THE INFLUENCE OF C18-FATTY ACIDS ON CHEMICAL STRUCTURE OF CHITOSAN DERIVATIVES AND THEIR THERMAL PROPERTIES

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Abstract

Chitosan derivatives with a series of fatty acids (FA) have been developed using simultaneous N- and O-acylation reaction by the combination of two ways of conducting the reaction, i.e. a carbodiimide catalysis and ionic amino group protection. The chemical structure of chitosan derivatives as well as the characterization of the FA substitution degree were done by the IR spectra analysis. The correlation between the substitution of the chitosan functional groups as well as the saturation of FA and the changes of structural and thermal properties of the derivatives has been presented.

Key words: *chitosan, fatty acids, N,O-acylation, chemical structure, thermal properties*

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1. Introduction

Chitosan, as a natural polysaccharide, is characterized by the strong inter- and intramolecular chain interactions due to the presence of three kind of functional groups i.e. hydroxyl, amide and amino groups, occurring in β(1→4)-2-amin- (GlcN) and β(1→4)-2-acetamid-2-deoxy-D-glucopyranose (GlcNAc) units. The final physico-chemical properties of chitosan are governed by three factors: deacetylation degree (ratio GlcN to GlcNAc), molecular mass and structure arrangement. However another way of changing chitosan properties is its chemical modification. For that purpose the -OH and $–NH_2$ groups might be used, and the selectivity of the substitution depends on the reaction conditions [1, 2].

One of the most interesting routes is chitosan modification with fatty acids resulting in amphiphilic products suitable for different biomedical applications [3, 4]. The derivatives properties strongly depend on the fatty acids substitution degree and on the type of chitosan functional group which undergoes the reaction.

The aim of our study was to synthesize and characterize the chitosan derivatives based on fatty acid series, containing C18 aliphatic chain with different number of double bonds, namely stearic, oleic, linoleic and α-linolenic acids. The particular goal was to define the type of substituted functional groups (-OH and/or $–NH_2$) and to determine the fatty acid substitution degree in order to provide the correlation between the derivatives chemical structure and the analyzed properties.

2. Materials and Methods

2.1. Materials

Chitosan (CH000) ChitoClear® 43000 – hqg10 from Primex ehf Iceland Company (deacetylation degree 96%); stearic (SA), oleic (OLA), linoleic (LA), and α-linolenic (ALA) acids from Sigma Aldrich; 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) from GenScript Corporation; and acetic acid (AA), methanol (MeOH), ethanol (EtOH), isopropanol (IP) and ammonium (NH_4OH) solvents from POCh Gliwice were used in this study.

2.2. Synthesis of C18-Fatty Acids Chitosan Derivatives

Chitosan-unsaturated fatty acids syntheses were carried out according to individual two stage procedure, as followed:

Firstly, fatty acids (OLA, LA, ALA) and EDC catalysis (1:1 mol/mol) were dissolved in methanol and stirred at a room temperature for 3 hours. Separately, CH000 (1 wt.%) was dissolved in 1% AA and MeOH mixture (2:1 v/v). The FA-EDC solution was slowly added dropwise to the chitosan solution and the reaction was conducted at a room temperature, for 24 hours at 4.5-5 pH range. The amount of fatty acid was 30 wt%. The product was precipitated with methanol/ammonia solution (7/3 v/v), centrifuged, washed with distilled water to neutralize pH and methanol in order to remove unreacted fatty acid, and finally dried for 48 hours under vacuum at 60°C.

For the synthesis of chitosan with stearic acid, due to the low solubility of SA in MeOH, the different reaction conditions were developed. The mixture of SA and EDC (1:1 mol/mol) in EtOH was heated to 30°C and sonicated (in ultrasonic bath, Polsonic company) for 30 minutes to ensure the complete dissolution of the acid, and then stirred at 50°C for 2.5 hours. Separately, CH000 (1 wt.%) was dissolved in 1% AA and EtOH mixture (2:1 v/v) and heated to 80°C. The SA-EDC solution was slowly added dropwise to the chitosan solution, then the temperature was raised to 90°C and the reaction mixture was stirred for 2 hours. Next, the temperature has been gradually reduced by 10°C per hour to 60°C. The whole reaction time was 24 hours. The amount of fatty acid was 30 wt%. The product was precipitated with methanol/ammonia solution (7/3 v/v), centrifuged, washed with distilled water to neutralize

pH and methanol in order to remove unreacted fatty acid, and finally dried for 48 hours under vacuum at 60°C.

The molecular structures of FA series are shown in Figure 1.

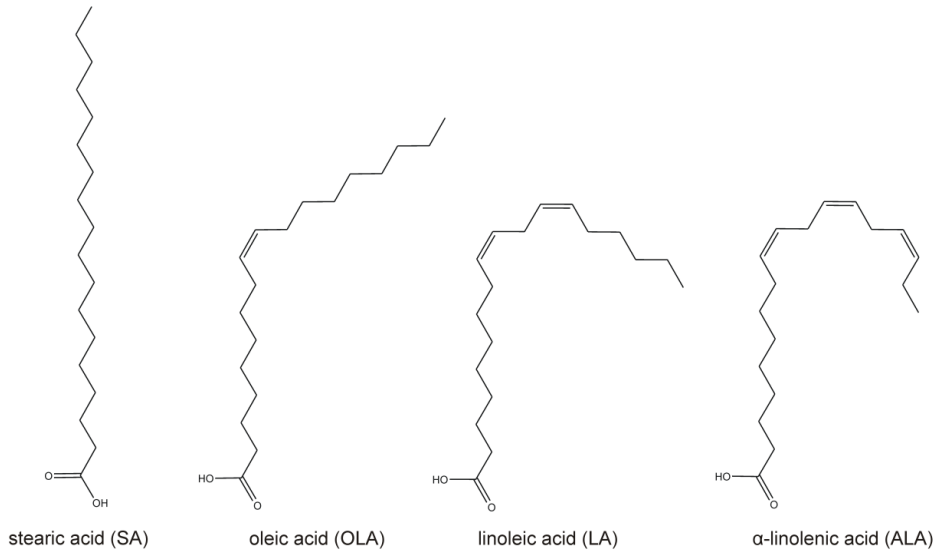

Figure 1. The molecular structures of fatty acids.

2.3. FTIR-ATR analysis

The chemical structure of chitosan derivatives was assessed by the Fourier transform infrared-attenuated total reflection spectroscopy on a Bruker ALPHA FT-IR ATR spectrometer. Prior to the analysis, samples were dried at 60°C under vacuum for 24 hours. For each sample, 32 scans with 2 cm^{-1} resolution were averaged across the spectral range of 400-4000 cm^{-1}.

Additionally, to characterize the substitution degree of the chitosan derivatives the absorption intensities of the characteristic bands were determined. For that purpose the reference band (R) and probe bands (A, B, C) were selected. The band at 1150 cm^{-1}, which is assigned to asymmetric bridge oxygen stretching, was chosen as the reference one. A key criterion for the R band is the constancy of the absorption intensity regardless to the progress of the reaction and the external environmental conditions. The selected R band changes only in the case of a chitosan molecular mass reduction, which does not occur in the mild conditions of reaction. The selected probe bands describe amides (A), esters (B) and alkyls (C) functional groups. The assigned absorption wavelengths of peaks and the baselines (appointed according to the literature [5–8]) are listed in Table 1.

Table 1. Wavelengths of reference band, probe bands and base lines.

	Peak [cm^{-1}]	**Baseline range [cm^{-1}]**
Reference band (R)	1150	1190-770
Probe band (A)	1555	1785-1485
Probe band (B)	1740	1785-1485
Probe band (C)	2867	3000-2545

2.4. Viscosity

Intrinsic viscosity $[\eta]$ was performed by using an Ubbelohde viscometer (K = 0.01) at 25°C in 1% AA. Chitosan and derivatives were dissolved at 37°C in order to obtain the concentration around 0.2 g/dL. The solutions were filtered through a Buchner funnel and intrinsic viscosity was calculated using the Solomon–Ciuta equation (1):

$$[\eta] = \frac{\sqrt{2*(\frac{t}{t_0})-\ln(\frac{t}{t_0})-1}}{c} \quad (1)$$

where c is the concentration of the solution (g/dL); t, the flow time of the solution and t_0 is the flow time of the pure solvent.

2.5. Thermal properties

Thermal analysis of the chitosan and its derivatives were carried out using a differential scanning calorimeter equipped with an internal cooling accessory (TA Instruments DSC-Q100). For each measurement, a sample of about 10 mg was placed in an aluminum pan and cooled to -90°C in an inert atmosphere at rate of 10°C/min. The samples were kept in this temperature for 3 min in order to achieve the uniform temperature in the whole material. The sample was then heated up to 400°C, using the same rate as before.

A thermal gravimetric analysis was used to evaluate the thermal stability of the obtained materials. Thermogravimetric measurements were made using a TGA Q5000 (TA Instruments). The samples were heated from 40°C to 900°C at a heating rate of 10°C/min under a nitrogen atmosphere.

3. Results and Discussion

Within the presented research chitosan derivatives with a series of fatty acids (FA) have been developed. The aliphatic fatty acids chains have the same length but vary in the number of double bonds. The FA molecules can be grafted onto chitosan macrochains by the *N*,*O*-acylation reaction, using both amino and hydroxyl functional groups of chitosan. Due to some statistical character of the *N*,*O*-acylation reaction, there are several possibilities of substitution of the chitosan glucopyranose units. The GlcN chitosan units, with free amino and hydroxyl groups can undergo *N*-acylation, *O*-acylation and *N*,*O*-acylation reactions, forming derivatives with structures (1A), (1B), and (1C) respectively, as presented in Figure 2.

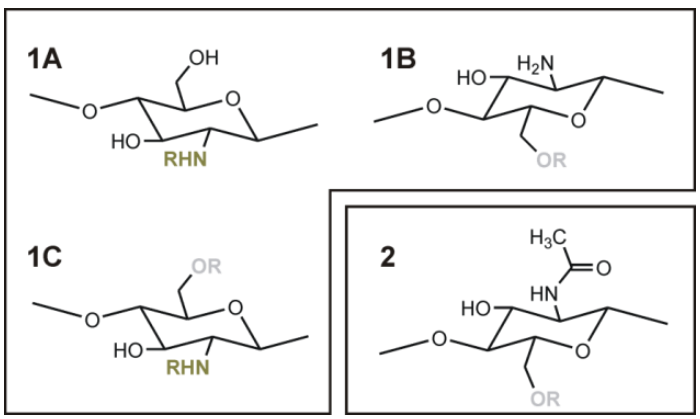

Figure 2. Possible structures of glucopyranose chitosan units after *N*,*O*-acylation reaction

The GlcNAc units can react only by *O*-acylation (**2**). Owing to the low reactivity of the secondary hydroxyl group of the chitosan, it is assumed that the *O*-acylation of this group does not occur or is not sufficient.

The conducting of reaction while leveraging both functional groups of chitosan might lead to achieving the high substitution degree and additionally preserves some (unreacted) free amino groups, considered a key factor in chitosan biofunctionality.

The IR spectra analysis of the FA-chitosan derivatives confirmed the predetermined *N*,*O*-acylated structure. Apart from the typical absorption bands for chitosan, a new band at 1739 cm^{-1}, corresponding to C=O stretching vibration of ester groups, is noticeable. Also, the intensity of the peak at 1555 cm^{-1} of C=O stretching vibration of amide groups, and bands in 2950-2840 cm^{-1} region of C-H stretch from aliphatic long fatty acid chains, are observed.

The simultaneous amino and hydroxyl acylation of chitosan was achieved by the combination of two ways of conducting the reaction with chitosan, i.e. application of a carbodiimide catalysis and ionic amino group protection. The EDC catalysis enables to activate the carboxylic groups (of fatty acids compounds) to react with a nucleophile. If the amino groups are present, they react to form the amide bonds, nevertheless if there are hydroxyl groups, they react to form the ester bonds. However, due to the fact that $-NH_2$ groups are stronger nucleophiles than –OH groups, without blocking amino ones the *O*-acylation reaction of chitosan is almost impossible. Unfortunately, the traditional way of amino groups protection is usually associated with the use of hazardous chemicals (such as hydrazine), which is a serious drawback from the biomedical point of view. An interesting method for amino groups blocking implemented for chitosan modification, based on the presence of methanesulfonic acid ($MeSO_3H$), or 2M sulfuric acid (VI) (H_2SO_4) as the reaction media was proposed by Sashiwa [9] and Badawy [10, 11]. Anions of these compounds can bind to amino groups forming ammonium salts, thus protonating them and preventing the subsequent *N*-acylation reaction. However, important disadvantages of these procedures are the decrease of the molecular weight of chitosan during the reaction and the limited substitution degree in the case of the chitosan modification by the compounds with long alkyl chains [9–11].

Therefore, to achieve our research aim, we developed the method which provides partial protonation of amino groups and accordingly partial protection using slightly acidic environment (pH 4.5-5) of the reactions. Worth mentioning is the fact that applied conditions (dilute acetic acid) do not promote the hydrolysis of the oxygen bridges, so the decrease of the molecular weight of the derivatives should not take place.

For the quantitative analysis of the chitosan derivatives, the absorption ratios of three probe bands to the reference one were calculated (Table 2).

Table 2. The absorption bands ratios of chitosan and its derivatives

	Alkyl chain (C/R)	**Amide bond (A/R)**	**Ester bond (B/R)**
CH000	**0.44**	0.58	-
CH_SA	**1.12**	0.55	0.38
CH_OLA	**1.34**	0.72	0.77
CH_LA	**0.73**	0.69	0.42
CH_ALA	**0.81**	0.82	0.89

Due to the occurrence of long aliphatic chains in the structure of fatty acids the substitution degree of the derivatives can be represented by the ratio of the band at 2867 cm^{-1} (C) to the reference band (C/R). The ratio for the unmodified chitosan is 0.44 and it is increased for all derivatives being at least twice as large. Nonetheless, between the individual derivatives the values are different. Considering the fatty acids structure in terms of the number of double bonds, the correlation between the substitution degree and the unsaturation of FA is noticeable. The derivatives with the two and three double bonds (CH_LA and CH_ALA) have shown minor substitution degree than two others. It might be due to the mobility of fatty acid molecules, which decreases with the increased number of double bonds in molecules – the *cis* conformation of double bonds results in more curved chain and a specific spatial arrangement.

Considering two possible reactions of FA and chitosan i.e. *N*-acylation forming the amide bond or *O*-acylation forming the ester bond, by the analysis of 1555 cm^{-1} and 1740 cm^{-1} bands respectively, the favored way of the reaction might be described. The amide band intensity ratio to the reference band for the unmodified chitosan is 0.58 and for the derivatives is only slightly higher, hereby *N*-acylation of chitosan occurs only marginally (as a result of a partial protection of amino groups by the acetic acid ions) contrarily to the *O*-acylation. The intensity of the new absorption band at 1740 cm^{-1} for all derivatives as well as the ester bonds ratio (B/R) is substantial.

An interesting result is that the ratio of newly created amide and ester bonds for CH_ALA derivative is higher than for CH_SA or CH_OLA, even though the total amount of fatty acid for CH_ALA is lower. It implies the different way of the FA molecules attachment. The detailed analysis of the infrared spectra in the region 1800-1600 cm^{-1} points out an additional absorption band at 1705 cm^{-1} for both derivatives (CH_SA, CH_OLA) with the highest substitution degree (Figure 3). The peak indicates the formation of the ionic bond (NH_3^+ COO^-) between the FA and the chitosan amino group [12].

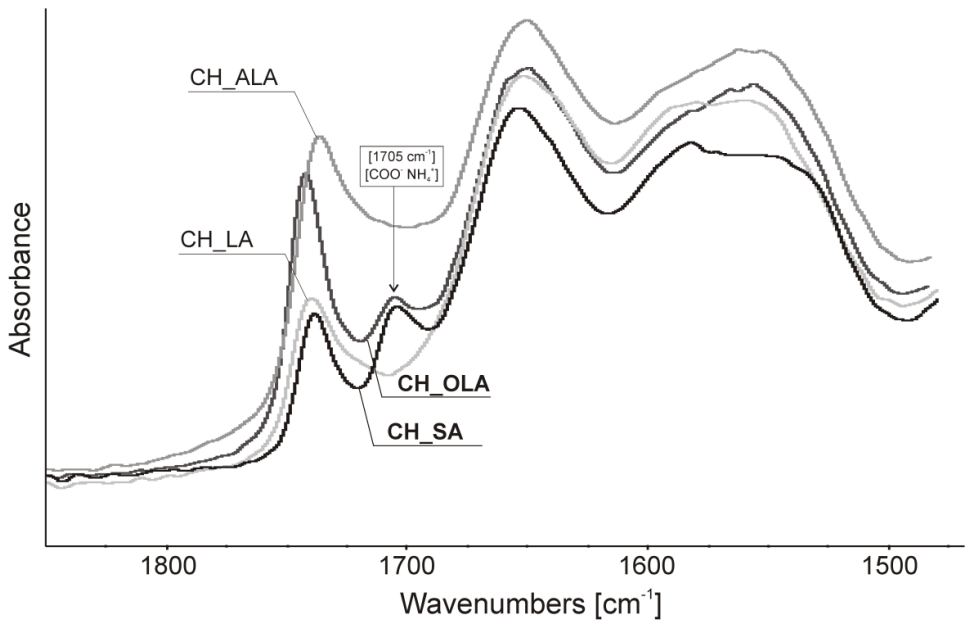

Figure 3. The IR spectra (1500-1850 cm^{-1} region) of FA-chitosan derivatives

In general the ionic derivatives of chitosan are known as the amphiphilic materials with specific properties, e.g. the ability to formation micelles, which emphasizes the fact the ionic interactions between chitosan and selected FA are strong enough to ensure the stability of the product [13, 14]. For the CH_LA and CH_ALA derivatives no ionic interactions occur, due to the previously mentioned limited mobility of LA and ALA, caused by the spherical hinders.

The intrinsic viscosity (*IV*) measurements show significant changes between the unmodified chitosan and its derivatives (Fig. 4). The intrinsic viscosity of the chitosan depends not only on the molecular weight but also on the charge density between the macromolecules in the solutions. The intra- and intermolecular interactions, mainly represented by the hydrogen bonds, stabilized the conformation of the chitosan chains in the solution [15]. The observed decrease of *IV* for all the derivatives, compared to the unmodified chitosan, is mainly related to the lower number of free amine or hydroxyl groups in chitosan derivative chains, which leads to weaker macromolecules interactions (less hydrogen bonds). Within derivatives, the lowest viscosity was observed for the modification with the stearic acid, while the highest relates to the α–linolenic acid modification, containing three double bonds. In general, the viscosity of the derivatives increased with the number of double bonds in the aliphatic chain. Referring the values of intrinsic viscosity to the results of the FTIR analysis, it could be noticed that the higher substitution degree (C/R ratio) with selected fatty acids, the lower values of the *IV*, independently on the type of bond between the fatty acid and the chitosan (ionic or covalent). Some steric hindrances could also affect the capillary flow of the analyzed solutions, due to the stiffening of the fatty acid side chains with the increased amount of double bonds, but such analysis is beyond the scope of this paper.

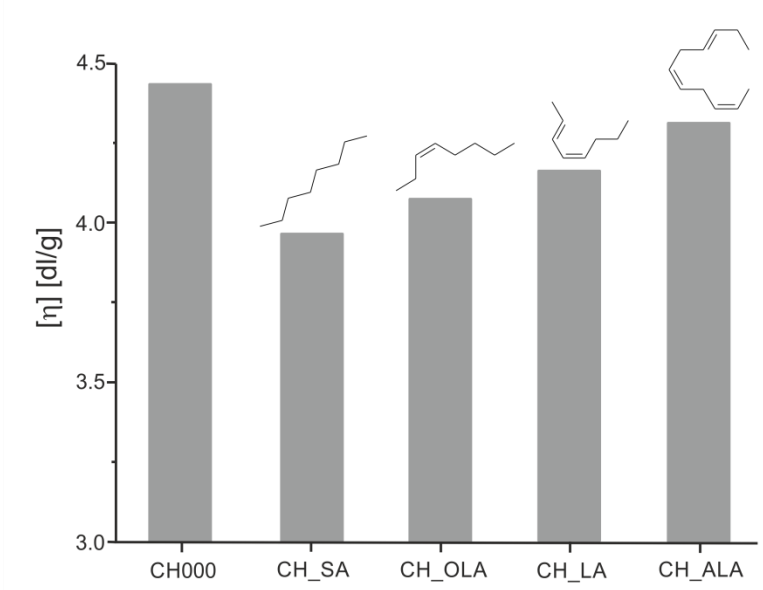

Figure 4. Intrinsic viscosity of chitosan CH 000 and its derivatives with schematic representation of FA saturation.

The analysis of thermal results (Table 3) corresponds to the FTIR observations. The highest amount of grafted fatty acid for CH_SA and CH_OLA derivatives, allowed to observe the characteristic transitions for the FA: the glass transition temperature (T_g) and the

melting temperature (T_m) for OLA and T_m of SA, respectively as pointed in Figure 5. The melting points of the FAs are 33.4 and -33.3°C for SA and OLA, respectively, which is notably lower than for the neat ones 70.9°C, 5.5°C [16] respectively, arising from the chemical bonds and physical (ionic) interactions.

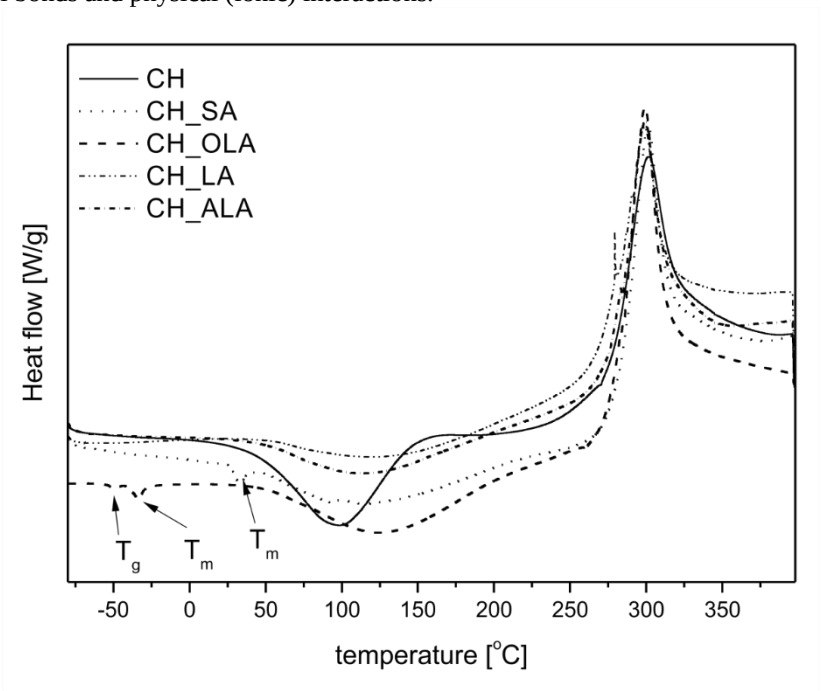

Figure 5. DSC thermograms of chitosan and its derivatives

The meaningful difference is observed also for the endothermic peak related to the bounded water. Since no melting of water was observed, those endothermic transitions could be attributed to the evaporation of the non-freezable water, entrapped between the polysaccharide chains. The evaporation temperatures (T_v) are higher for all the derivatives, which was also observed for the modification with shorter acidic chains [17]. As it was pointed out by Qu et al. the water molecules could more easily penetrate between the polymer chains due to the lower number of hydrogen bonds. However considering the fact that water binds to the free hydrophilic functional groups, which are consumed for the binding of FA in discussed derivatives, lower evaporation enthalpy (ΔH_v) is observed.

The degradation temperature (T_D) is comparable for all the materials and this process is heterogeneous, what is represented by the additional shoulder (T_D 1st) on the peak in range 282- 286°C. The decrease of the degradation enthalpy (ΔH_D) observed for all the derivatives could be explained by the lower amount of the hydrogen bonds meaning, less energy is required to destroy the polymer.

The TGA analysis (Table 4) confirmed the previous observations from the DSC measurements. The modification of chitosan with fatty acid sufficiently changed the character of the thermal degradation curves, therefore the evaporation temperatures (T_v) were much higher in comparison with the unmodified chitosan. The relatively low weight loss confirmed that in the materials non-freezable water occurred [18]. The subsequent stages of thermal degradation indicated the increase in the thermal stability of the chitosan derivatives. The enhanced thermal stability of the derivatives (even up to 100°C) was caused by the higher acylation degree, due to the *N*-acylation reaction with the fatty acid. This observation

corresponded to the FTIR results. The increase of the thermal stability of the derivatives was proportional to the amount of new amide bonds formed (A/R absorption ratio). According to the literature data, chitosan with higher acetylation degree has a higher thermal stability, due to the strong and stable hydrogen bonds between the amide groups. Therefore, the CH_ALA derivative, where the amide ratio is almost two-fold higher than the unmodified chitosan, exhibits much higher T_{max2} temperature.

Table 3. Thermal transition of unmodified chitosan and its derivatives

	FA				Bounded water		Degradation temperature		
	T_g [ºC]	ΔC_p [J/(g·°C)]	T_m [ºC]	ΔH_m [J/g]	T_v [ºC]	ΔH_v [J/g]	T_D 1st shoulder [ºC]	T_D 2nd shoulder [ºC]	ΔH_D [J/g]
CH	not observed				100.6	199.2	n.o.	302	309
CH_SA	not observed		33.4	3.15	132	128	n.o.	301.8	264
CH_OLA	-53.1	0.092	-33.3	3.12	123.9	183	285.4	300	258.8
CH_LA	not observed				128.1	130,7	282.8	299.7	218.8
CH_ALA	not observed				119.5	127.2	286.4	300.2	230.4

Table 4. Thermal degradation data of chitosan and its derivatives

	water		stage I		stage II			
	T_v [ºC]	weight loss [%]	T_{max1} [ºC]	weight loss [%]	T_{max2} [ºC]		weight loss [%]	
CH	49.4	7.4	288.6	53%	507.6		38.8	
CH_SA	150.3	11	292.4	45.6%	560.3		43	
CH_OLA	124.5	10.8	291.7	43.5%	442.6	582.1	6.5	39.1
CH_LA	131.8	8.9	290.8	43.8%	459.7	581.4	6.3	40.7
CH_ALA	151.3	8.4	288.2	45.2%	457.4	602.8	6.4	39.8

T_v – evaporation temperature;
T_{max} – maximum temperature of decomposition corresponding to each stage

4. Conclusions

The chitosan derivatives based on the series of C18 fatty acids were synthesized by the simultaneous *N,O*-acylation reaction, using EDC catalyst and partial protonation of the amino groups. The IR spectra analysis confirmed the predetermined structure and allowed to describe quantitatively the fatty acids substitution degree, indicating the superiority of *O*-acylation over *N*-acylation. Presented results pointed out the correlation between the fatty acid saturation, thereby FA chains mobility, and the amount of FA substitution. The SA and OLA based derivatives with the additional ionically bounded FA compounds exhibited the highest substitution degree. Thermal properties were discussed in terms of the fatty acid location along chitosan chains and the amount of hydrogen bonds. All the derivatives demonstrated increased thermal stability as a result of the covalently attached long FA chains.

5. Acknowledgments

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