Preparation of *N*-alkylamides of Highly Methylated (HM) Citrus Pectin

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Abstract

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N-Alkylamides of highly methylated (HM) citrus pectin (DM = 73%) were prepared using heterogeneous amino-de-alkoxylation (aminolysis) with five selected *n*-alkylamines. The reaction was carried out in *N*,*N*-dimethylformamide at 8°C (*n*-butylamine), 25°C (*n*-hexylamine), and 45°C (*n*-octylamine, *n*-dodecylamine and *n*-octadecylamine). All the derivatives were converted into acid forms by washing with acidic water-ethanol mixture. The products were analysed by organic elemental analysis, diffusion reflection FT-IR spectroscopy and by ^{13}C CP/MAS NMR. Both FT-IR and NMR spectra indicate the presence of alkylamide substituents bound to pectin. The degrees of amidation (*DA*) of the derivatives and molar and mass reaction yields (Y_m and Y_n) were calculated based on the results of elemental analysis. The *DA* values of the N-alkylamides were 39–55% that corresponded to Y_n of 54–75%.

Keywords: HM citrus pectin; amino-de alkoxylation (aminolysis); *N*-alkylpectinamides

Pectin is presently widely used in the food industry. It is a component of fruit and vegetables and is used on a large scale as an excellent jellying agent. Pectin is a cholesterol-reducing dietary fibre (Judd & Truswell 1985; Hexeberg *et al*. 1994), and it has also affinity to heavy metals ions (Dronnet *et al*. 1996; Kartel *et al*. 1999). Pectin is a well fermentable substrate; as such, it is totally degraded in the proximal colon. Digestible pectins cannot prevent colorectal carcinomas and inflammations, which are developed in the distal colon. Chemical modification of pectin, however, may decrease its availability for intestinal microorganisms. In contrast to starch, pectin has not been often used for the preparation of derivatives that can be applied in the food industry. Nevertheless, pectin has some advantages over starch, first of all the presence of partially esterified carboxyls.

The introduction of non-polar groups increases hydrophobic character of macromolecules, and it was reported that alkyl esters of pectin and pectic acid adsorb bile acids, fat and cholesterol (Müllner *et al*. 1993; Klavons & Bennet 1995). The chemical modification of pectin (amidation, trans-esterification) is relatively easy and it modifies in a significant way physiochemical and biological properties of

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pectin. Crescenzi and Callegaro (1993) prepared a number of highly substituted alkyl- and aryl-esters of pectin, practically insoluble in water. Acylation and methylation of pectins (Damian *et al*. 1999) were used to lower the solubility of these polysaccharides to make them usable as drug carries (drug delivery systems). Müllner *et al*. (1993) prepared partially depolymerised (n = 10–500), hydrophobic derivatives of pectin (amides and esters) and recommended them as bile acid sequestrants.

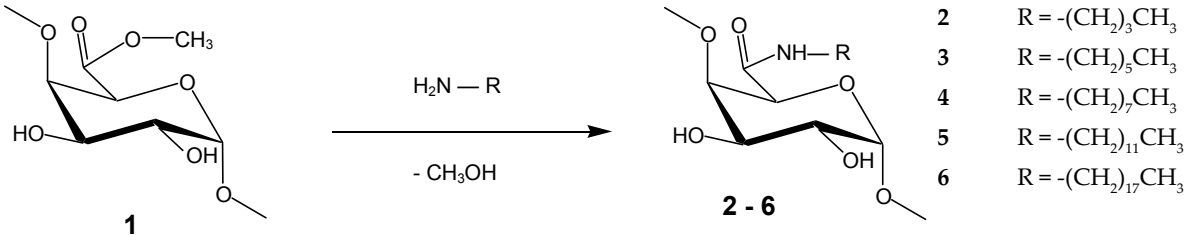
N-Alkylpectinamides have some advantages in comparison with other alkylated derivatives of pectin. First, the preparation of them does not require extreme conditions. Second, the amide bond is sufficiently resistant to hydrolysis by acids or alkali. Yields of *N*-alkylamides prepared by the reaction of pectin with aliphatic non-branched amines are relatively high. The highest yield was observed for the reaction of pectin with methylamine (Sinitsya *et al*. 2000). Physicochemical characteristics of these derivatives, especially their solubility in water, depend on the degree of substitution, i.e. on the content of hydrophobic groups bound to pectin. Even very low content of hydrophobic groups enable interactions of pectin macromolecules with lipids and proteins in water environment (Tribet 1998).

In this paper we describe the preparation of partially amidated pectins by means of the reaction of highly methylated (HM) citrus pectin with five chosen *n*-alkylamines. The derivatives obtained were analysed by organic elemental analysis and by spectroscopic methods, i.e. diffuse reflectance FT-IR (DRIFT) spectroscopy and ^{13}C CP/MAS NMR spectroscopy.

MATERIALS AND METHODS

HM citrus pectin. Highly methylated citrus pectin **1** (Genu, HM type B, Denmark) with the degree of methylation (DM) of 73% and anhydrogalacturonic acid (AGA) content of 89.7% was used for the preparation of derivatives. Commercial pectin was purified and converted into H-form by washing with 0.1 mol/l HCl dissolved in ethanol-water mixture (1:1 v/v). Pectin subsequently was washed several times with ethanol-water mixture followed by 96% ethanol until the chloride reaction was negative, and finally dried at 60°C. The content of AGA, which expresses the total content of uronic carboxyls, was measured by photometric method with *m*-hydroxybiphenyl at 520 nm (Blumenkrantz & Aboe-Hansen 1973). The degree of methylation (DM) was determined by photometry with chromotropic acid at 570 nm (Filippov & Kuzminov 1971).

Preparation of N-alkylpectinamides. The reactions of **1** with five *n*-alkylamines were carried out in a heterogeneous system in *N*,*N*-dimethylformamide (DMF) medium:



2 R = -$(CH_2)_3CH_3$
3 R = -$(CH_2)_5CH_3$
4 R = -$(CH_2)_7CH_3$
5 R = -$(CH_2)_{11}CH_3$
6 R = -$(CH_2)_{17}CH_3$

As a good suspending agent (Yevdakov *et al*. 1972), DMF was applied before the addition of *n*-alkylamine to prepare a fine suspense of HM pectin. This improvement led to a significant decrease of the reaction time in comparison with the reaction in methanol medium (Sinitsya *et al*. 2000).

Pectin powder (2 g) was weighed into a 200-ml flask and suspended in 50 ml of DMF. An amount of 5 g of each *n*-alkylamine (Fluka) was dissolved in 150 ml of DMF. The solution was gradually added into the flask under stirring. The reaction was carried out in cupped flasks at 8° C (**2**), 25° C (**3**) and 45° C (**4–6**) during 8 (**2**, **3**), 12 (**4**) and 36 (**5**, **6**) hours under continuous mixing. The solids were washed with DMF at appropriate temperature to remove free amine and then washed with 0.1 mol/l HCl in ethanol-water (1:1 v/v) mixture to convert ionised carboxylic groups into the protonated form. This step is necessary to hydrolyse alkylammonium salt that can be formed in the reaction of HM

pectin with alkylamines. Finally, the products were washed with 80% aqueous ethanol, filtered and dried at 60°C.

The degrees of amidation (*DA*), the mass and molar yields of reaction (Y_m and Y_n, respectively) were calculated on the results of elementary analysis according to the following equations (Sinitsya *et al*. 2000):

$$DA = \frac{M_N}{M_C}\left[6 + \frac{73}{100} + (K-1)\frac{M_N}{14}\right] \times 100$$

$$Y_m = \frac{M_N M_A}{14}$$

$$Y_n = \frac{DA}{73} \times 100$$

where: DA – degree of amidation (%)

Y_m – mass yield of the reaction, i.e. the relative mass of bonded amine (%) in the reaction product

Y_n – molar yield of the reaction, i.e. the relative content of ester groups substituted by amine (%)

M_N – nitrogen content (%)

M_C – carbon content (%)

M_A – molar mass of *n*-octadecylamine (g/mol)

14 – the nitrogen atomic mass (g/mol)

6 – the sum of carbons in galacturonic unit

$K = 18$ – the sum of carbons in amine molecule

73 – the DM of original pectin (%)

Spectroscopic methods. Diffusion reflectance FT-IR spectra were measured on Nicolet 740 (Nicolet Analytical Instruments, USA) spectrometer with DCT 680, 256 scans were accumulated with a spectral resolution of 4.0 cm^{-1}. ^{13}C CP/MAS NMR spectra of solid samples were measured on 75.46 MHz Mercury Plus 300BB (Varian, Australia) NMR spectrometer.

RESULTS AND DISCUSSION

The carbon and nitrogen contents, the calculated values of amidation degrees, and the reaction yields are shown in Table 1. The degrees of amidation (*DA*) were in the range of 39–55%, while the molar yields of amidation (Y_n) were 54–75%. Therefore, organic elemental analysis confirms that, in all cases, great amounts of methyl ester groups of initial HM pectin were converted into *N*-alkylamides.

DRIFT spectra of the original HM pectin **1** and the products of HM pectin amidation with *n*-alkylamines (**2–6**) are shown in Figure 1. The spectra of *N*-alkylpectinamides have two intense bands at 1665 ± 5 cm^{-1} (amide I) and 1552 ± 6 cm^{-1} (amide II). The presence of these two bands and the absence of intense carboxylate stretching bands confirm our suggestion that the substituents are bound to pectin as covalent amide and not by an ionic salt linkage. The possible ionic bonds between amine and free carboxyls of pectin were destroyed by the acid treatment during the sample purification, so most of nitrogen atoms observed by elemental analysis originate from *N*-alkylamide substituents bound to pectin.

Amidation of pectin with *n*-alkylamines results in an appearance of new IR bands from alkyl substituents. The samples of *N*-alkylpectinamides **2–6** showed more intensive C-H absorption in the region of 2970–2930 cm^{-1} than the initial pectin **1**. This effect can be explained by an increased C-H bond content after amidation. The spectra of samples **5** and **6** had two very intense bands at 2933 ± 5 cm^{-1} and 2858 ± 4 cm^{-1} that were assigned to antisymmetric and symmetric C-H stretching vibrations of methylene groups. The bands at

Table 1. Characterisation of *N*-alkylamides of HM citrus pectin

No.	Name	w_C (%)	w_H (%)	w_N (%)	*DA* (%)	Y_m (%)	Y_n (%)
2	*N*-butylpectinamide	42.2	6.6	2.9	43	15	58
3	*N*-hexylpectinamide	47.5	7.6	3.8	55	27	75
4	*N*-octylpectinamide	49.1	7.9	3.1	45	29	62
5	*N*-dodecylpectinamide	54.2	8.4	2.8	39	37	54
6	*N*-octadecylpectinamide	53.2	8.3	2.6	41	50	57

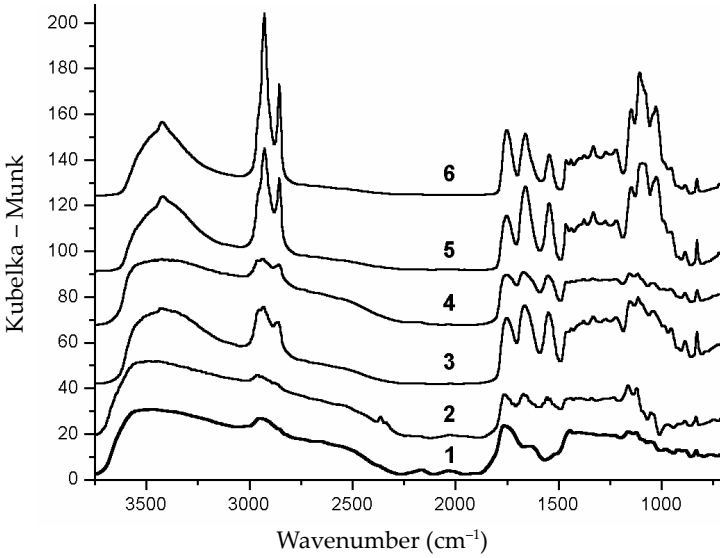


Figure 1. DRIFT spectra of HM citrus pectin **1** and *N*-alkylpectinamides **2–6**

1464 ± 4 cm^{-1} (scissoring of CH_2) and 726 ± 4 cm^{-1} (rocking of CH_2) are also indicative to methylene units of *n*-alkyls.

Solid state ^{13}C CP/MAS NMR spectra of *N*-alkylpectinamides have intense signals of $NHCH_2$ (39–42 ppm), CH_2 (35–20 ppm) and CH_3 (~15 ppm) carbons absent in the spectrum of the initial HM pectin. In contrast, the OCH_3 carbon signal at 53–54 ppm is very weak in all the derivatives (Figure 2). Therefore, the NMR spectra also confirm the conversion of methyl ester groups into *N*-alkylamides.

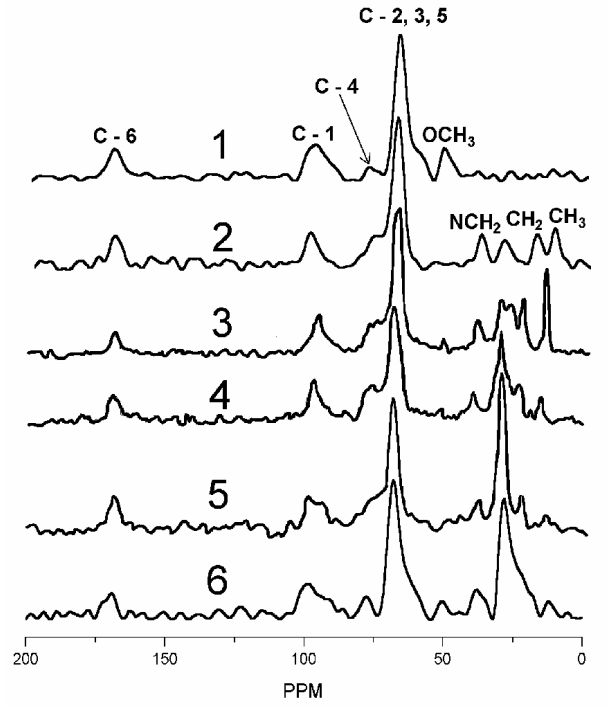


Figure 2. ^{13}C CP/MAS NMR spectra of HM citrus pectin **1** and N-alkylpectinamides **2–6**

Conclusion

Five *N*-alkylpectinamides were prepared by heterogeneous aminolysis in DMF with five selected *n*-alkylamines containing 4–18 carbon atoms in the alkyls. The amidation with *n*-alkylamines permits to link alkyl moieties to the pectin macromolecule which influences physical chemical properties of the derivatives and their possible application. Partially, *N*-alkylpectinamides could be more hydrophobic than the initial HM pectin due to the presence of long alkyl substituents. We suggest that these modified pectins could be interesting as bioavailable sorbents and drug delivery systems, which are able to absorb non-polar compounds, and, therefore, could be useful in different fields of application.

References

Blumenkrantz N., Aboe-Hansen G. (1973): New method for quantitative determination of uronic acids. Anal. Biochem., **54**: 484–489.

Crescenzi V., Callegaro L. (1993): Esters of pectic and pectinic acids, their manufacture and uses. WO 9314129. Chem. Abstr., **120**: 301518.

Damian F., Van den Mooter G., Samyn C., Kinget R. (1999): *In vitro* biodegradation study of acetyl and methyl inulins by bifidobacteria and inulinase. Eur. J. Pharm. Biopharm., **47**: 275–282.

Dronnet V.M., Renard C.M.G.C., Axelos M.A.V., Thibault J.-F. (1996): Characterisation and selectivity of divalent metal ions binding by citrus and sugar-beet pectins. Carbohydr. Polym., **30**: 253–263.

Fillippov M.P., Kuzminov V.I. (1971): Photometric determination of methoxyl groups in pectin substances. Zh. Anal. Khim., **26**: 143–146.

Hexeberg S., Hexeberg E., Willnmsen N., Berge R.K. (1994): A study on lipid metabolism in heart and liver of cholesterol- and pectin-fed rats. Br. J. Nutr., **71**: 181–182.

Judd P.A., Truswell A.S. (1985): The hypocholesterolaemic effects of pectins in rats. Br. J. Nutr., **53**: 409-425.

Kartel M.T., Kupchik L.A., Veisov B.K. (1999): Evaluation of pectin binding of heavy metal ions in aqueous solutions. Chemosphere, **38**: 2591–2596.

Klavons J.A., Bennet R.D. (1995): Preparation of alkyl esters of pectin and pectic acid. J. Food Sci., **60**: 513–515.

Müllner S., Weisner M., Ahlers M. (1993): Pectin derivatives as adsorbents for removal of bile acids. DE patent No. 41 36 322 A1, 6 May 1993.

Sinitsya A., Čopíková J., Prutyanov V., Skoblya S., Machovič V. (2000): Amidation of highly methoxylated citrus pectin with primary amines. Carbohydr. Polym., **42**: 359–368**.**

Tribet C. (1998): Hydrophobically driven attachments of synthetic polymers onto surfaces of biological interest: Lipid bilayers and globular proteins. Biochimie, **80**: 461–473.

Yevdakov V.P., Khorlina I.M., Khelemskaya H.M. (1972): Use of phosphorus(III) acid amides for preparing galacturonan amides. Zh. Obshch. Khim., **43**: 388–393.

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Souhrn

Synytsya A., Čopíková J., Marounek M, Mlčochová P., Sihelniková L., Blafková P., Tkadlecová M., Havlíček J. (2003): **Příprava *N*-alkylamidů vysokomethylovaného citrusového pektinu.** Czech J. Food Sci., **21**: 162–166.

Deriváty vysokomethylovaného (HM – highly methylated) citrusového pektinu (DM = 73 %) byly připraveny pomocí heterogenní amino-dealkoxylace (aminolýzy) s pěti vybranými alkylaminy. Reakce probíhala v prostředí *N*,*N*-dimethylformamidu při 8 °C (*n*-butylamin), 25 °C (*n*-hexylamin) a 45 °C (*n*-oktylamin, *n*-dodecylamin a *n*-oktadecylamin). Všechny deriváty byly převedené do kyselé formy promytím kyselým 50% ethanolem. Vzorky byly analyzovány pomocí elementární organické analýzy, difuzně reflexní FT-IR spektroskopie a 13C CP/MAS NMR. FT-IR a NMR spektra prokázala přítomnost alkylamidových skupin v derivátech. Stupeň amidace *N*-alkylpektinamidů (*DA*), molární (Y_n) a hmotnostní (Y_m) výtěžky reakce byly spočítané na základě výsledků elementární analýzy. Hodnoty *DA* derivátů byly v rozmezí 39–55 %, což odpovídá hodnotě Y_n 54–75 %.

Klíčová slova: HM citrusový pektin; amino-dealkoxylace (aminolýza); *N*-alkylpektinamidy

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