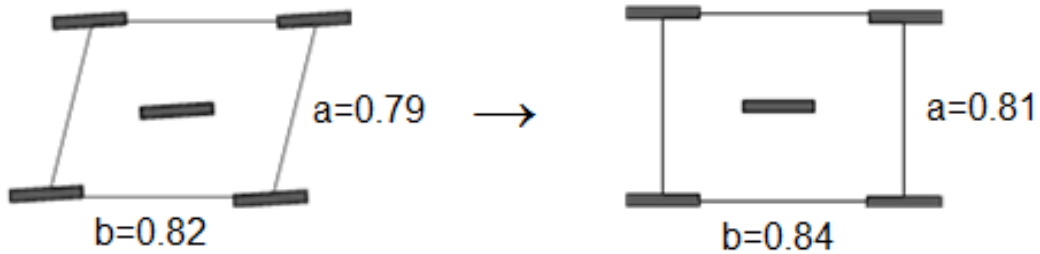
Figure 3. Transformation of monoclinic unit cell of CIβ into orthorhombic unit cell of α-CS

Introduction of large acetylamino group in AGU leads to transformation of monoclinic crystalline unit cell of CIβ into orthorhombic unit cell of α-chitin (α-CT) that is accompanied by decrease of γ angle from 97 to 90°, considerable contraction of **a** parameter and great expansion of **b** parameter (Fig 4).
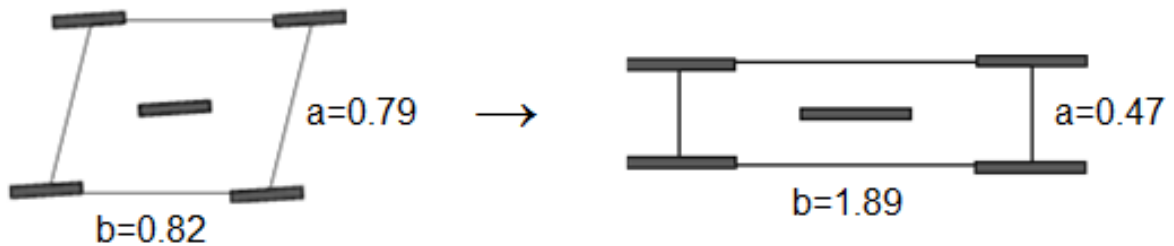

Figure 4. Transformation of monoclinic unit cell of CIβ into orthorhombic unit cell of α-CT

When the monoclinic crystalline unit cell of CIβ transforms into monoclinic unit cell of β-chitin (β-CT) a number of elementary links in the cell (n) reduces by half, from 4 to 2, which is accompanied by contraction of a parameter and increasing of parameter b (Fig. 5).
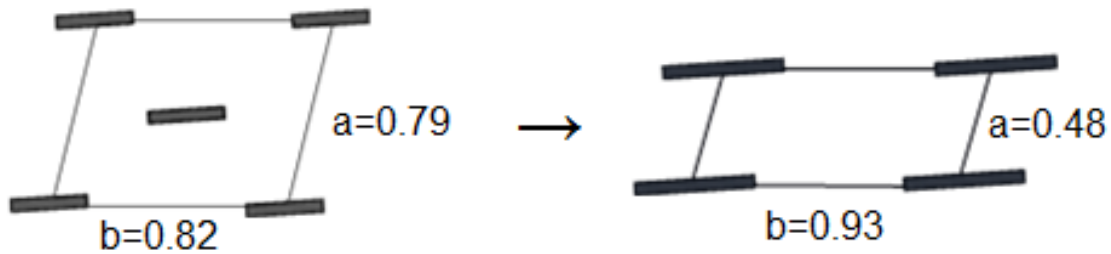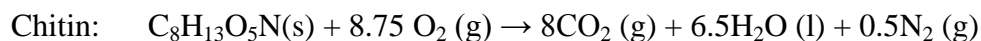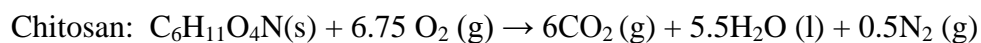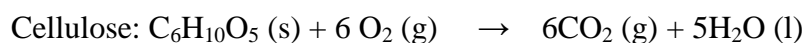
Figure 5. Transformation of monoclinic unit cell of CIβ into monoclinic unit cell of β-CT

Reducing the packing density of crystallites in the sequence: cellulose - chitosan - chitin (Table 2), causes the decrease of axial elastic modulus of crystallites, from 150 GPa for CIβ [4] to 65 GPa for chitosan and 41 GPa for chitin [21].

It was also found that introduction of nitrogen-containing groups in AGU of cellulose lowers the glass transition temperature from 493-500 K to 410-430 K [22, 23].

Study of thermodynamic properties of the biopolymer samples showed that the standard enthalpy of combustion ($\Delta_c H^o$) is little sensitive to change in degree of amorphicity (Y). Furthermore, exothermic value of $\Delta_c H^o$ increases when going from cellulose to chitosan and chitin (Table 3). This is due to the fact that cellulose contains lesser amounts of atoms C and H, and therefore the combustion of this polysaccharide requires lower oxygen amount than its nitrogenated derivatives.

The combustion process of one mole of the biopolymers can be written, as follows.

Cellulose: $C_6H_{10}O_5$ (s) + 6 O_2 (g) $\rightarrow$ 6CO_2 (g) + 5H_2O (l)

Chitosan: $C_6H_{11}O_4N$(s) + 6.75 O_2 (g) $\rightarrow$ 6CO_2 (g) + 5.5H_2O (l) + 0.5N_2 (g)

Chitin: $C_8H_{13}O_5N$(s) + 8.75 O_2 (g) $\rightarrow$ 8CO_2 (g) + 6.5H_2O (l) + 0.5N_2 (g)

As can be seen, the complete combustion of chitosan requires 1.12 times and of chitin 1.46 times more oxygen than the complete combustion of cellulose, which determines the higher exothermic value of the combustion enthalpy of nitrogenated polysaccharides.

Table 3. Standard enthalpy of combustion ($\Delta_c H^o$) of biopolymers having different degree of amorphicity (Y)

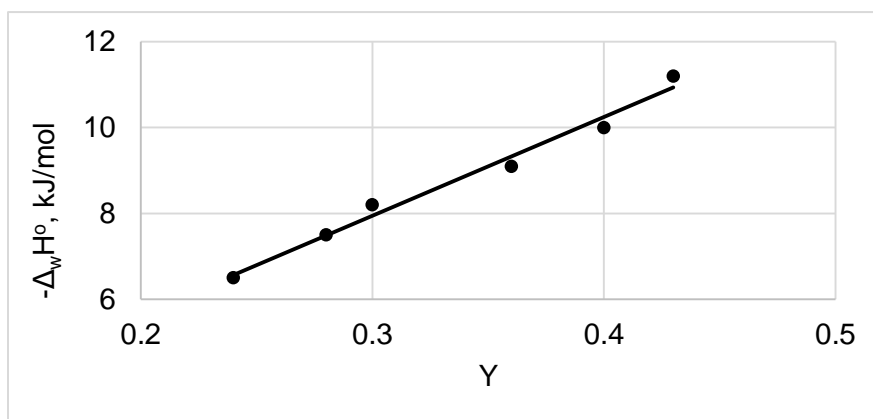| Sample	Y	$-\Delta_c H^o$ kJ/mol
MCC	0.24	2820
CC	0.30	2820
KP	0.36	2830
α-CS	0.43	3160
α-CT	0.28	3910
β-CT	0.40	4100

Figure 6. Dependence of wetting enthalpy on degree of amorphicity of biopolymer samples

The most pronounced physicochemical feature of cellulose and its nitrogenated derivatives is their hydrophility. Therefore, a great attention is paid to the interaction of hydrophilic biopolymers with water. The calorimetric investigations showed that the standard enthalpy of wetting ($\Delta_w H^o$) strongly depends on the degree of amorphicity of the hydrophilic samples (Fig. 6), because their interaction with water occurs in amorphous domains of the biopolymers [13, 15]. This allows calculate the degree of amorphicity by calorimetric method using results of determination of wetting enthalpy:

$$Y = \Delta_w H^o / \Delta_w H^o_a \qquad\qquad (7)$$

where $\Delta_w H^o_a \approx -27.2 \pm 0.2$ kJ/mol is an average standard wetting enthalpy of amorphous biopolymers.

Theoretical isotherms sorption of water vapor by completely amorphous cellulose, chitosan with DA=0% and chitin with DA=100% can be calculated using the method of additive functions of Van Krevelen [24]. As can be seen from Fig. 7, all isotherms have a sigmoidal shape.
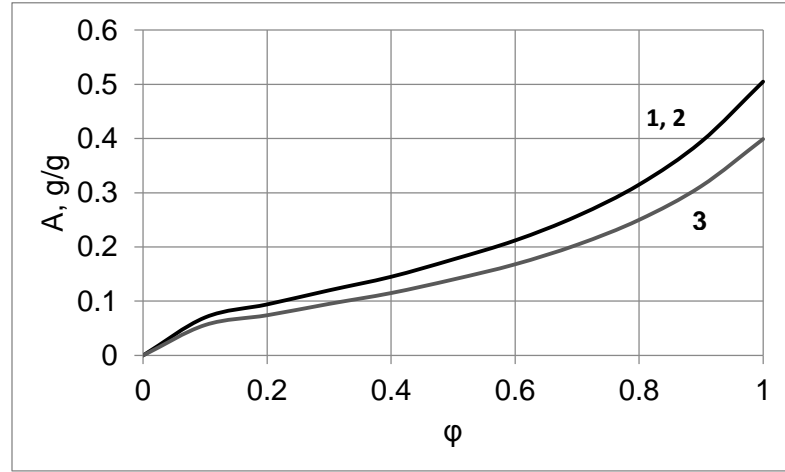

Figure 7. Theoretical isotherms sorption of water vapor by completely amorphous cellulose (1), chitosan (2) and chitin (3) at 298 K

Comparison of sorption isotherms for amorphous biopolymers showed that the sorption ability of cellulose and chitosan containing hydrophilic hydroxyl or amino groups was higher than of chitin containing also hydrophobic acetyl groups. Maximum equilibrium water sorption value at 298 K and relative vapor pressure φ =1 for amorphous cellulose and chitosan is $A_{m,a}$ = 0.5 (g H_2O per 1 g of dry sample), while for amorphous chitin it is less, $A_{m,a}$=0.4 (g/g) only. Besides, the maximum equilibrium water sorption value of amorphous nitrogenated polysaccharides is function of degree of acetylation (DA, %):

$$A_{m,a} \ (g/g) = 0.5 - 0.001 \ DA \qquad\qquad (8)$$

In the case when the biopolymer is not completely amorphous, along with DA also degree of amorphicity (Y) must be taken into account in order to calculate the isotherm for semicrystalline sample [16]:

$$A = A_{m,a}Y/(1 - K \ln\varphi) \qquad\qquad (9)$$

where coefficient K=2.7.

As can be seen from figures 8 and 9, experimental and calculated isotherms are very close, which proves the reliability of eq. (9).
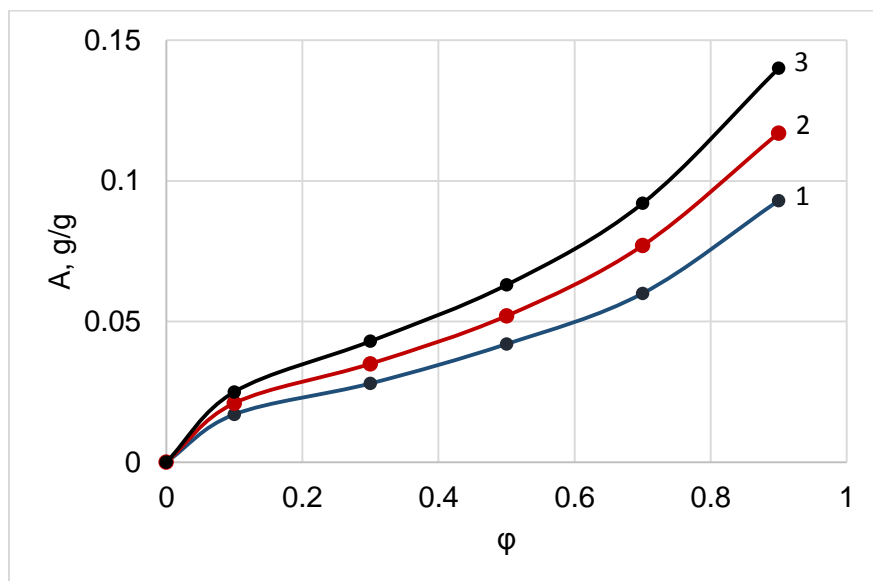

Figure 8. Theoretical (lines) and experimental (points) sorption of water vapor by samples of MCC (1), CC (2) and KP (3)
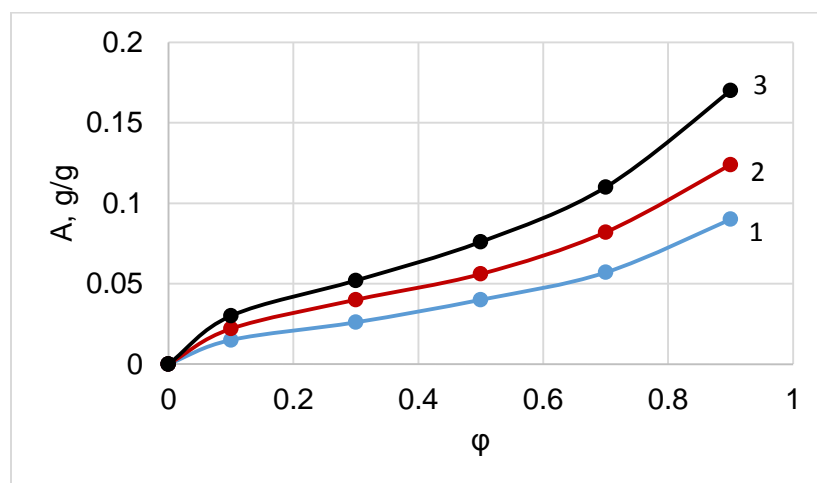

Figure 9. Theoretical (lines) and experimental (points) sorption of water vapor by samples of α-chitin (1), β-chitin (2) and α-chitosan (3)

Using the experimental sorption value, the degree of amorphicity (Y) or crystallinity (X) of the biopolymer sample with known DA can be estimated. For example if $\varphi=0.7$, the following equations can be used for calculations:

$$Y= k \, A_{0.7}/A_{m,a} \tag{10}$$

$$X= 1 - Y \tag{11}$$

where coefficient $k=1.963$; for cellulose samples $A_{m,a}=0.5$, whereas for its nitrogenated derivatives the value of $A_{m,a}$ can be obtained by means of eq. (8).

Three used methods, X-ray, calorimetry and sorption, gave the similar value of amorphicity degree (Table 4).

Table 4. Degree of amorphicity of the samples determined by various methods

Sample	X-ray	Calorimetry	Sorption
MCC	0.24	0.26	0.25
CC	0.30	0.29	0.31
KP	0.36	0.35	0.34
α-CS	0.43	0.42	0.44
α-CT	0.28	0.30	0.28
β-CT	0.40	0.38	0.41

Significant differences in the behavior of the biopolymers have been found in the study of acidic treatment (Fig.10).
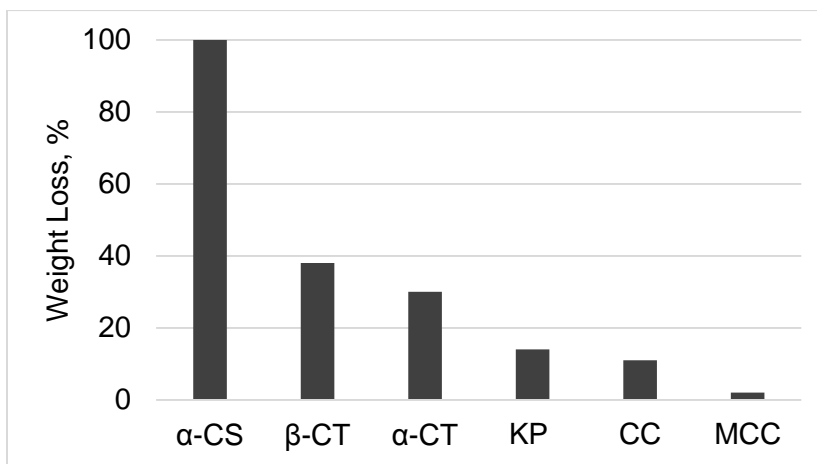

Figure 10. Weight loss of biopolymer samples after acidic treatment

After treatment of cellulose samples with dilute hydrochloric acid at boiling temperature for 1 h a relative low weight loss was observed (< 15%). Acidic treatment of chitins leads to higher loss in the samples weight, whereas treatment of chitosan causes complete hydrolysis to water-soluble monomers and oligomers that do not precipitates after neutralization of the acid. Thus, hydrolyzability of biopolymers increases in the sequence: cellulose-chitin-chitosan.

4. Conclusion

Comparative research of structure and properties of cellulose and its nitrogenated derivatives – chitin and chitosan, has been implemented. The obtained results showed, the substitution of hydroxyl group in C2 of AGU on nitrogen-containing groups leads to transformation of crystalline unit cell, increasing of interplanar distances and cell volume, as well as to reduction in packing density of crystallites. Reducing the packing density of crystallites in the sequence: cellulose - chitosan - chitin, causes the decrease of axial elastic modulus of crystallites. Moreover, the glass transition temperature of cellulose is higher than its nitrogenated derivatives.

Since cellulose contains lesser amounts of atoms C and H, the combustion of this polysaccharide requires lower oxygen amount than nitrogenated derivatives. As a result, the exothermic value of combustion enthalpy increases when going from cellulose to chitosan and chitin.

Study of hydrophilic properties revealed that wetting enthalpy and sorption of water vapor strongly depend on degree of amorphicity (Y) of the biopolymers. At the same Y-value the hydrophilic properties of cellulose and chitosan containing hydroxyl or amino groups were higher than of chitin containing also hydrophobic acetyl groups. In the sequence: cellulose-chitin-chitosan, the increase in hydrolyzability was observed.

Furthermore, three methods of determination degree of crystallinity and amorphicity such as X-ray, calorimetry and sorption, were compared in this paper.

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