HYDROPHOBICALLY MODIFIED PECTATES AS NOVEL FUNCTIONAL POLYMERS IN FOOD AND NON-FOOD APPLICATIONS

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Butyl and hexyl amides of pectate with various amidation degrees were prepared from citrus pectin by means of alkylamidation of methyl-esterified pectins, followed by the total alkaline pectin methyl esters hydrolysis. These water soluble derivatives were characterized chemically as well as by elementary analysis and FT-IR spectroscopy. All prepared pectate amides exhibited the excellent emulsifying efficiency, and pectate hexyl amide also the ability to form stable foam. As the results of the study on the effect of pectin with DE 66% on the function of small intestine in pectin fed rats, the increase of specific activity of alkaline phosphatase, maltase, and aminopeptidase and the decrease of food utilization was demonstrated. The pectin derivatives might serve as emulsifiers and foaming additives in food production and other areas as well as nutraceuticals for obesity treatment.

Keywords: Pectin, Modification, Pectate alkylamides, Surface activity, Intestine enzymes, Obesity

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INTRODUCTION

Pectin is one of the commercial polysaccharides produced from agricultural by-products- citrus peels and apple pomace. These pectins are complex acidic polysaccharides with a linear backbone of (1-4)-α-D-galacturonic acid units, which are partially methylesterified and bear as side chains neutral sugars of the arabinogalactan type (Thakur et al. 1997). There is a continuing interest in exploitation of the pectin component from other plant residues. The expanding uses of pectin within the food and pharmaceutical industries increases the demand for pectins and pectin-rich plant sources. Next to the citrus peels and apple pomace, various other agricultural by-products are available, such as sugar beet pulp (Michel et al. 1985), pumpkin peel (Jun et al. 2006), sunflower heads, grape and olive pomaces, etc. (Thakur et al. 1997).

Pectin is widely used in the food industry as a hydrocolloid additive with gelling, thickening and stabilizing properties, as well as recognized as a dietary fibre playing a significant role in reducing the risks of the high life style-related diseases - cardiovascular and gastrointestinal ones, obesity, diabetes, etc. (Prosky 2001). Among the many areas in obesity research, diet supplementation with pectin suggested as a fat-replacer, decreased

digestion and absorption of nutrients (Dvir et al. 2000; Drochner et al. 2004) and function of the small intestine (Chun et al. 1989). In relation to the prevention and treatment of obesity, there is a lack of knowledge on the effect of the structural features of pectin (such as the degree of methylesterification) as well as of partial hydrophobic modification, which are assumed to contribute to intermolecular interactions with the intestinal mucous membranes.

Hydrophobically modified pectins, such as alkyl esters of pectin and pectic acid (Klavons and Bennet 1995; Pappas et al. 2004) have been suggested as emulsifiers in cosmetic and pharmaceutical cream formulations, and as stabilizers or beverage clouding agents. Pectin amides (Reitsma et al. 1986; Anger and Dongowski 1988; Sinitsya et al. 2000) were reported to possess good gelling properties important in the low sugar diets, and useful as sorbents (Sinitsya et al. 2004). These pectin amides contain both methyl ester and amide groups. To our best knowledge, pectin amides without methyl ester groups (pectate amides) have not yet been studied.

The aims of the present paper were

- (i) to prepare a novel series of pectate derivatives with some carboxyl groups transformed into C_4- and C_6-alkylamides using the commercial citrus pectin as model pectin and characterize their structural and surface active properties, and
- (ii) to study the changes in the specific activity 'in vivo' of some enzymes of the small intestine and their possible role in the mechanisms that influence the rate of nutrient absorption under high fat diet feeding conditions using the model citrus pectinate.

EXPERIMENTAL

Materials

Commercial citrus pectin with degree of methylesterification DE = 66% (PE66) was from Danisco, Smiřice (Czech Republic). It contains 85% galacturonic acid and 15 % neutral sugars. The highly esterified pectin with DE 93% (PE93) was prepared from the commercial pectin at the Institute of Chemistry, Slovak Academy of Sciences (Bratislava, Slovakia) by esterification in acidic methanol. Both pectin preparations were used for the synthesis of amidated derivatives and physiological studies. The commercial citrus pectin amide (PE-NH2) was kindly donated by Danisco (Smiřice, Czech Republic). The butyl and hexyl amines were from Merck (Germany). Alkaline phosphatase (AP) and aminopeptidase (AMP) were from Sigma-Aldrich (USA), and maltase was from Glycosynth (UK). High-fat (HF) diet was from Research Diet (USA).

Analytical Methods

DE was determined by precipitation of the insoluble copper pectates and pectinates (Tibenský et al. 1963). The degree of amidation (DA) was calculated using the following equation (Sinitsya et al. 2000):

$$\% \text{ DA} = \%N / \%C \times [6 + DE/100 + (K-1) \times \%N / 14] \times 100, \quad (1)$$

where the % of carbon (C) and nitrogen (N) were determined by elementary analysis using analyser model 240 (Perkin-Elmer). Fourier-transform infrared (FT-IR) spectra of the samples (2 mg/200 mg KBr) were obtained on the NICOLET Magna 750 spectrometer with DTGS detector and OMNIC 3.2 software using 128 scans at a resolution of 4 cm^{-1}.

Synthesis of Pectate Alkyl Amides

The synthesis was carried out in two steps. In the first step the reaction was carried out with primary alkyl amines (butyl, hexyl) in a heterogeneous system using methanol at 5°C for 10–20 h according to Sinitsya et al. (2000). In a typical experiment, the pectin (15 g) was suspended in 150 ml dry methanol and hexylamine (90 ml) was added under stirring. The reaction proceeded at 5°C for 20 h. Then the products were washed with chloroform to remove the unreacted amine. The wet samples were treated with 0.1 M HCl in ethanol–water mixture (2:1, v/v) to convert carboxylates into their protonated form, washed with neutral ethanol, and air-dried yielding pectinic acid alkylamides. In the second step, these derivatives were further deesterified in alkaline medium in a suspension of 70% ethanol for 12 h, and the obtained pectate alkylamides were recovered by successive washing with ethanol and acetone, and drying on air.

Surface-Activity Testing

The emulsifying efficiency was tested on emulsions of the oil in water (O/W) type as described by Sroková et al. (2003). The emulsion was prepared by mixing a solution (0.05 g of the derivative in 9 ml water) and 1 ml of paraffin oil dyed with SUDAN IV in the laboratory mixer at 20,500 rpm for 1 min. The stability of the emulsion was estimated at three different time intervals after the emulsions had been prepared, i. e. 5 min (h_1), 1h (h_2) and 24h (h_3), and expressed in terms of the height (mm) of the oil and cream layers formed on the surface of the emulsion. The surface tension of aqueous polysaccharide solution in the concentration range 0.015-2.5 $g.l^{-1}$ was determined at 25°C using the Du Nouy ring apparatus.

Enzyme Activity testing

Male Sprague-Dawley rats (30 day old) fed with high fat/high energy (HF) diet (4.04 kcal/g; 14.5% energy as protein, 30% as fat and 55.5% as carbohydrate) were divided into three groups: 1. control group (C), which was allowed free access to HF diet, 2. pectin group (P) receiving the same diet containing 15% (w/w) of the citrus pectin (PE66), 3. PF group, pair-fed to food intake of pectin fed group for 10 days. The activities of alkaline phosphatase (AP), maltase and aminopeptidase (AMP) were tested using a modified simultaneous azo-coupling method (Lojda et al. 1979, Nachlas et al.1960). Enzyme activity in a cryostat tissue sections (8 μm) was histochemically (cytophotometrically) analysed with a Vickers M85a microdensitometer. The measurements were performed using a x 40 objective, an effective scanning area of 28.3 μm^2 and a scanning spot 0.5 μm in at least 30 brush border areas along the villus length in five sections of the jejunum. The integrated absorbance of enzyme activity was calculated as the absorbance values recorded by the instrument in min/mm^{-3} brush border ± SE and these mean values were referred to one animal.

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RESULTS AND DISCUSSION

The alkylamidation of the model pectins was performed in two-steps by: (i) introduction of alkylamide groups yielding the intermediate derivative of pectinic acid alkylamide and (ii) removal of the methoxyl groups yielding the pectate alkyl amides. All derivatives were water-soluble. The derivatives were characterized by FT-IR spectra (Fig. 1a). The vibrations of the Amide I and Amide II at 1655 cm^{-1} and 1550 cm^{-1}, respectively, as well as the C=O streching vibration of the ester and carboxylate groups at 1751 cm^{-1} and 1600 cm^{-1}, respectively, were differentiated using peak-fitting (Fig. 1b). The chemically determined degree of esterification (DE) and degree of amidation (DA) of both the intermediate and final products are sunmmarized in Table 1. As seen in Table 1, the chemical modification of pectins was accompanied by depolymerization, indicated by the decrease of the intrinsic viscosity.

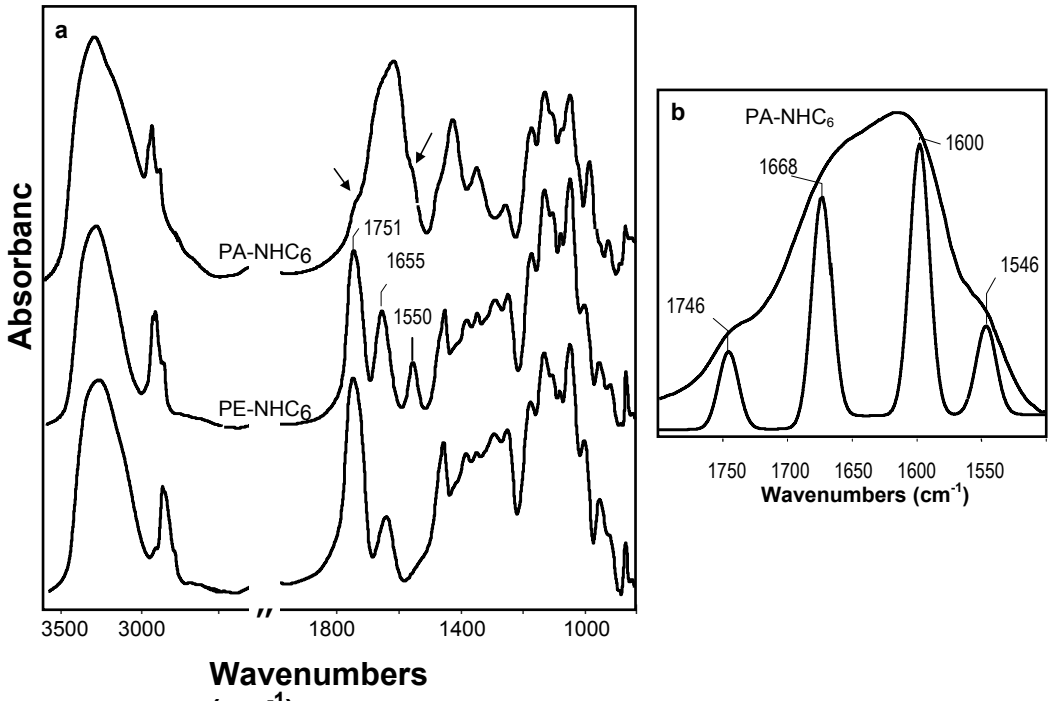

Fig. 1. FT-IR spectra in KBr of (a) the starting pectin (PE93), pectinic acid hexylamide (PE-NH_6) and pectate hexylamide (PA-NHC_6), and (b) the partial spectrum of PA-NHC_6 obtained by peak-fitting. The arrows indicate the Amide I and Amide II bands.

The surface activity of the original pectins, their butyl and hexyl amide intermediates and final pectate amides were tested for surface tension and emulsifying efficiency. All tested samples gave stable oil-in water emulsions.

The substitution of the methyl ester groups by alkyl amide groups resulted in very pronounced foaming, depending on the alkyl length and DA. With the hexyl derivatives the foam was stable after 24 h. Despite these properties, the surface-activity depressing effect of the derivatives was very low (69.7-59.8 $mN.m^{-1}$). Similar effects were observed with polymeric surfactants prepared from other polysaccharides such as from hydroxyethylcellulose (Sroková et al. 2003).

Table 1. Analytical Data and Surface Active Properties of Pectins and their Derivatives

Sample	DE	DA	[η]	Oil/Cream layers[a] (mm/mm)			γ_{min}	c.m.c.
	%	%	($ml.g^{-1}$)	5 min	1h	24 h	mN.m-1	$g.l^{-1}$
Original pectin								
PE66	66	0	434	0/0	0/0	0/12	nd	nd
PE93	93	0	132	0/8	0/11	0/11	nd	nd
Pectinate amide and alkyl amides								
PE-NH_2	31	24	395	0/0*	0/0*	0/10*	59.8	1.08
PE-NHC4	85	13	92	0/0*	0/8*	0/11	69.7	1.37
PE-NHC6	nd	nd	176	0/0*	0/0*	0/7*	62.7	0.62
Pectate amide and alkyl amides								
PA-NH_2	0	23	229	0/0	0/4	0/9	68.8	1.25
PA-NHC4	0	15	42	0/2	0/7	0/8	no	no
PA-NHC6	3	30	85	0/0*	0/10*	0/10	63.4	0.31
Tween 20				0/0	0/0	0/4		

[a]Oil/water emulsion: Height of oil and cream layers formed on the surface of the emulsion after 5 min, 1 h and 24 h; * Foaming; Tween 20, commercial synthetic emulsifier.

As a preliminary study of the effect of pectin and their derivatives on the function of the small intestine, the citrus pectin (PE66) was tested for changes in the activity of the small intestine enzymes AP, maltase and AMP in pectin fed rats. As illustrated in Table 2, the activity of these enzymes in the pectin group animals (P) significantly raised by 18%, 25%, and 23% respectively, in comparison with values of the control animals (C). These results are in accord with the reports about the increased activities of intestinal brush border bound enzymes using diet supplemented with pectin (Chun et al. 1989; Farness and Schneeman 1982).

The observed functional changes were also associated with significantly decreased food intake, food efficiency, as well as with significantly lowered epididymal plus retroperitoneal fat pad weight. These somatic changes were also associated with the alteration of the weight gain parameters (Control 48.0±2.1, Pectin 22.6±1.5*, PF±29.1*). Moreover, in the pectin fed animals (PF) as compared to C animals, the reduced intake of high fat (HF) diet did not change the intestinal enzyme and body fat parameters. This indicates that up-regulation of enzyme activities in P group is rather a consequence of specific effect of pectin than the decrease of luminal nutrition. Further studies with other pectins and pectin amide derivatives are in progress. The obtained results about the effect of pectin on the function of the small intestine can expand the knowledge on the

participation of the small intestine in the mechanisms that might play a key role on influence development of obesity and associated feeding and body fat disturbances.

Table 2. Effect of Citrus Pectin (PE66) on the Function of Small Intestine in Pectin-Fed Rats

Group	AP	Maltase	AMP	Body fat %	Food intake g/day	FE
Control	13.4±0.6	14.2±0.5	14.3±0.5	0.73±0.07	12.3±0.5	0.39±0.01
Pectin	15.8±0.2*	17.8±0.9*	17.6±0.9*	0.27±0.03*	9.6±0.8*	0.24±0.02*
PF	13.1±0.1#	14.6±0.4#	13.7±0.5#	0.64±0.03#	10.1±0.4*	0.30±0.01*#

Values are means ± SE (n = 8 animals/groups); Enzyme activity is given as a density values in jejunal enterocytes at wavelength of 520 nm; AP (Alkaline phosphatase), AMP (Aminopeptidase) and Maltase are intestinal enzymes; Body fat (%) represents epididymal plus retroperitoneal fat pads weight; FE (food efficiency) is given as the weight gain/food intake; * significantly different from C group; # significant differences between P and PF groups at $P<0.05$ by Tukey's test after ANOVA.

CONCLUSIONS

1. The results suggested that the novel butyl and hexyl amide pectates with DA ranging between 15-30% represent polymeric surfactants with excellent emulsifying efficiency, and in the case of the hexyl derivatives the ability to produce stable foams.
2. The derivatives are potential polysaccharide-based biodegradable surfactants useful in various technical applications and industrial processes.
3. The results of the effect of the pectin on the energy homeostasis of rats suggested that the enhanced activity of enzymes and decreased food utilization observed in pectin-fed rats might play an important role in prevention the development of obesity on high fat diet.

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