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Physico-chemical evaluation of hydrophobically modified pectin derivatives: Step toward application

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Abstract

Present study reports synthesis and physico-chemical evaluation of hydrophobically modified pectin derivatives, obtained by reacting of pectin with di-acyl chlorides (glutaryl and sebacoyl chloride). Depending on length of the inserted carbon chains, the acylation resulted in possible formation of mono-grafted (isolated chains) and bi-grafted (chemical gels) structures. The structural features of obtained derivatives were investigated using FTIR spectroscopy, confirming the successful synthesis. The concentrated aqueous solutions of modified pectin showed interesting rheological properties, having lower values of apparent viscosity compared to neat pectin. Since the GPC analysis indicated that no degradation occurred, the viscosity decrease was explained by more heterogeneous organization within modified pectin solutions (microparticles together with sticky polymer entanglement). A shift in particle size distribution proved that proposed modifications also affected pectin solution properties in diluted regime. The modified samples turned to be more sensible to thermal degradation than neat pectin, whereby the increasing size of flexible acyl chains attached to a polymer backbone reduced the glass transition temperature. The hydrophobicity of obtained derivatives was evaluated by sessile drop and du Nouy ring methods. It was found that acylation enhanced hydrophobicity of the pectin molecule, while hydrophobically associative character turned to be inconsistent in aqueous and non-aqueous environment.

Keywords: pectin esterification, hydrophobicity, surface tension

1. Introduction

Polysaccharides belong to a special class of biopolymers with peculiar features such as biocompatibility, biodegradability, bioadhesivity and nontoxicity. The usefulness of these water-soluble carbohydrate polymers undoubtedly relies on the wide range of their functional properties coupled with wide availability and usually low costs. The research activities related to the exploitation of polysaccharides are today spanning many new avenues, which concern the development of novel materials to be implemented in a number of domains [1]. Pectin forms the most complex class of polysaccharides, mainly composed by high molecular weight heterogeneous groups of glycanogalacturonans and acidic structural polysaccharides with diverse structures. Pectin backbone consists of (1→4)-α-D-galacturonic acid residues, some of which are partially methylesterified. The degree of esterification (DE) of the galacturonic acid residues has a determinant influence on the physico-chemical properties of pectins, mainly in terms of complexation and gel formation [2]. Having excellent gelling, stabilizing and film forming properties, pectin has been recognized as an attractive novel biopolymer material, which can be successfully employed in food and pharmaceutical industry, health promotion and cosmetic applications [3]. Being recognized as "green'', pectin represents a suitable polymer for the development of bio-based packaging films. Furthermore, the FDA regards pectin as generally safe (GRAS), so this polymer can afford low-calorie edible films and coatings. Still, pectin based materials are far from being adequately exploited due to certain limitations when applied in some specific areas. For example, the tendency of forming lumps and agglomerations causes a serious problems during dissolving [4]. Extremely high hydrophilicity of pectin molecule causes rapid hydration, swelling and erosion and reduces the ability to control drug release efficiently in different dosage forms [5]. The hydrophilic nature also has undesirable influence on the barrier properties of pectin based films due to ineffective moisture transfer [6].Therefore, the functionality of pectin often needs to be strengthened or altered to satisfy specific applicative requirements. Numerous hydroxyl and carboxyl groups distributed along the backbone, as well as a certain amount of neutral sugars present as side chains make this polysaccharide a suitable candidate for chemical and physical treatments. Based on distinct reaction methods, chemical modification of pectin may be conducted in various ways, including substitution (alkylation, amidation, quaternization, thiolation, sulfation, oxidation, etc.), chain elongation (cross-linking and grafting) and depolymerization (chemical, physical, and enzymatic degradation) [3].The properties of pectin could be also improved by physical modification which involves noncovalent bonding, such as ionic interactions, hydrogen bonds or hydrophobic interactions[7]. Physical cross-linking represents one of the prime techniques used for the modification of pectin and it has been successfully applied for the enhancement of its mechanical and barrier properties as well as its water resistance [8].

Hydrophobically modified derivatives of pectin have been gaining ground for the last decade. An introduction of non-polar residues increases hydrophobic character of pectin macromolecules, offering divergency in physicochemical properties. Alkylation of carboxyl functions was proved to be a successful method for such modifications [9, 10]. It was found

that derivatives carrying long alkyl chains showed interesting behaviour which can be used for control of aqueous phases' rheological properties [11]. Polymers bearing shorter alkyl groups may also present some interesting properties notably at interfaces [12]. Moreover, it was evidenced that intramolecular and/or intermolecular (aggregates) specific 'hydrophobic' interactions could exist in dilute solution [13]. Slightly substituted polymers with surface-active properties can be valorized in different ways, so deeper understanding of the specific features adopted by hydrophobic derivatives of pectin could be essential from application point of view.

Seeking after improved functionality, the aim of this work was synthesis and detail evaluation of structural (FTIR, GPC), bulk (DSC, TGA) and macroscopic properties (particle size distribution and surface properties) of hydrophobically modified pectin. Targeting saccharide oxygen as a nucleophile, modifications were performed via esterification of alcoholic functions using chlorides of dicarboxylicacids: glutaric and sebacic. The insertion of the di-acyl residues of different carbon chain length was expected to enhance hydrophobic nature of the molecule, whereby mono-grafted (isolated chains) or bi-grafted (chemical gels) products are possible to obtain. Calculated per single galacturonic acid unit, the extent of modification is given in terms of the molar ratios of the reactants, 1:3 and 1:15, meaning that every third and fifteenth unit was acylated. The special emphasis is put on how the resulted structural changes affected water-polymer interactions.

2. Experimental

2.1 Materials

High-methoxyl apple pectin (70–75% DE), glutaryl chloride and sebacoyl chloride were purchased from Sigma-Aldrich Company. Dimethyl sulfoxide, absolute ethanol, acetone and pyridine were supplied by Merck. All chemicals were analytical reagents and used as received.

2.2 Synthesis of acylated derivatives of pectin

Synthesis of pectin derivatives was carried out via conventional esterification using di-acyl chlorides [14]. Prior use, all chemicals were dried under vacuum at 40°C until constant weight was obtained. The solution of 1.5 % w/v was prepared by dissolving pectin in DMSO for 24h at 60°C. The calculated amount of esterification agent was added dropwise into pectin solutions, which previously had been cooled down to 5°C. The pyridine, used to remove hydrogen chloride by product and to catalyze the reaction, was added in a stoichiometric amount. The mixture was left to react under stirring for 12h at 50°C. When the reaction was completed, the mixture was treated with absolute ethanol, used as precipitant. The separated precipitate was washed three times with absolute ethanol under stirring for 1h. Finally, the products were washed with acetone. The resulting derivatives were left to dry under vacuum at 40°C, to reach constant weight.

2.3 Fourier Transform Infrared Spectroscopy (FTIR)

The FT-IR spectra were recorded on a spectrophotometer Bomem MB100. The thin films, obtained by vacuum drying of 1% w/v aqueous solutions, were placed between ZnSe windows and spectra were recorded in transmittance mode for the wavelength range of 600–4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.4 Gel Permeation Chromatography (GPC)

The measurements were performed by using a GPC Malvern equipped with a Viscotek TDA apparatus with three detectors (refractive index, right angle laser light scattering (90°), low angle laser light scattering (9°) and a viscometer). The eluent was water solution containing NaN_3 0.02% and $NaNO_3$ 0.1 M. The flux was 0.5 ml/min with the injection volume of 100 μl. The used columns were a pre-column TSK and a mixed $TSKgelGMPW_{xl}$ (Tosoh Corporation). The samples were analysed by using a conventional calibration with 9 samples of Pullulan with M_P ranging between 1220000 and 1000 Da. The concentration of samples was about 1.5 – 2.0 mg/mL. All the samples, standard and modified pectins were analysed in duplicate.

2.5 Determination of apparent viscosity

The investigated solutions (4 % w/v) were prepared by dissolving samples in 0.025 M NaCl via gentle stirring for 18h at room temperature and centrifuged (3000 g, 15 min) in order to avoid entrapped air bubbles [15]. Polymer solutions had been stored at 4 °C overnight before measurements were carried out. Experiments at imposed shear stress were performed on Rheometrics, model RMS-605 fitted with a parallel plate geometry (25 mm diameter, 1.0 mm gap). Temperature control of a bottom plate (25 ± 0.1°C) was achieved with a Peltier system.

2.6 Thermal analysis

2.6.1 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry measurements were performed using a TA DSC-Q2000 instrument equipped with a TA Instruments DSC cooling system under a nitrogen purge gas flow of 30 $mLmin^{-1}$. Indium was used to calibrate the calorimeter in temperature and energy. Crystallization is an exothermic process, and the heat evolved during the phase transition may cause some thermal gradients within the sample. As a consequence, transitions can occur at temperatures that do not correspond to those detected by the instrumentation [16]. The thicker the sample, the more critical this problem is. In order to reduce these issues, sample mass was limited to 6.0 ± 0.5 mg. All the specimens were equilibrated at 0°C and heated up to 100°C at 20°C min^{-1}. Then an isotherm step was performed at 100°C for 30 min in order to remove free and bounded water. Afterwards, the samples were cooled to 0°C at 10 °Cmin^{-1}, thermally stabilized for 1 min and then re-heated up to 200°C at 20°C min^{-1}. Each sample was tested three times to confirm repeatability of measurements.

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2.6.2 Thermogravimetric analysis (TGA)

Thermogravimetric analyses were performed on a Mettler-Toledo TG-SDTA 851 thermobalance, under nominal nitrogen flow of 30 mLmin^{-1}, in a temperature range of 25 - 600 °C, at a heating rate of 10 °C min^{-1}. The measurements were performed on 5 ± 0.5 mg samples placed in ceramic crucibles. Each sample was tested three times in order to confirm repetition of measurements.

2.7 Particle size distribution and Optical microscopy

Particle size distribution was measured by a laser light-scattering particle size analyser (Mastersizer 2000; Malvern Instruments). Prior to measurement, the samples were dispersed in 0.025 M NaCl solution (25°C), in an ultrasonic bath (at a frequency of 40 kHz and power of 50 W), for 3 min. The concentration of samples was 0.5% w/v, and the pH was found to be around 3.0. The same solutions were investigated afterwards by means of optical microscopy (LEICA DC 150).

2.8 Scanning Electron Microscopy (SEM)

SEM analyses were performed using a Jeol JSM 5800 scanning electron microscope with an acceleration voltage of 20 kV. Prior to SEM analysis, the 4% w/v solutions of neat and modified pectin were freeze-dried in order to conserve the structure without collapsing. The samples were cut in half in frozen state. Before analysis, samples' cross-sections were covered with platinum vapour in a LEICA SCD005 nebuliser.

2.9 Surface properties measurements

Surface properties of neat and modified samples were determined in two different approaches: water droplet contact with solid film surface (dynamic contact angle measurement) and from 0.5 % w/v solutions of the investigated samples (du Nouy ring method).

2.9.1 Dynamic contact angle measurement

Surface properties of the samples were investigated by contact angle (CA) measurements using the sessile drop method. Contact angle is defined as an angle between film surface and tangent line at the contact point of water droplet with surface. Measurements were carried out in constant temperature using a Theta Optical Tensiometer (Attension, KSV). Contact angles were measured by dispensing an approximately 4μL droplet of ultrapure water onto film substrate and repeated several times to control reproducibility. The films were casted form 2% w/v water solutions of referent (neat pectin) and modified samples. Prior to measurements, the films were dried under vacuum at 40°C until a constant weight was reached [17].

2.9.2 Du Nouy ring method

Surface tension of all samples was tested using a du Nouy ring tensiometer (SEO DST 30 Surface tension metre). For each test, 20 ml of 0.5 % w/v solution was placed in a 50 ml beaker. Distance between an immersed ring and liquid surface was fixed at 5 mm to ensure a clean meniscus break on the immersed platinum–iridium ring. The circumference (R) of a

ring and its dimension ratio (R/R0) were 0.5960 and 53.3906 cm, respectively. Based on the input parameters, i.e. ring dimension, sample density and temperature (25°C), calculations were made by a tensiometer pre-programmed software [18].

3. Results and Discussion

3. 1 Preparation of acylated pectin derivatives

The pectin derivatives (carboxylate esters) with different acyl chain lengths and extent of modification were prepared via reaction of pectin hydroxyl groups and di-acyl chlorides (Scheme 1) [19]. Since the reactivity of OH at C2 and C3 atoms in nonaqueous mediums was found to be approximately similar, the ester bond could be formed via one or another OH group with the same probability [20]. The pectin derivatives were named as G or S, depending on the type of acylating agent (G - glutaryl chloride; S- sebacoyl chloride), and numbered as 1/3 or 1/15 accordingly to the molar ratio between acylating agent and galacturonic acid unit. The proposed esterification mechanism with two possible reaction patterns is presented in Figure 1.

Figure 1

3.2 FTIR analysis

The FT-IR spectra of pectin modifications (G1/3, G1/15, S1/3 and S1/15) are shown in Figure 2. A spectrum of neat pectin is reported on each plot for comparison.

Figure 2

The neat pectin spectrum showed a broad band around 3400 cm^{-1} deriving from OH stretching vibrational modes due to inter- and intramolecular hydrogen bonding of galacturonic acid. A presence of moderately intense bands in the range of 2830-2995 cm^{-1} was ascribed to C-H stretching vibrations. Strong absorption bands at approximately 1746 cm^{-1} were attributed to stretching of C=O groups from non-ionized carboxylic acid (methylated or protonated). Its ionization led to their disappearance and caused the appearance of stretch modes of COO^{-} in the range of 1616-1650 cm^{-1} and 1438-1443cm^{-1}. Bands at 1357 cm^{-1} and 1228 cm^{-1} corresponded to -CH$_2$ wagging vibrations, while bands at 1368 cm^{-1} and 920 cm^{-1} corresponded to -CH$_3$ scissoring and rocking vibration of methyl ester groups, respectively [21]. Strong bands at 1146 cm^{-1} and 1105 cm^{-1} were indicative for stretching vibrations of ether R-O-R and cyclic C-C bonds in a ring structure of pectin molecules. The band at 830 cm^{-1} related to the out of the plane deformation of COOH groups [22].
Going downfield, the first change in spectra of modified samples when compared with neat pectin spectrum was evidenced by a pronounced decrease in peak intensity ascribed to OH stretching vibrations (3400 cm^{-1}). Since the OH groups located at C-2 and C-3 atoms of

galacturonic acid were targeted as esterification centres, the above could be correlated with exhaustion of these groups throughout the modification process. Also, it could be closely related to restricted hydrogen interactions due to more hydrophobic nature of the modifications [23]. The appearance of discrete peak indicative for aliphatic C-H stretching modes at 2995 cm^{-1} could be reasonably explained by insertion of - $(CH_2)_n$ segments into a pectin backbone. An increase in ratio of peak area at 1746 cm^{-1} over sum of peak areas at 1746 and 1620 cm^{-1}, indicated formation of additional ester bonds between pectin hydroxyl groups and acyl group originating from esterification agents. An opposite trend, i.e. a decrease in the ratio, is observed in spectra of alkylated pectins, which were obtained in reaction between alkyl halides and carboxylic groups of pectin targeted as esterification centres. The above was explained by a translation of protonated carboxylic group to carboxylic anion, as a result of modification procedure [24]. Taking this into account, the results presented in Figure 2 could be interpreted as follows: a decrease in relative area, attributed to COO$^-$ vibration linkages, suggested that carboxylic groups of acylated derivatives mainly exist in their protonated form. This is more pronounced for the samples with higher acylation degree (G1/3, S1/3).

3.3 GPC

The GPC analysis was performed to determine the molecular weight distribution of modified pectin. Pectin molecule was found to be very prone to degradation under aggressive chemical agents, leading to a decrease in pectin molecular weight and loss of pectin functionality [25].

Figure 3

No significant differences in retention volume between the samples of neat and modified pectins were found, indicating no changes in molecular weight patterns as well as a lack of degradation during modification (Figure 3).

3.4 Viscosity

The amphiphilic systems exhibit original rheological properties in aqueous solution, which are mainly governed by inter- and intramolecular associations in specific system [26]. The behavior of the neat pectin and resulting derivatives in aqueous solution (4% w/v) was investigated and presented in Figure 4.

Figure 4

Within the range of investigated shear rates**,** all the modified pectin solutions showed a decrease in apparent viscosity when compared to the neat pectin. In the series where every third galacturonic unit of pectin chain was subjected to acylation, viscosity of corresponding solutions decreased in the following order: neat pectin>S1/3>G1/3 (Figure 4a). The same decreasing tendency was found for the samples modified with lower concentrations of acylating agent (S1/15, G1/15, Figure 4b).

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Since GPC analysis indicated that degradation did not occur, the described rheological behaviour could be explained by more heterogeneous organization within modified pectin solutions (microparticles together with sticky polymer entanglement) [27].

Interestingly, under a low share rates (0.1 - 0.3 s^{-1}), the G1/3 and G1/15 solutions behave in a similar way, but different with respect to S1/3 and S1/15. A sharp viscosity drop observed in this range could be correlated with breakdown of the specific hydrogen-bonded structures, formed probably due to favoured hydrogen interactions in these systems [28]. This phenomenon, affected by derivative structures, will be discussed in more detail in the next sections.

3.5 Particle size distribution and Optical microscopy

Interactions with water are essential for the physical stability of pectin and its application [29]. Different aspects of pectin/water interactions can be tested by a wide variety of analytical methods. Particle size distribution in specific solvent is a useful approach to obtain deeper insight into molecular interactions in investigated systems. Figure 5 shows particle size distribution of diluted solutions of neat and modified pectin.

Figure 5

The solution of neat pectin had a very broad distribution wherein the particles were ranging from 0.26 to 214 μm in size (Figure 5a). Particle size with cumulative distribution of 50% is known as medium particles diameter ($d_{V, 0.5}$)[30]. According to the calculated statistical data, the diluted pectin solution had $d_{V, 0.5}$ with 50% of the particles under 11.04 μm. The particles in smaller size range (0-1.21 μm) occupied less than 10% w/v, while 90% w/v was occupied by particles smaller than 81.17 μm. When compare to neat pectin, particle size distribution was found to be narrower and shifted towards lower size ranges for G1/3 and G1/15 (Figure 5b). Interestingly, the size ranges obtained for diluted solution of S1/3 and S1/15 were translated to higher size values (Figure 5c). The values are summarized in Table 1.

Table 1

For better understanding of the results, it is necessary to consider all inter- and intramolecular interactions that may be possibly formed in given colloidally dispersed systems. According to the structures of neat pectin and acylated derivatives (Figure 1), particle size distribution could be dictated by following phenomena: electrostatic repulsion forces due to high content of ionisable COOH groups; hydrogen bonding; hydrophobic attractive interactions caused by insertion of - $(CH_2)_n$ segments into pectin backbone; and/or cross-links possibly formed by bridging of pectin chains . As polyelectrolyte, pectin is very prone to an influence of pH. At pH < 3.5 (below the pKa), free carboxyl groups are mainly undissociated, while at pH > 4.5 (above pKa) they are mainly dissociated [31]. Dissociated carboxyl groups at higher pH are negatively charged and cause electrostatic repulsion of the macromolecules. The obtained acidic pH of 3.0 suppressed carboxylic groups ionization, so charge–charge repulsions were negligible for the investigated system.

In the neat pectin molecule structure, hydrogen bonds may be established between free carboxyl groups or hydroxyl groups of neighbouring molecules, while hydrophobic interactions mainly occur between methyl esters. The evidenced changes in particle size distribution clearly indicate that considered interactions were perturbed due to chemical modification. As previously stated, size ranges obtained for S1/3 and S1/15 solutions, were shifted to higher values due to coalescence of the particles and intensified hydrophobic interaction caused by insertion of di-acyl residues. Interestingly, this trend was more pronounced for the S1/15. Since di-acyl residues from sebacoyl chloride were found to be long enough to bridge chains of pectin molecule, this behaviour could be explained by formation of bi-grafted structures (chemical gels) with reduced swelling ability. The size ranges for G1/3 and G1/15 were found to follow the opposite distribution. The observed decrease in particles' diameter could indicate formation of mono-grafted structures of G1/3 and G1/15 derivatives. The presence of isolated chains with additional terminal carboxyl groups in these structures may hinder hydrophobic interactions and cause predomination of the hydrogen bonding. Presumably, carbon chain length of gutaryl chloride was insufficient to forefront hydrophobic effect as well as to bind together pectin chains. At low pH, an internal distribution of carboxylic groups within polymeric backbone becomes dominant and dictates an intensity of hydrogen interactions and therefore, properties of a solute. Stabilized by intramolecular hydrogen bonds, the particles did not show a tendency towards coalescence, hence the size distribution is more uniform [32]. The evidenced distributions are visualized by means of optical microscopy and resulting images are presented in Figure 6.

Figure 6

3.6 TGA

Figure 7a, b shows the TGA/DTG curves of neat and modified pectin samples.

Figure 7

Analysing the curves, two pronounced mass loss steps can be distinguished: the first, from ambient temperature up to 180 °C, and the second between 180°C and 600°C. The first degradation step was related to water loss [23]. It has been reported that there are three kinds of absorbed water in hydrophilic polymers: free, freezing bound and non-freezing water or bound water [33, 34]. Free water has the same phase transition temperature as bulk water and crystallizes at 0°C. Freezable bound water crystallizes below 0°C due to weak Van DerWaals interactions between polymeric chains and water molecules. Non-freezable bound water does not crystallize even when swollen sample is cooled down to −100°C because of strong physical interactions between polar moieties of hydrophilic polymers and hydroxyl groups of water molecules via hydrogen bonds [35]. Neat pectin degradation curve exhibit intense water evaporation step up to 100 °C, while the modified samples showed different thermal pattern of continuous mass loss in a temperature range of 30-180°C. The differences in water loss for

neat and modified pectin (Figure 7 a, b) may indicate that the proposed modifications affected water-pectin interaction. It can be assumed that a presence of hydrophobic domains in modified samples reduced a quantity of unbound water in the same storage conditions as for pure pectin. The second stage of mass loss was attributed to pectin degradation in the temperature range of 180–600°C. It was previously reported that degradation of about 60% of mass loss primarily derived from pyrolytic decomposition [23]. It consists of primary and secondary decarboxylation involving acid side groups and carbon in the ring. The most intense degradation step in a temperature range of 180-285°C (reaching maximum at 230°C) could be observed for both, i.e. neat and modified samples. Moreover, degradation of modified samples started at slightly lower temperatures when compared to neat pectin. This can be attributed to higher number of ester bonds in pectin derivatives resulting from pectin modification [36].

3.7 DSC

Glass transition (Tg) temperatures of neat and modified pectin samples are reported in Table 2. The data were collected from the second heating. A glass transition temperature (Tg) is a complex phenomenon which is dependent on many factors including intermolecular interactions, molecular weight, chain flexibility, branching and/or cross-linking density [37]. The results summarized in Table 2 indicated that structural changes caused by modification affected Tg values. When compared to neat pectin, all modified samples showed lower Tg values. Differences in Tg were more pronounced for formulations where every third galacturonic unit of pectin chain was acylated (G1/3, S1/3), while in case of G1/15 and S1/15 they were negligible. This is probably due an increase in free volume caused by insertion of di-acyl residues into a pectin backbone. Generally, the more free volume, the lower Tg values [38].

Table 2

3. 8 Surface properties

The hydrophobicity of pectin modifications was interpreted in terms of their surface properties, obtained by applying two different measurement approaches. Firstly, the surface properties were determined by dynamic contact angle measurement, as presented in Figure 8. It is well-known [39] that the water contact angle will increase with increasing surface hydrophobicity. The presented results (Figure 8) revealed that for the proposed modification water contact angle values increased in the following order: neat pectin<G1/15<G1/3≈S1/15<S1/3. Presumably, loss in free hydroxyl groups and insertion of hydrophobic domains into a pectin backbone resulted in decreasing hydrophilicity of modified samples [40]. Moreover, it was also proved that surface properties were affected by both acylation degree and length of inserted carbon chains.

Figure 8

When measured by the ring method (Table 2), the surface tensions increased in following order: S1/3<S1/15<Neat pectin<G1/15<G1/3. As a consequence of intensified hydrophobic interaction caused by a presence of sebacyl residue (C10), surface tensions of S1/3 and S1/15 solutions decreased as expected. On the contrary, surface tensions of G1/3 and G1/15 solutions increased when compared to neat pectin solution. This can be correlated with the assumed mono-grafted structure of G1/3 and G1/15 derivatives (Figure 1a). An increasing number of carboxylic groups, distributed along polymeric chains in aqueous environment, favoured hydrogen interactions causing an increase in surface tension [29]. Interestingly, the surface tension trend is turned to be inconsistent when different approaches were used.

3.9 SEM

Micrographs of cryogenically fractured cross-sections of freeze dried 4% w/v solutions of neat pectin and G1/3, G1/15, S1/3 and S1/15 modifications, are presented in Figure 9.

A sponge-like inner structure, rich in voids and wrinkles, was formed for all investigated samples probably due to migration of water molecules [41]. However, a comparative analysis of the presented morphologies evidenced some differences for modified samples. When compared to neat pectin, the microstructures of pectin modifications were stiffer and more wrinkled. This may be correlated with polymer-water interactions which had been affected by insertion of hydrophobic segments into highly hydrophilic pectin backbone. The modification with higher acylation degree, i.e. G1/3 and S1/3 (Figure 9 a, c), resulted in a microstructure with more compact arrangement and less prominent voids in comparison with G1/15 and S1/15 samples (Figure 9 b, d), probably due to poorer interaction with polar solvent.

Figure 9

4. Conclusions

Pectin was hydrophobically modified by covalent attachment of acyl chains of various lengths (C4, C10) at different degrees of acylation. The acylation involved possible formation of mono-grafted (isolated chains) or bi-grafted (chemical gels) derivatives structure. The modified samples showed interesting behavior in aqueous solutions useful for controlling rheological properties. Depending on the derivative structure, the water-polymer interactions were preferentially governed by one of the following phenomena: hydrogen bonding, hydrophobic attractive interactions, and/or cross-linking. The acylation affected thermal properties of neat pectin, which was confirmed from TGA and DSC analyses. Hydrophobic nature of the derivatives was determined both on the surface of solid the films and in the aqueous environment. The obtained results revealed a certain discrepancy when applying two

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methods of measurement, which was correlated by predomination of the specific interactions with respect to aqueous and non-aqueous environment.

In summary, the reported study represents a step toward overcoming limitations imposed by the hydrophilic nature of native pectin. The detailed evaluation of new-created functional properties of obtained derivatives provides the starting line for better control and fine-tuning of its properties with respect to the possible application (emulsifying agents, matrices for food packaging films, microparticulate and polysoap systems).

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Figure captions

Figure 1. Esterification mechanism (m=3 or 8)

Figure 2. FT-IR spectra of neat and modified pectin (lower part) with calculated areas of carbonyl region peaks (upper part): a) G1/3 and G1/15; b) S1/3 and S1/15.

Figure 3. Chromatograms of neat and modified pectins

Figure 4. Apparent viscosities of neat and modified pectin solutions a) G1/3 and S1/3; b) G1/15 and S1/15

Figure 5. Particle size distribution of diluted solution a) neat pectin; b) G1/3, G1/15; c) S1/3, S1/15

Figure 6. The visual display of diluted solution of neat and modified pectin a) G1/3; b) G1/15; c) S1/3; d) S1/15

Figure 7. Thermal degradation curves of neat and modified pectins, in a form of weight loss versus temperature curves (TGA) (a), and their derivatives (DTG) (b)

Figure 8. Contact angle measurements in a given time conducted with water drop on film surface

Figure 9. SEM micrographs of fractured cross-sections of freeze-dried solution (4% w/v) of neat and modified pectin a) G1/3; b) G1/15; c) S1/3; d) S1/15.

Figure 1
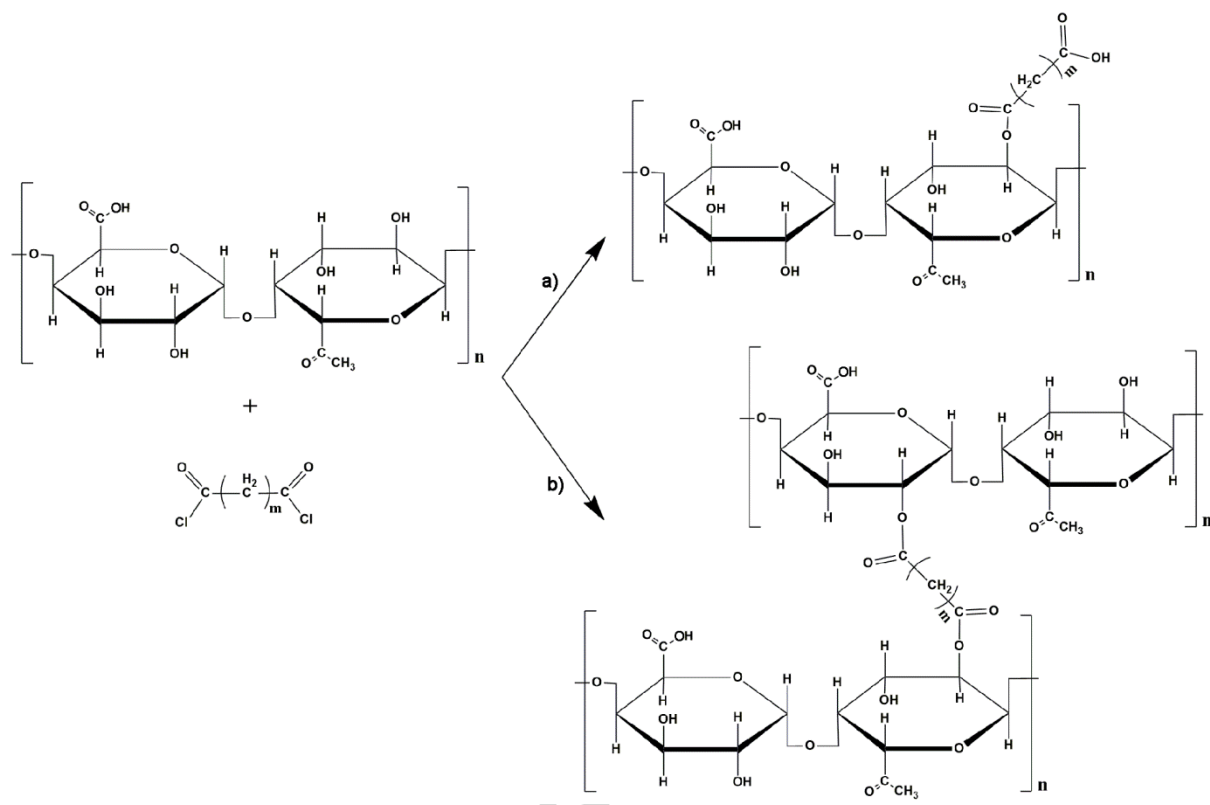
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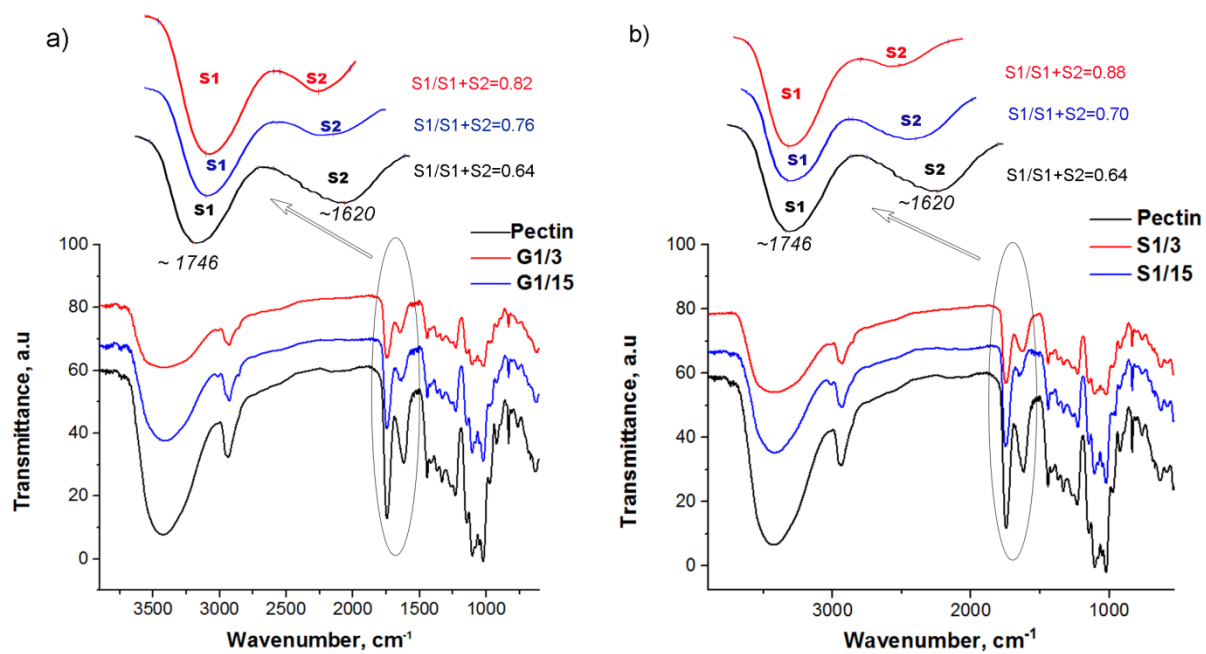
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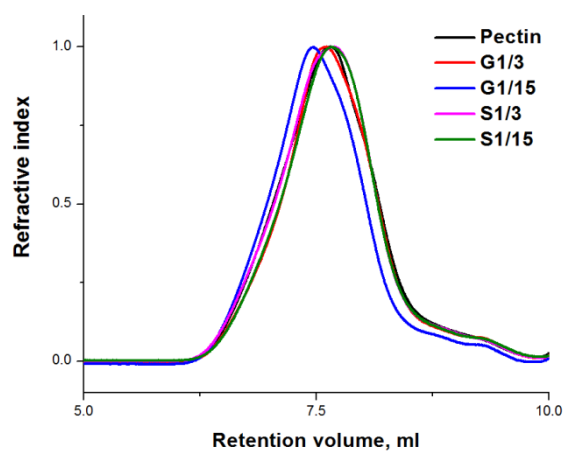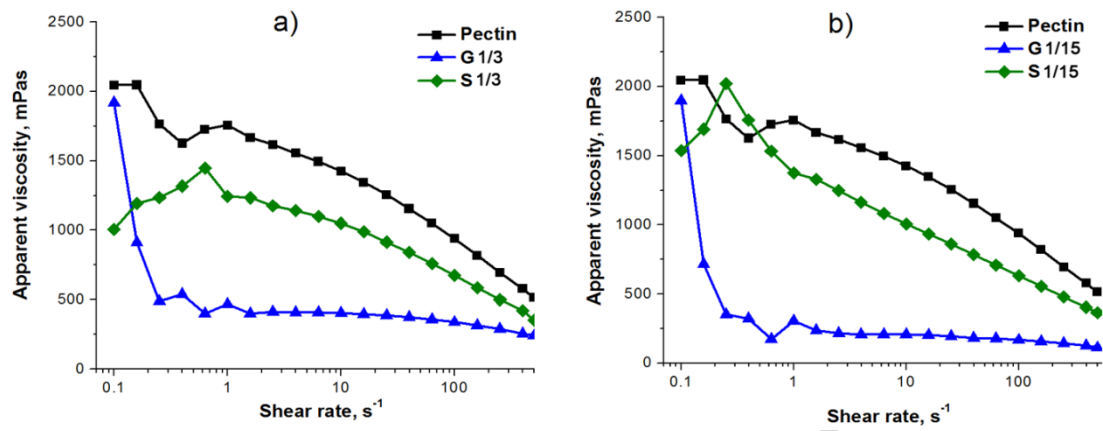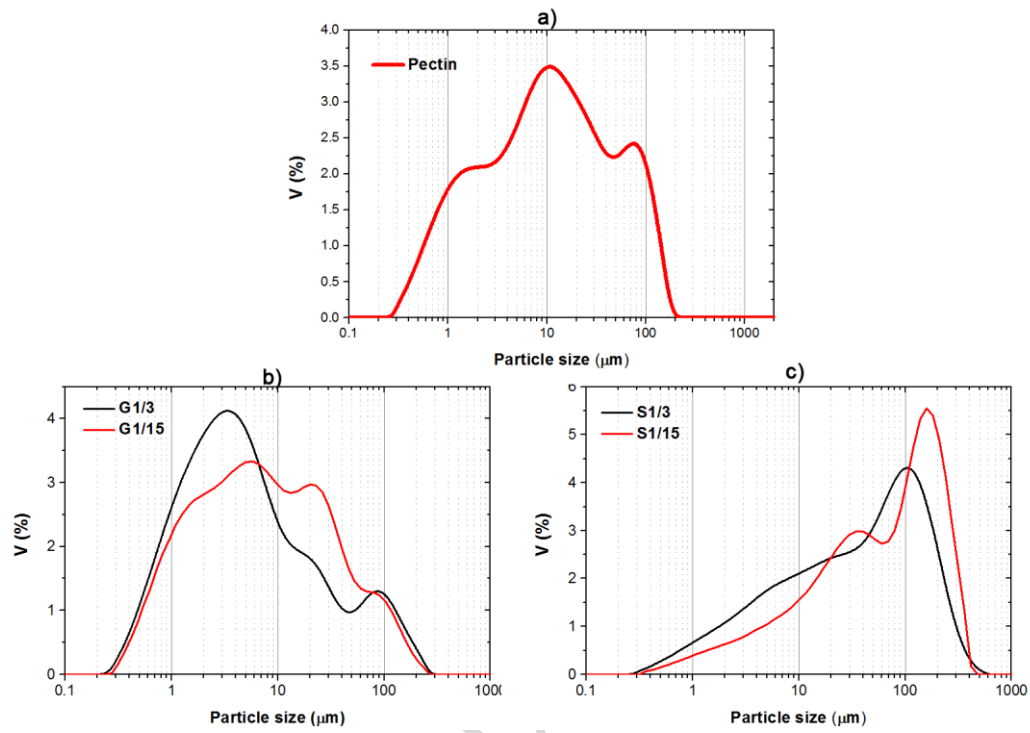
Figure 4

Figure 5

Figure 6
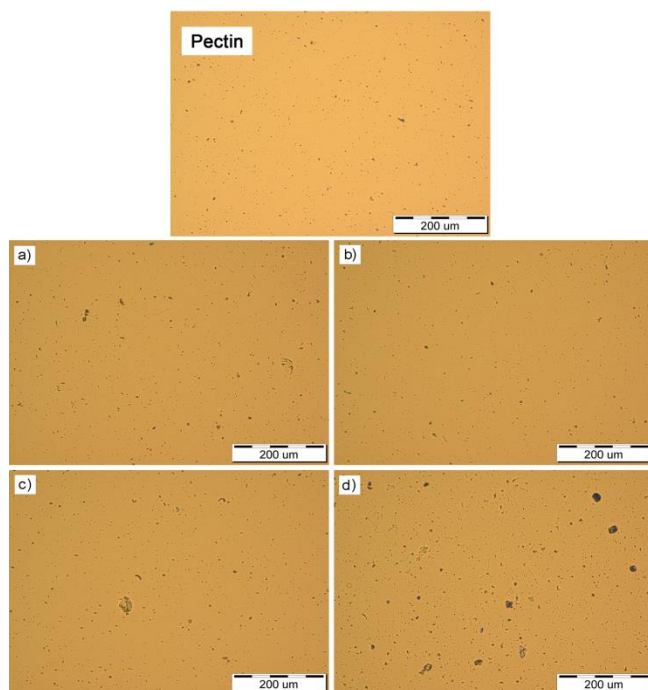
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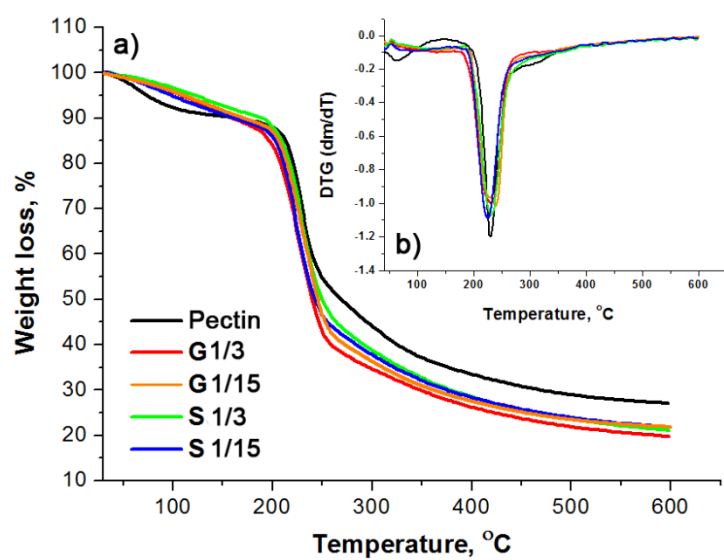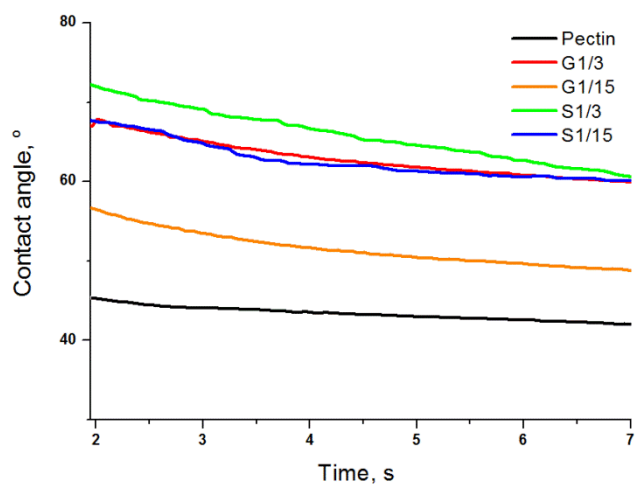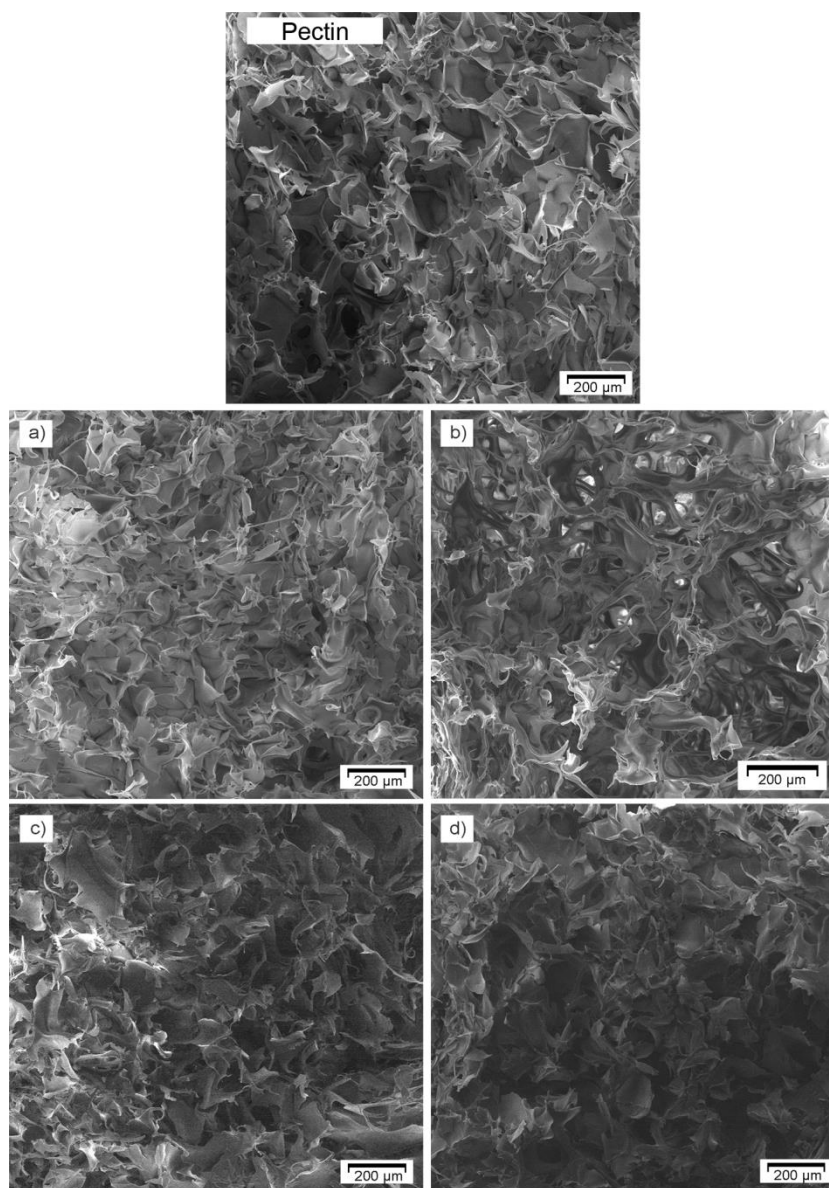
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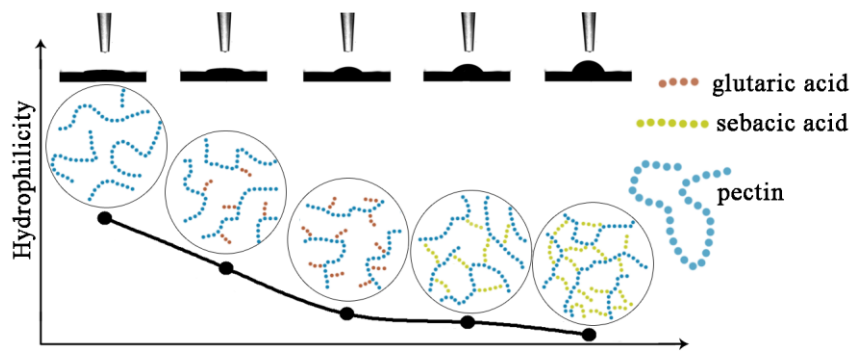
Figure 9

Table 1. Particle size diameters

Sample ID	$d_{V,0.1}$	$d_{V,0.5}$	$d_{V,0.9}$
Pectin	1.21	11.04	81.17
G1/3	0.98	4.57	59.70
G1/15	1.10	7.22	55.59
S1/3	2.95	41.82	185.75
S1/15	5.63	69.90	247.05

Table 2. The glass transition (Tg) temperatures and surface tension values of 0.5% w/v solutions (du Nouy ring method) of neat and modified pectin

Sample	Tg, °C	Surface tension, mN/m
Pectin	131	48.3
G1/3	94.65	51.7
G1/15	118.26	50.0
S1/3	94.20	45.3
S1/15	121.04	46.0

Graphical abstract

Highlights

- Pectin was modified by reacting with glutaryl and sebacoyl chloride.
- The acylation enhanced the hydrophobic nature of the pectin molecule.
- Hydrophobic character was affected by acylation degree and acyl chain length.
- Modification offered improvement in functionality of neat pectin.