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Amidated pectin derivatives with *n*-propyl-, 3-aminopropyl-, 3-propanol- or 7-aminoheptyl-substituents

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ABSTRACT

Highly methylated (HM) pectin (*DM* = 90%) was amidated with four amines under anhydrous conditions. The goal was to prepare modified pectins that could be useful components of composites with new properties. The yields and degree of amidation (*DA*) of the products were, respectively, 67–92% and 67.3–72.0%. Maximal values were obtained for pectin propylamide (**1**) and minimal for pectin 7-aminoheptylamide (**4**). While products **1** and pectin 3-propanolamide (**2**) showed 95 g/100 g solubility in water, pectin 3-aminopropylamide (**3**) was 46 g/100 g and **4** only 3 g/100 g water-soluble. The TG/DTA data indicate that the thermal properties were not dramatically altered under inert environment and improved as far as thermooxidation resistance. From the overall prospective product **3** seems to be the most valuable component for further composite preparation.

1. Introduction

Amidated pectins, i.e. those containing primary amino groups $CONH_2$, are common pectin derivatives of good gelling properties at low-sugar conditions. The usual method of their preparation is ammonolysis of methyl ester groups of HM pectins. Introducing the primary aliphatic amines instead of ammonia in this reaction leads to *N*-alkylamidated pectins containing secondary amide groups (CONHR). Introduction of alkyl groups onto pectin macromolecules, similar like in case of pectin esters (Pappas et al., 2004), changes physical and chemical properties of the polysaccharide depending on the number of amide groups formed and the alkyl structure.

N-alkylamidated pectins could be prepared under anhydrous conditions (Sinitsya, Čopíková, Prutyanov, Skoblya, & Machovič, 2000) or in presence of water and protease using amines or amino acids (Cheng, Gu, & Nickol, 2000). In both cases the C6 carboxyls were modified with amine groups to form amides. Some pectin derivatives containing alkyl groups are water insoluble or only partially water-soluble (Synytsya et al., 2004). For this reason vibration spectroscopy and solid state ^{13}C CPMAS NMR are preferential tools for their study (Sinitsya, Čopíková, & Pavlíková, 1998). FTIR and FT-Raman are vibrational spectroscopic methods possessing complementary information about the structure and origin of pectins (Synytsya, Čopíková, Matejka, & Machovič, 2003). Particularly, FTIR is especially useful for detection of functional groups, while FT-Raman is sensitive to skeletal vibrations mainly. In the case of native and modified pectins FTIR spectra has been used for quantification of methoxy, amido and *O*-acetyl substitution as well as the free carboxyls (Filippov, 1992). Amidated pectins can be used as gelling substances for food industry, hydrophobic sorbents (Synytsya et al., 2004), components for composites suitable for drug delivery (Liu, Fishman, & Hicks, 2007) or for other applications when combined with other derivatives (Šimkovic, 2008; Šimkovic, Uhliariková, & Mendichi, 2008).

Our goal was to find out how the used amine functionality could affect the structure of formed product as well as their properties. The main interest was oriented on water-soluble products applications. We have used *n*-propylamine, 3-amino-1-propanol, 1,3-diaminopropane and 1,7-diaminoheptane to prepare amidated pectin derivatives. While for 1,7-diaminoheptane dimethylsulfoxide (DMSO) was used as a solvent, the rest of amines were liquids and no solvents were used and needed. This could be considered as an advantage due to absence of solvent impurities and related costs. The products were characterized by spectroscopic methods (2D NMR, FTIR and FT-Raman), thermal (TG/DTA) and elemental analysis.

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2. Experimental

2.1. Preparation of amidated pectins

Highly esterified pectin (**0**) from citrus peel (Sigma P9561; *DM* = 90%; N, 0.63; C, 39.50; H, 5.70; vacuum dried overnight at RT) was mixed with amines (*n*-propylamine, 3-amino-1-propanol, 1,3-diaminopropane, or 1,7-diaminohepate; Sigma; samples **1**–**4**) in a ratio as listed in Table 1 and stirred for 24 h/RT. Because 1,7-diaminohepate (1.30 g; 10 mmol) was solid compound, it was solubilized in 40 g of DMSO and equimolar amount of **0** (1.90 g; 10 mmol) was added and the mixture stirred until complete dissolution (9 days/RT; sample **4**). The reaction was stopped by dilution with a surplus of water (samples **1** and **4**) or ethanol (samples **2** and **3**). In the former cases the mixture was dialyzed through 1 kDa dialysis tubing (Spectra/Por®) and lyophilized; in the latter cases solids were filtered and dried to constant weight under vacuum. After dialysis the mixture containing product **4** was separated to water-soluble part and insoluble residue and then lyophilized.

2.2. Analytical methods

The elemental analyses of all the samples was performed in duplicate using a Fisons EA-1108 instrument. The water-solubilities were determined by mixing 30 mg of sample in 20 ml of water and after 24 h/RT stirring filtered through 0.2 μm syringe filter (PALL; Acrodisc®; Gelman Lab.), the soluble part was lyophilized in prebalanced vial. The degrees of amidation (*DA*) of the products were calculated from the elemental composition according to the formula:

$$M_{CR} = M_N \times \frac{12}{14} \times \frac{K}{I}$$

$$M_{CP} = M_C - M_{CR}$$

$$DA = \frac{M_{CR}}{M_{CP}} \times \frac{6}{K} \cdot 100,$$

where M_N and M_C are contents of nitrogen and carbon (%), M_{CR} and M_{CP} are contents of carbon (%), respectively, in amine substituents and pectin, 12/14 is the ratio of the carbon to the nitrogen atomic mass, 6 is the sum of carbons in the galacturonic unit of pectin, *K* and *I* are the numbers of carbons and nitrogens in the amine molecule, respectively ($K = 3$ for products **1**–**3**, $K = 7$ for product **4**; $I = 1$ for products **2** and **3**, $I = 2$ for products **1** and **4**).

NMR measurements were performed in D_2O at 25 °C on Varian 600 MHz UNITY INOVA spectrometer equipped with 5 mm 1H {13C, 15N} PFG Triple Res IDTG600-5 probe head. ^1H and ^{13}C chemical shifts are referred to 3-(trimethylsilyl)-propionic acid (TSP) internal standard. Multiplicity edited ^1H-^{13}C HSQC spectra were recorded in phase-sensitive pure absorption mode with optimization on one bond coupling constant $^1J_{CH} = 165$ Hz. ^1H–^1H COSY spectra with gradient selection and ^1H–^{13}C HMBC spectra were measured in absolute intensity mode. The spectral widths employed in 2D NMR experiments were typically 6000 Hz (^1H) and 20000 Hz (^{13}C), respectively.

Infrared spectra (4000–400 cm^{-1}) of the samples (KBr discs) were measured on Nicolet 6700 FTIR spectrometer (Thermo Scientific, USA); 64 scans were accumulated with a spectral resolution of 2.0 cm^{-1}. Obtained spectra (region 1820–1490 cm^{-1}) were analysed by the normalised least-squares curve fitting procedure (Microcal Origin 6.0 software) using multiply Voigt curves and linear baseline. The second derivative algorithm assisted the curve fitting procedure. The areas of the separate peaks at 1710–1760 cm^{-1} ($A_{C=O}$, stretching C=O vibration in acids and esters), 1650–1670 cm^{-1} (A_{CONH}, amide I) and 1600–1630 cm^{-1} (A_{COO^-}, asymmetric stretching vibration of COO^-) were used for the quantification of the degree of methylation (*DM*) of the initial pectin (sodium pectinate form) and the degrees of amidation (*DA*) of the products (pectinic acid form) as the molar ratio (%) between substituted and total carboxyls:

$$DM = \frac{A_{C=O}}{A_{C=O} + A_{COO^-}} \cdot 100;$$

$$DA = \frac{A_{CONH}}{A_{C=O} + A_{CONH}} \cdot 100.$$

FT-Raman spectra (4000–200 cm^{-1}) of powder samples were recorded by using Bruker FT-Raman (FRA 106/S, Equinox 55/S) spectrometer equipped with a quartz beam splitter, liquid nitrogen-cooled germanium detector and excitation at 1064 nm from a Nd: YAG laser. The laser power was set at 100 mW, and 256 scans were accumulated with a spectral resolution of 2.0 cm^{-1}.

Thermogravimetric measurements were run on SDT 2960 (TGA–DTA module; TA Instruments) thermobalance at 90 cm^3/min nitrogen or air flow and 10 K/min heating rate from 20 to 1000 °C using 3–24 mg sample amounts in platinum pan.

3. Results and discussion

3.1. Pectin propylamide

As seen from Table 1 the use of liquid amines without a solvent for amidation of esterified pectin is an effective way of modification. Treatment of pectin methylester with *n*-propylamine resulted in excellent yield (sample **1**; 90%) of propylamide calculated on fully substituted derivative. The product was 95 g/100 g water-soluble and according to ^{13}C NMR (data not shown) there were two carbonyl signals observed at 173.20 and 178.08 ppm with intensities at the ratio 8.5:1. We assume the less intense signal belongs to de-esterified carboxyls. There was only one carboxyl signal in the ^{13}C NMR spectrum of the starting material **0** at 173.57 ppm, which indicates according to determined *DM* that the chemical shifts for

Table 1
Reaction conditions and results of pectin amidation.

Sample	m_p/m_a [g/g][a]	Amine	Solvent	*N* [%][b]	Yield [%][c]	*DA* [mol%][d]		[e]Solubility [g/100 g]
						EA[f]	FTIR	
1	1.9/10	1-propylamine	–	5.21	90	71.4	71.9	95
2	1.9/10	3-amino-1-propanol	–	4.91	92	71.2	70.8	95
3	1.9/10	1,3-diaminopropane	–	9.07	94	73.2	72.0	46
4	1.9/1.3	1,7-diaminoheptane	DMSO	6.12/1.66[f]	67/1[f]	46.8	67.3	3/100[g]

[a] Amounts of pectin and amine used.

[b] Nitrogen content of the initial HM pectin was 0.63%.

[c] Yield was calculated on fully amidated product.

[d] Degree of amidation (*DA*) was calculated on fully amidated galacturonic units using elemental composition and peak fitting of FTIR spectra.

[e] Solubility in water (see Section 2).

[f] Determined by elemental analysis.

[g] Water-insoluble/water-soluble part.

Table 2
NMR data of prepared derivatives (ppm).

Sample	C_1/H_1	C_2/H_2	C_3/H_3	C_4/H_4	C_5/H_5	C_6/H_6	C_1'/H_1'	C_2'/H_2'	C_3'/H_3'
0	102.98/4.94	70.72/4.44	70.84/3.98	81.68/4.44	74.83/3.68	173.45/–	56.36/3.82*	–	–
1	101.20/5.03	70.70/3.77	71.70/3.97	79.60/4.45	73.80/4.85	173.20/–	44.00/3.18⁺	24.5/1.54	13.7/0.93
2	102.30/5.01	70.55/3.76	71.35/3.97	79.50/4.45	73.50/4.86	173.60/–	62.20/3.67⁺	33.85/1.78	38.33/3.33
3	102.00/5.01	69.00/3.75	69.80/3.97	76.96/4.43	72.31/4.88	173.50/–	63.69/3.60⁺	38.84/2.94	28.42/1.86
4	104.75/4.57	72.21/3.71	73.66/3.73	74.80/3.76	78.05/4.09	176.81/–	61.03/3.64⁺	30.25/3.16	20.11/1.43$^{\gamma}$

Key: *Methylester; ⁺-NH-CH_2-signal; $^{\gamma}$The remaining CH_2 signals are at 28.66/1.24 (C_4'/H_4'); 29.00/1.31 (C_5'/H_5'); 27.30/1.60 (C_6'/H_6') and 40.20/2.90, (C_7'/H_7') ppm.

methylester and amides are identical. There were no OCH_3 signals of methyl ester at 55.79 ppm for sample **1** as observed in the initial HM pectin (Table 2; sample **0**). The above ratio calculated from NMR intensities corresponds to *DA* of 89%.

According to HSQC and COSY (not shown) there are except of polygalacturonic acid signals also signals of arabinose units at 107.00/4.64 (C_1/H_1), 79.60/4.45 (C_2/H_2), 71.70/3.97 (C_3/H_3), 76.30/3.79 (C_4/H_4) and 64.00/3.84 (C_5/H_5) ppm. Additionally there are three new signals which were not present in the starting material. Two of them are according to multiplicity edited ^1H–^{13}C HSQC spectra CH_2 groups and the third is CH_3. The CH_2 signal with higher chemical shift is splitted to doublet, which indicates that it has blocked rotation due to linkage to nitrogen. As there are no additional signals in ^{13}C, HSQC and HMBC spectra we assume that the primary groups of arabinose units are not modified as well as the hydroxyls of galacturonic units.

In this study FTIR spectra were applied for quantification of *DA* based on the $\nu(C{=}O)$ and amide I peaks decomposition and as assay of product purity. FT-Raman spectra could confirm the detection of *N*-alkyls in the products. Determination of *DA* by the

Table 3
Bands positions (cm^{-1}) in the FTIR spectra.

Samples										Assignment[a]
0s	**0a**	**1s**	**1a**	**2s**	**2a**	**3s**	**3a**	**4s**	**4a**	
3411	3423	3416	3416	3410	3410	3377	3397	3399	3401	$\nu(OH)$
		3100sh	3100sh			3100sh	3100sh	3100sh	3100sh	amide B
	3018									
2954	2955	2965	2964							$\delta_{as}(CH_3)$
2935	2937	2936	2929	2930	2926	2933	2927	2932	2930	$\delta_{as}(CH2)$, $\delta(CH)$ring
		2878		2890						$\delta(CH)$ring
2860sh	2860				2859			2858	2859	$\delta_s(CH_3)$
1749	1750		1740		1738		1734		1736	$\nu(C{=}O)$
		1653	1652	1652	1657	1654	1653	1653	1652	amide I
	1638									$\delta(H_2O)$
1618										$\nu_{as}(COO^-)$
		1550	1551	1553	1551	1551	1549	1549	1549	amide II
						1494				
		1462	1460			1470sh	1466	1464	1460sh	$\delta(CH_2)$
1443	1444	1443	1443	1445	1447	1440	1443	1440	1443	$\delta_{as}(CH_3)$
1418		1413						1407		$\nu_s(COO^-)$
						1398	1401		1406	$\nu,\delta(C{-}OH)_{COOH}$
1373	1373	1387	1385					1377		$\delta_s(CH_3)$
1333	1334	1335	1333	1333	1335	1332	1334	1334	1333	$\delta(CH)$
1269	1271	1287	1287				1282		1281	$\nu(COC)$ester
		1247	1249				1251			$\delta(CH)$
1235	1233			1239	1237			1233		$\nu(COC)$ester
1148	1151	1153	1155	1152	1156	1150	1151	1149	1150	$\nu(COC)_{glycosidic\ bond}$
1105	1105	1102	1104	1106	1110	1103	1103	1101	1102	$\nu(CC)(CO)$
		1078	1081	1077	1077	1078	1076			$\nu(CO) + \delta(OH)$
1050	1051									$\nu(CC)(CO)$
1015	1015	1022	1024	1017	1015	1016	1015	1020	1019	$\nu(CC)(CO)$
			984							$\delta(COO^-)$
957	968	953	952	961	957			957		$\gamma(C{-}OH)_{ring}$
921	920									$\rho(CH_3)$
892	890sh	890	886	886	882	887	887	889	886	$\gamma(C{-}OH)_{ring}$
850	847						848			skeletal
833	831	828	828	828	828	828	829	827	826	$\gamma(C{-}OH)_{ring}$
809	808									
763	763	759	754	765	766	761	759	759		ring “breezing”
								721		$\rho(CH_2)$
710sh	711				709					
688	685									Skeletal
665	669			676	678					Skeletal
638	632	632	622	635	630	632	631	633	630	Skeletal
593	592	580	581	585	581	583	586	591	587	Skeletal
536	533	534	533	533	529	534	533	533	533	Skeletal
		515	511							Skeletal
453	452			468	458					Skeletal
427	427	429	427		427				437	Skeletal

[a] γ-stretching; δ-in-plane bending; γ-out-of-plane bending; as-asymmetric; s-symmetric; a-carboxylic acid; e-methylester; s-salt.

Table 4
FT-Raman data.

Samples					Assignment
0a	**1s**	**2s**	**3s**	**4s**	
2958					$\nu_{as}(CH_3)$
	2938	2937	2939	2932	$\nu_{as}(CH_2)$
2860	2877	2886sh		2860	$\nu_s(CH_3)$, $\nu_s(CH_2)$
1751					$\nu(C{=}O)$
	1655	1649	1657	1655	amide I
	1599		1591		$\nu_{as}(COO^-)$
	1553	1556	1552	1555	amide II
1456					$\delta_{as}(CH_3)$
	1448	1441	1443	1442	$\delta(CH_2)$
1376				1370	$\delta_s(CH_3)$
1335					$\delta(CH)$
1306	1302	1302	1305	1310	$\tau,\omega(CH_3)$; $\tau,\omega(CH_2)$
1259	1251	1248			$\delta(CH)$
1147	1146	1146	1144	1142	$\nu(COC)_{\text{glycosidic bond}}$
	1112		1113		$\nu(CC)(CO)$
1079	1083	1076	1079	1083	$\nu(CO) + \delta(OH)$
1051	1040				$\nu(CC)(CO)$
976	988	990		982	$\nu(CC)(CO)$
	956	960		954	$\delta(CCH)$, $\delta(COH)$
923			922		$\rho(CH_3)$
848	848	848	849	851	skeletal
830, 806	806				$\gamma(C\text{-}OH)_{ring}$
	768				ring “breezing”
747					$\gamma(C\text{-}OH)_{COOH}$
711		710	713	715	$\gamma(C\text{-}OH)_{ring}$
682	674	683	679	682	skeletal
636	635	635	635	636	skeletal
593	577	589	588		skeletal
536	534	537	538	534	skeletal
	503		503	502	skeletal
		465	464	474	
441	440	441	441	440	T(COC)def
374			356		
329	322	326	329	334	T(COC)def

Table 5
Thermal analysis features of samples.

Sample	Atmosphere	DTA peaks		DTG peaks		Residue [%] at [°C]	
		[°C]	Δ [°C/mg]	[°C]	max. rate [mg/min]	500	1000
0	Nitrogen	293	−0.075	273	2.10	21.94	15.93
	Air	292	0.348			0	0
		333	0.157	272	2.40		
		461	0.495	461	0.88		
		923	−0.072				
1	Nitrogen	215	−0.612	269	0.34	23.87	17.08
		277	−0.549				
	Air	272	0.639	261	0.51	10.60	2.56
		343	−0.166				
		497	−0.315				
		922	−0.398				
2	Nitrogen	253	−0.047	253	1.42	27.05	19.42
		650	−0.151				
	Air	257	0.155	251	1.56	22.70	0
		361	0.093				
		525	0.217				
		558	0.117				
		631	−0.044				
		914	−0.097				
3	Nitrogen	229	−0.068	229	0.42	28.60	16.34
		663	−0.124	400	0.24		
	Air	249	0.093	230	0.46	22.27	2.14
		313	0.152				
		520	0.048				
		572	0.040				
		649	−0.113				
		914	−0.132				
4	Nitrogen	245	−0.170	243	0.33	26.19	17.22
		359	−0.213	445	0.33		
		445	−0.250				
		613	−0.215				
	Air	249	0.096	236	0.34	20.46	1.9
		333	0.330	322	0.22		
		511	0.046	511	0.22		
		920	−0.209				

latter method is difficult because the amide I band is not so pronounced. According to FTIR spectra (Table 3), the carbonyl stretching band $\nu(C{=}O)$ was observed near 1750 cm^{-1} both for acidic (sample **0a**; COOH and methylesters) and salt (sample **0s**; only methylesters) forms of initial HM pectin. Conversion of HM pectin into its sodium salt led to decrease of this band and appearing of a new one at 1618 cm^{-1} assigned to asymmetric stretching vibration of COO^- (Synytsya, Čopíková, Matejka & Machovič, 2003). Several bands centred near 2955, 2860 (shoulder), 1444, 1373 and 920 cm^{-1} were assigned to CH_3 vibrations in methylester groups. The $\nu(C{=}O)$ band of sample **1** is shifted to 1740 cm^{-1}. This band is pronounced only for the acidic form **1a**; the salt form **1s** has only a weak shoulder at this position. The ester bands mentioned above decreased or disappeared in sample **1**, while several bands assigned to propyl substituent arise near 2965, 2878, 1462 and 1387 cm^{-1} (**1s**). The CH stretching bands of **1a** are less pronounced due to overlapping of intense OH stretching bands of COOH; the other ones have similar position. New amide bands appeared near 3100 (shoulder), 1653 (**1s**), 1652 (**1a**), 1550 (**1s**) and 1551 cm^{-1} (**1a**). This confirms the expected structure of propylamide derivative **1**. Analogical situation was also observed for FT-Raman spectra (Table 4) with new amide, COO^- and propyl bands at 2938, 1655, 1553, 1448 and 1302 cm^{-1} not observed or weaker for the initial pectin.

From the thermal analysis results it is clear that the initial pectin (sample **0**) was more thermally stable than pectin propylamide **1** in the inert environment (Table 5). While the DTG peaks were close for both derivatives and environments there were residues observed in air atmosphere at 500 and 1000 °C for sample **1** while for unmodified pectin **0** all sample was thermo-oxidized already at 500 °C. The maximal rates of degradation have reached bigger values for the unmodified sample which confirms greater over all thermal stability of **1**.

3.2. Pectin 3-propanolamide

Treatment of pectin with 3-amino-1-propanol resulted in formation of the corresponding 3-propanolamide derivative (Table 1; Sample **2**). According to the NMR analysis (Table 2) there is only one ^{13}C signal at the amide region (172.6 ppm). This indicates that the methylester was quantitatively converted to amide. The substituent signals were assigned similarly as above for sample **1**.

The FTIR data (Table 3; sample **2**) indicate $\nu(C{=}O)$ band at 1738 cm^{-1} for the acidic form (**2a**); the salt form (**2s**) has a shoulder at this position. Intense amide I and amide II bands were found for both **2a** (1657 and 1551 cm^{-1}) and **2s** (1652 and 1553 cm^{-1}). Bands at 2926–2930 and 2859 cm^{-1} were assigned to stretching vibrations of CH_2 in amine residue. Also for the FT-Raman data the amide band at 1649 and 1556 cm^{-1} and CH_2 bands at 2939, 2886 (shoulder), 1441 and 1302 cm^{-1} was observed (Table 4; sample **2**).

According to TG/DTA data (Table 5; Sample **2**) the DTG peak in inert atmosphere was at lower temperature than for propylamide derivative and the starting material. The maximal rate of degradation was higher than for propylamine substituent and the residues at 500 and 1000 °C are bigger than for previously mentioned product and substrate. The DTG values are similar also in oxidative environment, indicating the thermooxidation did not take place before reaching the DTG peak at 251 °C. The residue at 500 °C was 22.70% and 0% at 1000 °C. According to DTA the first exotherm at 257 °C was close to the peak measured in inert environment. The oxidation took place in several steps at higher temperature as indicating by five exotherms with peaks observed at 361, 525, 558, 631 and 914 °C.

3.3. Pectin 3-aminopropylamide

When the pectin reacted with 1,3-diaminopropane the highest yield from all the run experiments (94%) was achieved (Table 1; Sample **3**). The solubility in water was only 46 g/100 g and the water-soluble part was used for NMR analysis. The spectrum was similar like for the previous two samples and the signals were assigned in similar way (Table 2; sample **3**). According to the multiplicity edited ^{1}H–^{13}C HSQC spectra experiment there were four CH_2 signals related to amide substituent. Except those three listed in table, the forth was at 37.84/3.32 (C/H) ppm. Due to the fact that this signal was splitted to doublet similarly like the signal at 63.69/3.60 ppm, we assume that these two signals belong to the CH_2 groups linked to nitrogen. This could be explained by the formation of the diamide bridge group (R-$CONHCH_2CH_2CH_2NHCOR$) between two carbonyl groups of two galacturonic units. It means that part of the 1,3-diamine reagents is linked bifunctionally. This hypothesis was confirmed by the HMBC experiment showing correlation between the multiplet at 173.00/3.32 and the CH_2 signals at 38.84 and 28.42/3.32 ppm.

The FTIR data (Table 3; Sample **3**) of the whole product (before separation to water-soluble and insoluble part) exhibited similar amide bands as the two previous derivatives. There was one additional $\delta(CH_2)$ band at 1470 (**3s**) and 1466 (**3a**) cm^{-1} due to the presence of different amine residues. FT-Raman bands (Table 4; sample **3**) at 2939, 1657, 1552, 1443 and 1305 cm^{-1} also indicate substitution. These data are only complementary and could not be used for explanation of solubility differences of pectin derivatives.

The TG/DTA results (Table 5; Sample **3**) indicate slightly better overall thermal stability as the maximal rates are smaller than observed on two previous derivatives. The DTG maxima in inert and oxidative environment were observed at 229 or 230 °C, which are the lowest values from all studied samples. It means that the degradation of amide groups takes place at lower temperature but the amide bridges are crosslinking the residue, representing 26% or 20% from the starting material under inert or oxidative environment.

3.4. Pectin 7-aminoheptylamide

The last derivative was prepared by amidation with 1,7-diaminoheptane in DMSO at lower yield than the previous three derivatives although equimolar amounts of **0** and 1,7-diaminoheptane were used (Table 1; Sample **4**). This was considered as a sufficient amount although the yield was lower than for previous derivatives. We assume that it was due the presence of DMSO which caused partial de-esterification of **0**. Additionally also a small water-soluble fraction was produced with smaller nitrogen content than the insoluble product. According to ^{13}C NMR data of the water-soluble fraction there are signals for all seven CH_2 groups of the substituent (Table 2; Sample **4**) with the most intense signal of CH_2 linked to amide nitrogen at 61.03 ppm. Also in this case the individual signals were assigned by 2D NMR.

As the main part of sample **4** was not soluble in water or DMSO we have used FTIR and FT-Raman data for its complementary characterization (Tables 3 and 4; sample **4**). According to FTIR the ester bands are absent and amide I and II bands are present. Also the CH_2 vibration bands of substituents were found at 2930, 2858 and 1460 cm^{-1} (shoulder) for **4a** and at 2932, 2859 and 1464 cm^{-1} for **4s**. It indicates that part of the carboxyls were not amidated due to $\nu_s(COO^-)$ band at 1407 cm^{-1} for salt form and no band in acid form. In the FT-Raman spectrum there was no δ_{as} (CH_3) band and amide I was at 1655 cm^{-1} for salt form. Intense peaks at 1442 and 1310 cm^{-1} were assigned to vibrations of CH_2 groups in the substituent.

According to the TG/DTA data the product had two equally intense DTG maxima at 243 and 445 °C in inert environment (Table 5). Under oxidative conditions the second DTG splitted to several smaller peaks similar to the shape of DTA curve due to oxidation. The residues in nitrogen or air at 500 and 1000 °C were close to previous samples. It indicates that the thermal properties were not dramatically altered under inert environment and improved as far as thermooxidation resistance.

4. Conclusions

Amidated pectin derivatives were prepared in acceptable yields and *DA* values by treatment HM pectin with liquid amines without solvent or with 1,7-heptyldimaine in DMSO. While *n*-propylamide- and 3-propanolamide-derivatives were 95 g/100 g water-soluble, 3-aminopropylamide-pectin was 46 g/100 g water-soluble and for 7-aminoheptylamide-pectin the solubility was only 3 g/100 g of water. According to 2D NMR, FTIR and FT-Raman data only carboxyls were amidated up to 72.0% with the lowest value for 7-aminoheptylamide-pectin (67.3%). The modification did not affect the thermal stability under inert environment but improved the resistance toward thermooxidation. Sample **3** seams to be the most thermally stabile under inert environment.

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References

Cheng, H. N., Gu, Q.-M., Nickol, R. G. (2000). Amine modified polysaccharides, US Patent 6,159,721.

Filippov, M. P. (1992). Practical infrared spectroscopy of pectin substances. *Food Hydrocolloids, 6*, 115–142.

Liu, L. S., Fishman, M. L., & Hicks, K. B. (2007). Pectin in controlled drug delivery. *Cellulose, 14*, 15–24.

Pappas, C. S., Malovikovà, A., Hromádková, Z., Tarantilis, P. A., Ebringerová, A., & Polissiou, M. G. (2004). Determination of degree of esterification of pectinates with decyl and benzyl ester group by diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) and curve fitting deconvolution method. *Carbohydrate Polymers, 56*, 465–469.

Šimkovic, I. (2008). What could be greener than composites made from polysacchraides? *Carbohydrate Polymers, 74*, 759–762.

Šimkovic, I., Uhliariková, I., & Mendichi, R. (2008). Modification of polygalacturonic acid hydroxyls with trimethylammonium- and/or sulfonate-2-hydroxypropyl group. *Carbohydrate Polymers, 74*, 611–616.

Sinitsya, A., Čopíková, J., & Pavlíková, H. (1998). ^{13}C CP/MAS NMR Spectroscopy in the analysis of pectins. *Journal of Carbohydrate Chemistry, 17*, 279–292.

Sinitsya, A., Čopíková, J., Prutyanov, V., Skoblya, S., & Machovič, V. (2000). Amidation of highly methoxylated citrus pectin with amines. *Carbohydrate Polymers, 42*, 359–368.

Synytsya, A., Čopíková, J., Matejka, P., & Machovič, V. (2003). Fourier transform-Raman and infrared spectroscopy of pectin. *Carbohydrate Polymers, 54*, 97–106.

Synytsya, A., Čopíková, J., Marounek, M., Mlčochová, P., Sihelníková, L., Skoblya, S., et al. (2004). *N*-octadecylpectinamide, a hydrophobic sorbent based on modification of highly methoxylated citrus pectin. *Carbohydrate Polymers, 56*, 169–179.