

Article

Structural Determination of Pectins by Spectroscopy Methods

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Abstract: Plant polysaccharides include pectins, which are responsible for an important role in plant physiology and are part of the plant cell wall. These compounds are known as gelling and stabilizing agents, which are widely used in the food industry. The scientific literature lacks precise information on the spectroscopy of apple pectin and citrus pectin. Therefore, the aim of this work was to test and compare the physicochemical properties of these compounds. The curves of FT-IR, NMR, ESI-MS, and thermogravimetric analysis (TGA) of pectin samples were measured and discussed. The analysis of the spectroscopic results confirms that the isolated pectins using various enzymes (xylanase and cellulase) have a structure similar to the commercially available pectin (PectaSol-C), with a noticeable change in morphology. These characteristics are helpful for further basic research and application.

Keywords: pectins; structural properties; morphology analysis; mass spectroscopy; FT-IR; TG analysis; NMR



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1. Introduction

Pectins are natural substances that were first detected in 1790 in tamarind fruit [1]. In 1825 Braconnot isolated pectins from apples, described their physicochemical properties, and gave them a name that comes from the Greek—*pektos*, meaning “solidify, harden” [2–4].

The cell wall is the outer coatings of a plant cell. Pectins, next-door celluloses, and hemicelluloses are the main group of polysaccharide compounds that make up this structure. Pectins are responsible for maintaining and regulating the mechanical parameters inside the cell wall. Pectin modifications caused by enzymes during fruit development are among the most important processes leading to a change in the firmness and texture of the parenchyma tissue and the stiffness of the cellular wall [1,2].

Pectins, being linear and branched polymers, are a complex mixture of sugars, which mainly include oligo- and polysaccharides. The most important polysaccharides forming pectins are arabinan, arabinogalactan-I and arabinogalactan-II, homo-galacturonan, xylogalacturonan, rhamnogalacturonan-I, and rhamnogalacturonan-II [5,6]. In the pectic substance molecule, they alternate, forming in ten areas consisting of branched, heterogeneous chains, which contain various structural and linear polysaccharides, homogeneous (smooth region) *D*-galacturonic acid monomers. Pectins mainly contain *D*-galacturonic acid (GalA) and saccharides such as *D*-galactose (Gal), *L*-rhamnose (Rha), *D*-xylose (Xyl),

L-arabinose (Ara), and *L*-fucose (Fuc) [6–8]. The pectin polymer chain consists of 290 to over 1000 fragments of *D*-galacturonic acid, which relate to each other by α -1→4 glycosidic bonds [9]. Some of the *D*-galacturonic acid carboxyl groups in the pectic substance molecule are esterified with methyl groups [10].

Pectins are used in the food and cosmetics industries. Pectin derivatives are also interesting research material in terms of their biological activity. The properties of pectins arouse interest in the extraction of these polysaccharides from natural ones while maintaining the native pectin structure [11]. Many of the fruit's by-products are a source of pectin; however, because of the wide range of applications in various fields, new plants are now being explored as potential sources [11]. Extracted pectins show structural differences related to their origin resulting from their molecular structure [12–15].

Pectin substances are connected by a plant component. They perform many functions in plant tissue, including structure formation and regulation of water management. They perform another, a not less important function in plants, namely, they are intercellular adhesives. Healing properties are also attributed to pectin substances. It has been proven that the biological activity of pectin prevents atherosclerosis by lowering blood cholesterol levels, chelating metals harmful to health, and regulating peristalsis [16]. The content of pectic substances in plant tissues varies and depends on such factors as species, variety, and anatomical part of the plant. Knowledge of pectin content in plant tissues is of great importance in food industry. Knowledge allows for economical maceration of fruit and vegetable pulp in the production of nectars and naturally cloudy juices, juice clarification and winning, as well as in pectinolysis of fruit before concentration. In their nature, pectin compounds have an affinity for water; they form gels [17]. The degree of methyl esterification (DM) of pectin can be divided into high methoxy (HM; DM > fifty percent) and low methoxy (LM; DM < fifty percent) [17]. High methoxylated pectin can form gels under sugar or acidic conditions, while low methylated pectin can form a gel by ionotropic gelation with low valence ions such as calcium (Ca^{2+}) [18]. In the cell wall, pectin methylesterase can remove the methyl group from homogalacturonan, creating new carboxyl groups, with the Ca^{2+} source interacting to form pectin [17]. These arrangements and the number of methyl ester groups play an important role in gelling; these factors significantly influence the properties of the gel [18].

Pectins show health-promoting properties. Scientists are particularly interested in the use of pectin's modified citrus in the fight against cancer. Pectin works by inhibiting galactose binding by galectin-3 (Gal-3), which can reduce tumor cell aggregation (clumping) and adhesion (adherence) to the vascular endothelium and stimulate their apoptosis (death). Pectin inhibits angiogenesis (the formation of capillaries) in the course of cancer and reduces the formation of metastasis. They effectively inhibit the development of cancer of the prostate, colon, breast, multiple myeloma, and melanoma. They also limit the development of atherosclerotic lesions by inhibiting galectin-3 and lowering blood cholesterol levels [19–24]. New research is being conducted that is investigating the effects of what is ambiguously called PectSol-C, which has been broken down into smaller fragments that can theoretically be absorbed by the body. These processes are unclear currently; however, studies have shown that low molecular weight galactan-rich pectin fragments may bind to the carbohydrate recognition domain on the prometastatic Gal-3 protein. This binding can inhibit Gal-3 interaction with other proteins and peptides, arrest its ability to promote cell adhesion and migration, and prevent apoptosis. There is a possibility that modified pectins such as PectaSol-C may be used in a potentially safe, nontoxic approach to preventing or reducing carcinogenesis [24,25].

The research conducted on cytostatic activity proved that the obtained apple pectin method has better antitumor activity compared with the modified citrus pectin PectaSol-C. Enzymatically obtained apple pectin has a better antitumor activity in colon cancer compared with modified citrus pectin PectaSol-C. This activity is due to the induction of apoptosis and increased intracellular production of reactive oxygen species. In addition, the new apple pectin has been shown to possess strong antibacterial properties and prevent

Escherichia coli from adhering to colon cancer cells compared with PectaSol-C [25]. The pharmacological actions of pectin are also related to the degree of methyl esterification. Studies have shown that low DM compounds, with less than fifty percent methyl esterification, can significantly lower the concentration of cholesterol in the plasma and the liver of the hamster to high DM pectin [10]. Pectin additionally supports the detoxification of the body. They quickly form salts with metal cations, including lead, cadmium, and arsenic; therefore, they reduce their absorption and facilitate their excretion. By lowering the content of heavy metals in the thyroid or liver, they can also normalize the work of these organs. They also have antibacterial, anti-inflammatory, and antioxidant properties. They limit the multiplication of *Staphylococcus aureus* and inhibit liver fibrosis [11,26].

Studies have shown that the obtained apple pectin has a greater antimicrobial and neoplastic activity than PectaSol-C [25]. The study aimed to analyze the structure of apple pectin and citrus pectin to facilitate further research on the response to the relationship between the structure and biological activity. Pectins isolated with the innovative enzymatic method from apples are molecules characterized by a greater mass because of the use of enzymes compared with PectaSol-C. We wanted to find out about the differences between the already known citrus pectin, isolated on an industrial scale, and apple pectin, which has not been analyzed by spectroscopic methods so far. FT-IR has been frequently used to determine pectin DM in recent years, as this approach does not require sample pretreatment. Characterization of pectin includes not only the analysis of sugar composition but also DM. We want to use this knowledge for further chemical modifications to obtain new compounds that can be used in the industries. The analysis used the techniques of FT-IR, NMR, and ESI-MS spectroscopy to scratch the structure of the obtained pectin. The performed thermogravimetric analysis, also called TGA, was carried out to determine the change in the mass of the tested sample depending on the temperature change.

2. Materials and Methods

2.1. Material and Chemical Reagents

2.1.1. Enzymes

Pectins from apple pomace were extracted with xylanase endo- β -1,4-xylanase (EC 3.2.1.8) from *Trichoderma viride* (Sigma/Aldrich Chemical Co., Darmstadt, Germany, Cat. No. X3876) and endo-cellulase (endo- β -1,4-glucanase, EC 3.2.1.4) from *Trichoderma viride* (Sigma/Aldrich Chemical Co., Darmstadt, Germany, Cat. No. C9422). The enzymatic preparations did not exhibit any additional off-target activities, Wikiera et al. 2015 [27] (1:1) (C). Distilled water was used for the hydrogel preparation and swelling measurements.

2.1.2. Apple Pomace

Apple pomace (Pektowin S.A., Jasło, Poland) is a solid residue after pressing the juice without enzymes, used for the commercial production of pectins. The pomace was dried to constant weight (60 °C, 24 h), and its humidity was determined. The extrudate was ground into particles passing through a 40 mesh sieve (0.47 mm) prior to the extraction process.

2.1.3. Citrus Pectin

The comparative material is industrial citrus pectin (ecoNugenicsINC PectaSol-C Modified citrus pectins, Santa Rosa, CA, USA).

2.1.4. Pectin Extraction

Enzymatic extraction of pectin from apple pomace (solid/liquid ratio 1 g/15 mL) was performed using endo-cellulase, endo-xylanase, and a mixture of both preparations (50 U/g each). Extraction was carried out for 10 h at pH 5.0 at 40 °C with constant shaking (200 rpm). All extraction processes were performed in 5 replications. After extraction, the samples were chilled to 20 °C and centrifuged (4100 rpm, 10 min, 4 °C). Then the obtained solutions were filtered through paper, and the pellets were washed with distilled water, centrifuged again, and the second supernatant was also filtered. Then, 96% chilled EtOH

at 4 °C was added to the solutions. The volume of ethanol was calculated to reach a final concentration of 70%. The precipitated pectin was collected by centrifugation (4000 rpm, 20 min), washed with 70% EtOH, centrifuged as before, and the pellets were dried at 60 °C for 24 h until a constant weight was obtained. The resulting pectins: **A** (pectin extracted with cellulase), **B** (pectin extracted with xylanase), and **C** (pectin extracted with cellulase and xylanase), were ground to a particle passing.

2.1.5. Instrumental Analysis

Morphology analysis was performed by microscopic analysis of the pectins using an Eclipse Ti2 inverted microscope (Nikon Instruments Inc., New York, NY, USA). The dispersed powders on the microscope slide were analyzed from a series of photos to determine shape and size.

The spectra of the obtained pectins were observed on a Nicolet iS50 FT-IR spectrometer system (Thermo Scientific Inc., Madison, WI, USA) with a single reflectance ATR diamond. The FT-IR spectra were obtained in the frequency range 4000–400 cm^{-1} with a resolution of 4 cm^{-1} at room temperature.

The methyl esterification (DM) content of pectin was found to be proportional to the ratio of the area of the band at 1730 cm^{-1} over the sum of the areas from the bands at 1730 and 1630 cm^{-1} in the FT-IR spectrum. To calculate the DM content of different pectin samples, standard curves were established based on pectin with known DM content. Then, the peak areas at 1730 cm^{-1} and 1630 cm^{-1} were fitted and integrated. To quantify the DM% of pectins, a calibration curve was constructed based on pectin standards with a known% DM. The calibration curve was obtained from the ratio $\text{DM}\% = A_{1730}/(A_{1730} + A_{1630})$. The linear correlation coefficient for the calibration curve so obtained was $R^2 = 0.9864$. The data were analyzed using OMNIC 9.3.30, 1992–2012 Thermo Fisher Scientific Inc., Waltham, MA, USA.

The ^1H NMR and COSY spectra were collected by the Bruker 30 UltraShield, Billerica, MA, USA, Magnet system 300 MHz/54 mm at room temperature. For NMR analysis of pectin (35 mg) dissolved in 1 mL of high-quality D_2O (99.96%), values were recorded at 300 MHz.

2.1.6. Mass Spectrometry

The ESI-MS experiments were performed on a compact mass spectrometer (Bruker Daltonics, Bremen, Germany) with a standard ESI source. Analyte solutions (150 μL) were injected at a flow rate of 180 $\mu\text{L}/\text{h}$. The parameters of the apparatus were as follows: scanning range: 50–3000 m/z , drying gas: nitrogen, flow rate: 4.0 L/min, temperature: 200 °C, potential between the spraying needle and orifice: 4.0 kV. The Bruker Compass DataAnalysis 4.2 software was used to analyze the MS spectra.

2.1.7. Thermogravimetry Analysis

Pectin stability was determined by the TGA technique, which was performed on TGA 4000, PerkinElmer, Billerica, MA, USA. The sample (mass ~5.00 mg) was measured in the presence of nitrogen in the furnace atmosphere with a heating rate of 10 °C min^{-1} in the range of 25–1000 °C.

3. Results and Discussion

3.1. Morphology Analysis

The extracted pectins, as well as those commercially available, show differences in morphology, mainly in shape. PectaSol-C (**A**) is composed to a large extent of spherical structures with a distinctly undulating surface. Both in **B** and **C**, the structures are irregular; in **B**, they are additionally characterized by a large variety in size and a jagged surface. All analyzed samples have a log-normal size distribution with a predominance of structures < 10 μm , which is well illustrated in Figure 1d,e. PectaSol-C (**A**) shows the highest roundness of the samples, where besides the irregularly shaped granule, there are many round ones, as

indicated by the sharp peak in the range > 0.9 in Figure 1f. Under a polarization microscope, all structures are assigned as amorphous, with no change of polarization.

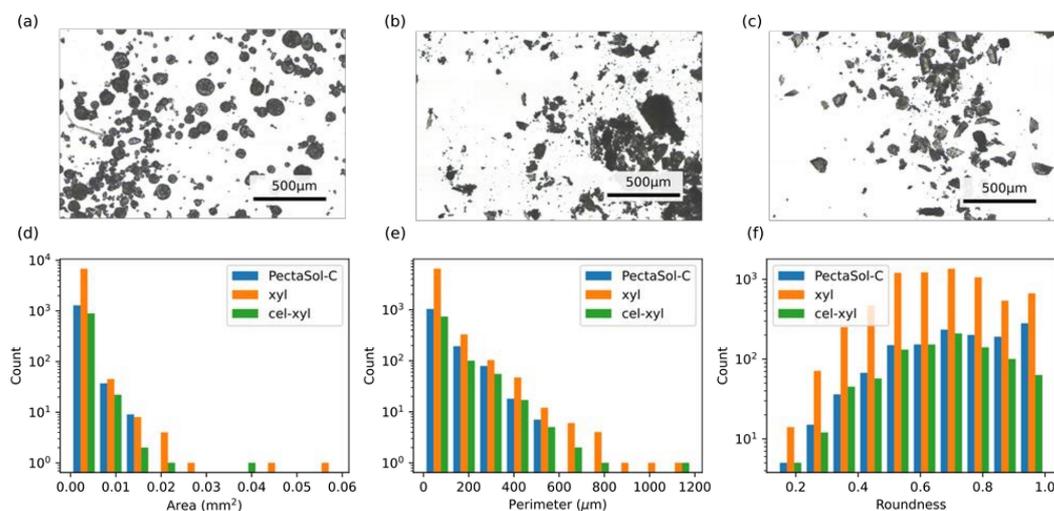


Figure 1. Comparison of pectin morphology (a) PectaSol-C, (b) xylanase, (c) cellulase and xylanase. Histograms of (d) surface, (e) perimeter, and (f) roundness of analyzed samples.

3.2. Physicochemical Characterization of Pectin

The dependence of the pectin structure is related to the origin of the plant and the method used to extract the pectin. This variability affects the physicochemical and hydrodynamic properties of pectins, and this is important for their functionality in specific cell layers, as well as in the area of pectin application in food. Currently, we use modern methods of spectroscopic techniques to find out the exact structure of this polymer.

NMR, FT-IR, ESI-MS, and TGA have been used to study pectin at a structural level. The use of spectroscopic techniques as tools for qualitative and quantitative analysis in the determination of carbohydrates is currently limited despite their enormous potential.

The dried pectin samples obtained from the fruit were subject to quantitative and qualitative tests to determine their physicochemical characteristics.

The FT-IR spectra of PectaSol-C and the pectins extracted from enzymes are presented in Figure 2.

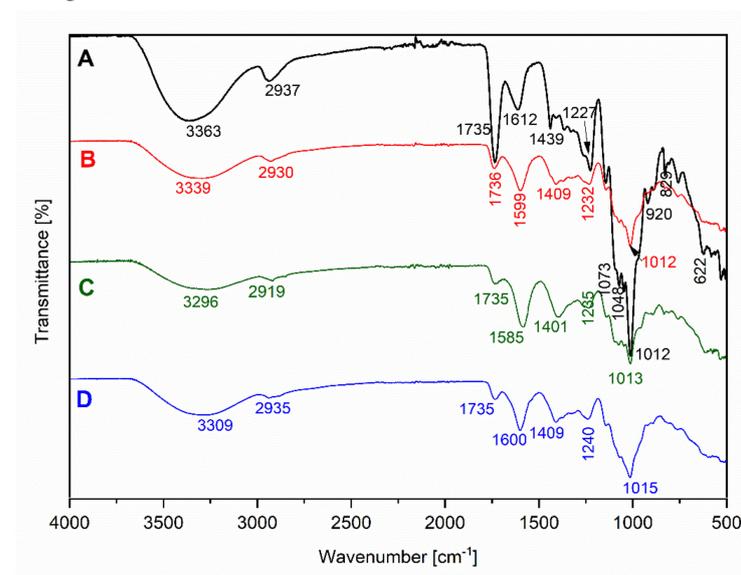


Figure 2. FT-IR spectra of commercial PectaSol-C preparation (A) endo-cellulase (B), endo-xylanase (C), and pectin extracted with cellulase and xylanase (D).

The main reason for the reported spectroscopic research is the detection of any possible changes in the structures of pectin after extraction of pectins from apple pomace (Table 1).

Table 1. Selected infrared bands with assignments.

FT-IR Frequencies [cm^{-1}]	Assignments
3296–3363	$\nu(\text{OH})$; intramolecular H-bonds
2933–2981	$\nu(\text{CH}_x)$
1600–1585	$\nu_{\text{as}}(\text{COO}^-)$ antisymmetric stretching vibrations, polygalacturonic acid; (H_2O)
1400–1440	$\nu_{\text{s}}(\text{COO}^-)$ symmetric stretching vibrations
1330–1320	Ring stretching vibrations
~1243	$\nu(\text{C-O})$
1125–1162	$\nu_{\text{as}}(\text{C-O-C})$
1100–1093	$\nu(\text{C-O})$
1019–1014	$\nu(\text{C-O})$, $\nu(\text{C-C})$, $\nu(\text{C}_2\text{-C}_3)$, $\nu(\text{C}_2\text{-O}_2)$, $\nu(\text{C}_1\text{-O}_1)$

Carbohydrates, a strong band of valence vibrations $\nu(\text{O-H})$ of the pyranose ring, are characteristic of pectin.

The major absorptions in the 3296–3339 cm^{-1} (3363 cm^{-1} in **A**) spectral range of **B–D** samples are caused by stretching the hydroxyl group, and the bands are shifted toward lower frequency in comparison with **A**. The C-H stretching vibrations of CH_2 groups are generating the medium bands around 2937 cm^{-1} in **A** (2930, 2919, 2935 cm^{-1} in **B–D**, respectively). It is well known that the bands in the 3000–2800 cm^{-1} frequency range [17,18] are related to $\nu(\text{C-H})$ stretching vibrations. The detailed assignment to $\nu(\text{C-H})$ stretching modes of methyl and methylene groups or the pyranose ring of the pectin can be difficult because the bands are overlapped by broad band $\nu(\text{O-H})$ stretching vibrations [28–32]. Generally, the HB interactions shift the stretching $\nu(\text{CO})$ and $\nu(\text{O-H})$ frequencies to lower numbers. This fact indicates that the pectin structures are stabilized by the intra- and intermolecular hydrogen bonds [33,34].

Next, the medium band at around 1735 cm^{-1} and strong near 1600 cm^{-1} (at 1612 cm^{-1} in **A**) are observed, corresponding to the $\nu(\text{C=O})$ ester stretching vibration of the C=O and the methyl esterified carboxyl group (COO-R) stretching vibration of ionic carboxyl groups (COO^-), respectively. It is worth emphasizing that the inversion of the intensity of these two bands compared with FT-IR spectra of **B–D** is found: the strong band at 1735 cm^{-1} and one medium at 1612 cm^{-1} in the FT-IR spectrum of **A** are observed. Carboxylate groups also show a weaker symmetric stretching band near 1410 cm^{-1} (1439 cm^{-1} in **A**). The weak band at 1240 cm^{-1} is assigned to the side-chain vibrations. Structural features arising with particular conformations around the glycosidic bonds of pectin are observable in the 1100–930 cm^{-1} spectral range. Moreover, the band near 1150 cm^{-1} corresponds to the ring vibration coupled with $\delta(\text{C-OH})$ bending vibrations [35,36]. In the presented research, the characteristic band with strong intensity at around 1015 cm^{-1} in the FT-IR spectra of pectins is mainly associated with the C-O stretching vibrations. The last remaining medium intensity bands below 920 cm^{-1} in the “fingerprint” region are mainly attributed to vibrations of the C-O-C bridges typically for polysaccharides [36–39].

In conclusion, it can be seen that regardless of the enzyme used to isolate pectin, the quality of pectin (**B–D**) does not change compared with commercial pectin (**A**), which is confirmed by the results of the FT-IR analysis.

Comparing the obtained DM by the FT-IR method for individual pectins, we can see that pectin (**C**) 72% has the highest and PectaSol-C (**A**) 21% has the lowest, which is caused by the production process. In addition, **B–D** pectins can be classified as high methoxy and PectaSol-C as low methoxy.

To create the FT-IR method, the DM content for A–D pectins is shown in the table below (Table 2).

Table 2. The degree of methylation in pectins.

PECTIN	%DM *
PectaSol-C (A)	21% ± 1.8
endo-cellulase (B)	64% ± 0.3
endo-xylanase (C)	72% ± 0.9
pectin extracted with cellulase and xylanase (D)	68% ± 0.5

* The results are shown with the associated standard error of means (\pm SEM) of the quadruplicate values from the average.

The degree of methyl esterification (DM) is defined as the mole percent of the carboxyl groups esterified with a methyl group. DM has a great influence on the gelling, stabilization, and thickening of the pectins and influences the conditions and the mechanisms of gel formation, i.e., B–D pectins as high methoxy can form gels under sugar or acidic conditions [8,9]. The pharmacological actions of pectin depend on the degree of DM formation.

3.3. Mass Spectrometry (ESI-MS)

In particular, the development of soft ionization techniques such as electrospray (ESI), along with the development of these MS techniques, have helped the application of mass spectrometry techniques to the structural characterization of compounds such as pectins.

Difficult to analyze in the mass spectroscopy method are glycoproteins and glycopeptides. This is due to the high molecular weight combined with high charge states and adduct accumulation which may affect the resolution limits of the mass spectrometer.

The method of mass spectroscopy was used for the analysis of enzymatically obtained apple pectins and PectaSol-C.

The binding defining fragments do not break the glycoside but are derived from a double pyranose ring cleavage. The ring breakage leads to the fact that parts of the ring are joined to reducing ions, and both signals are observed. Therefore, all these latter fragments are identical and of low construction value. The remaining nonreducing terminal fragments and the fragments inside vary in weight and appear as satellite peaks above their respective glycosidic ions because of the electron impact ionization linking on methylated di- and trisaccharides. Depending on the position of the sugars in the polymer, each fracture of the cross ring produces a rise to another hanging passage [40].

The MS spectra (Figure 3) were confirmed by an analysis with the major single charged ions $[M^*-H]^-$ at m/z 731 and 635 for $\text{GalA}_4\text{Me}_1\text{Ac}_1$ and $\text{GalA}_3\text{Me}_1\text{Ac}_1$, respectively. Other single charged ions at m/z 634 ($\text{GalA}_3\text{-Me}_1\text{Ac}_2$) and 555 (GalA_3Me_1) were also identified along with some cluster ions $[2M^*-H]^-$. To analyze the structure of the second MS segment, the ion spectrum at Rha m/z 983. The intense productions at m/z 935 and 731 indicate that Rha is at the reducing end and exposed to the glycosidic binding residue of $\rightarrow 2\text{Rha}$ [40].

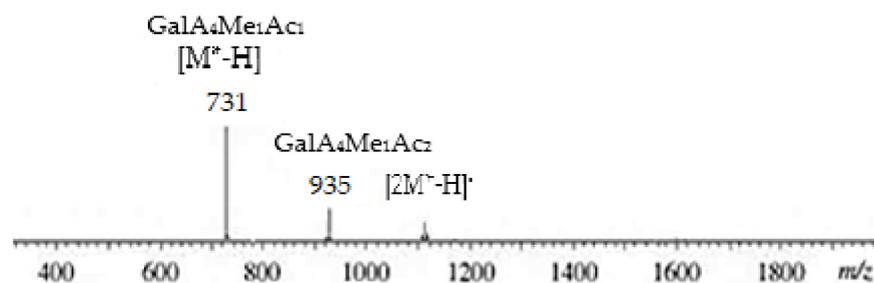


Figure 3. Mass spectrometry of apple pectin extracted.

3.4. Thermogravimetric Analysis (TGA)

Thermogravimetric analysis is a technique used to analyze chemical compounds. The change in the mass (a loss of weight) of the sample is detected during the temperature change. We present the decompositions processes of the three pectins in TGA curves (Figure 4).

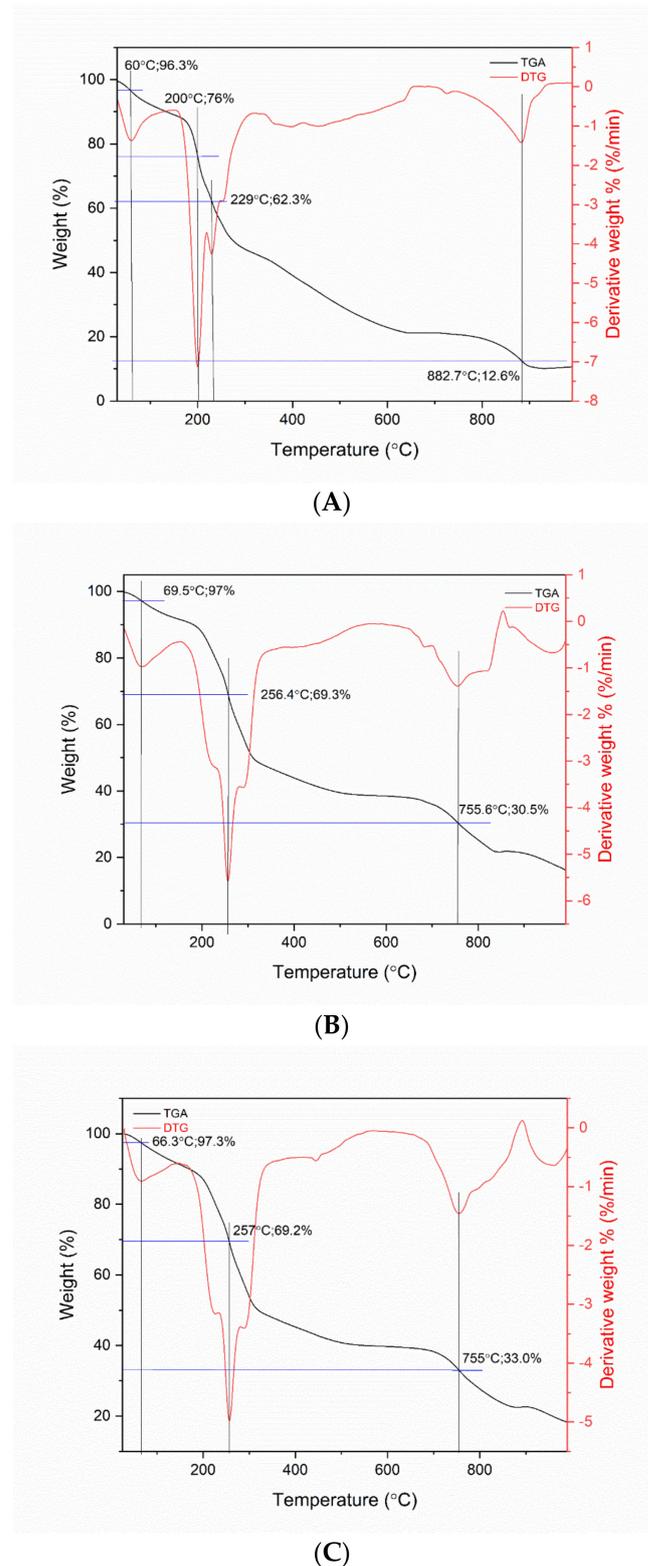


Figure 4. TGA curves of commercial pectin (PectaSol-C) (A), pectin extracted from xylanase (B), and a mixture of two enzymes (C).

The thermogravimetric analysis provides temperature or time data for weight change and a thermogram that graphically shows the percentage of weight change.

All discussed samples presented a four-step thermal decomposition in the 25–1000 °C temperature range characteristic of the pectin. In pure citrus pectin (available commercially, PectaSol-C, curve **A**), the first step at 60 °C corresponds to the water molecules and some volatile compounds loss, which confirms a loss of 3.7% of the weight of the PectaSol-C sample. The next steps with three endothermic effects at 200 °C, 229 °C, and 882.7 °C are associated with pyrolytic decomposition, consisting of primary and secondary decarboxylation with an acid side group and carbon in the ring [38,41–43]. The successive mass loss (87.4%) may lead to pectin residue formation.

The decomposition process of pectin extracted from xylanase (**B**) occurs in three main endothermic steps. The TG curve for **B** shows that the pectin is stable up to 69.5 °C with the first weight loss of 3.0%, which corresponds to the loss of water molecules as in PectaSol-C's TG curve. The similarity of the mass loss suggests a similar number of OH side groups in pectin with the total molar mass of pectin PectaSol-C 66.3 kDa, xylanase 899 kDa, respectively. The pyrolytic decomposition occurs at 870 °C and can be detected by two prominent endothermic peaks at 256.4 °C and 755.6 °C, respectively. The total mass loss in **B** is 69.5%.

The last TGA curve presents the decomposition of the pectin extracted with cellulase and xylanase mixture (**C**). The process occurs in the main endothermic steps, such as in **B**. In the first step at 66.3 °C, which corresponds to water molecules losing, we lose 2.7% of pectin. The next two endothermic processes are observed (257.2 °C and 755 °C) as in **A**, and pyrolytic thermal decomposition occurs. In that case, the total mass loss is 67.0%.

The thermogravimetric analysis provides the information that the analyzed pectins are stable up to around 70 °C, and the first endothermic step is characterized by the loss of water molecules.

3.5. NMR Spectroscopy

The NMR spectra of apple pectin were compared with modified chemical pectin (PectaSol-C). NMR spectra obtained from pectin samples are characterized by a broad signal chain (i.e., CH₃ and CH₂ groups) ranging from 0 to 2.5 ppm (Figure S1) [44].

In the ¹H NMR spectra of apple pectin, two signals of about 2.11 ppm are from the acetyl groups located at 3-O- and 2-O-galacturonic acid (GalA). For the first group, the peak observed is 1.91 ppm, and for the second group is 1.82 ppm. The signals at 1.23 ppm and 1.18 ppm are from the CH₃ group of rhamnose (Rha) and are assigned to the O-2.4 and O-2 combined Rha, respectively. The peak at 3.92 ppm is derived from the CH₃ group that is associated with the carboxyl groups of GalA. The remaining pectin signals are assigned to the 5 protons found in GalA (H₁, 4.98 ppm; H₂, 3.73 ppm; H₃, 3.93 ppm; H₄, 4.18 ppm, and H₅, 4.71 ppm).

Signals at 5.13 ppm and 4.89 ppm located in the anomeric region are assigned to H₁ Rha and H₁ Gal, respectively. Compared with intact pectin, the ¹H NMR spectra of PectaSol-C and apple pectin showed a visible decrease in the intensity of the anomeric peak at 4.98 ppm and an increase in the other two anomeric signals at 5.13 ppm and 4.89 ppm (Table 3).

Table 3. ¹H NMR chemical shifts.

Glycosyl Residue	H ₁	H ₂	H ₃	H ₄	H ₅	H ₆
Gal	4.89	3.67	4.10	3.92	4.41	3.67
Rha	5.13	4.31	3.87	3.67	4.07	nd *
GalA	4.98	3.73	3.93	4.18	4.71	nd *

* nd—not detected.

Because of the poor resolution of the ¹H NMR spectra, we used COSY for the analysis presented in Figure 5 to further determine the chemical structure of apple pectin by assigning the chemical shifts.

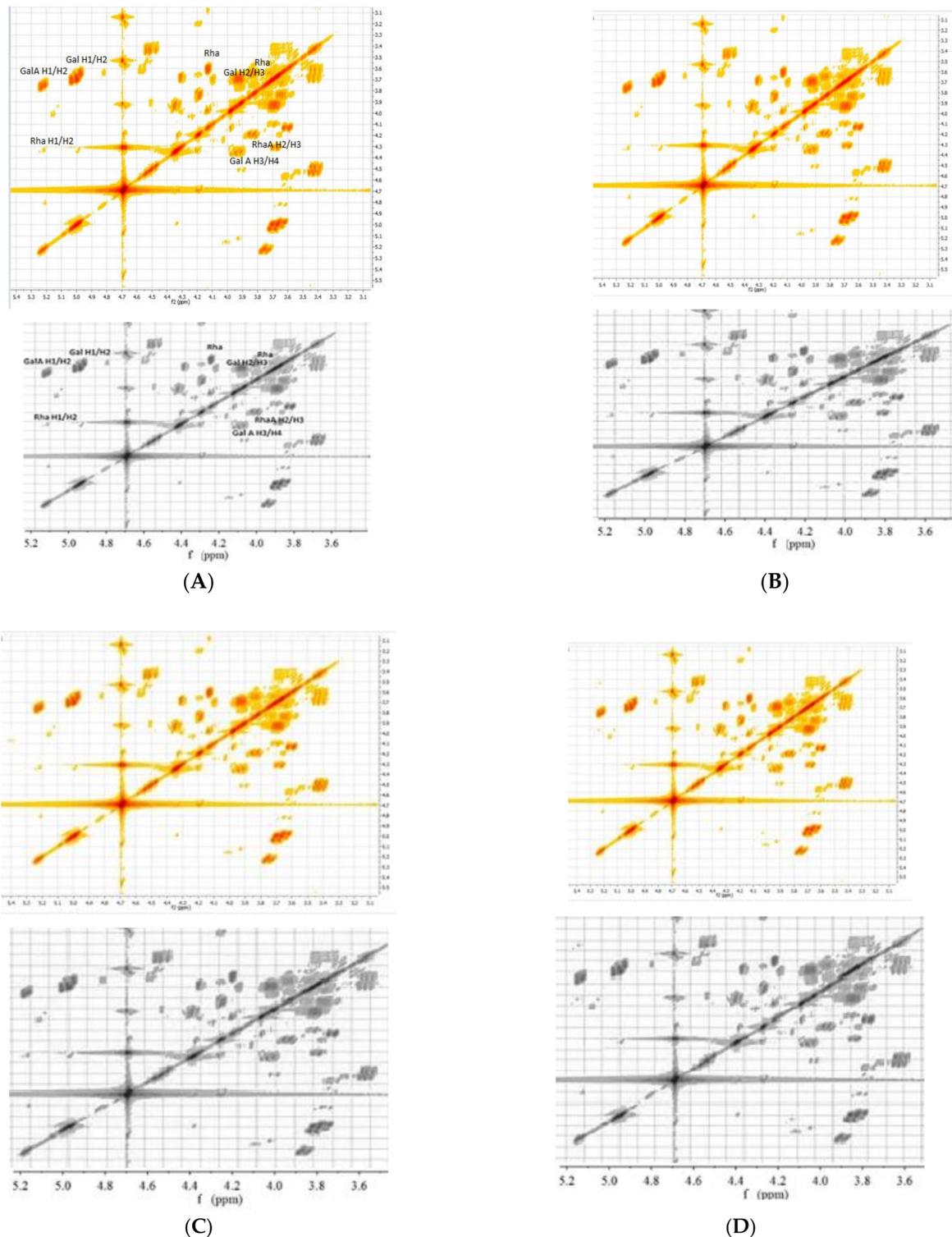


Figure 5. COSY spectra of pectin extracted from (A) PectaSol-C preparation, (B) xylanase, (C) cellulose, and (D) mixture of two enzymes.

Figure 5 shows the COSY spectra of the analyzed pectins, which showed three clear correlation signals in the anomeric region at 5.11 ppm, 5.92 ppm, and 4.89 ppm. These signals can be assigned to H-1 GalA, Rha, and Gal, respectively, based on the analysis of ^1H NMR spectra shown, respectively [45,46].

4. Conclusions

As compounds of natural origin, pectins are difficult to analyze in terms of the structure due to the complexity of this branched macromolecule, which has many structural domains. The exact structure of various heterogeneous oligosaccharides can be determined by NMR spectroscopy, and in combination with other techniques, a wealth of pectin structure information is provided, including monomer composition, branching, and substituent type, and femtomolar linkage.

The morphological differences of the extracted pectins are visible in microscopic analysis and may influence their use in the food industry. It also reflects the structural differences and domain structure of specific pectins.

Mass spectrometry methods used to solve isomeric structures provide better insight into the structure of pectins and the interrelationship of structural bonds, whether between pectin structures or between given polysaccharides located in the cell wall layers.

FT-IR and NMR spectroscopy are relatively demanding methods of pectin analysis. Regardless of the low resolution, the solid-state spectroscopy technique provides important information on pectin structure. The use of the FT-IR method to calculate the degree of methylation can be used in the food industry as well as in the pharmaceutical industry. Enzymatically extracted pectins have a high degree of methylation compared with PectaSol-C. The DM values obtained using this method were in agreement with values calculated using standard techniques.

The association of chemical analysis spectrometric methods was effective in identifying some features of the structure of pectins from apple pomace. ^1H NMR, COSY, FT-IR, and ESI-MS data confirm the presence of high contents of *D*-galacturonic acid (GalA) as seen in the linear polymeric region. Besides, the thermogravimetric analysis shows that the analyzed pectins, typical for polysaccharides, are stable up to around 70 °C.

The main reason for the reported spectroscopic research is the detection of any possible changes in pectin structures after the extraction of pectins from apple pomace. In conclusion, it can be seen that regardless of the enzyme used to obtain the pectin (B–C), it does not change compared to commercial pectin (A), as confirmed by spectroscopic methods.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/coatings12040546/s1>, Figure S1: ^1H NMR spectra of pectin extracted from (A) PectaSol-C preparation, (B) xylanase, (C) cellulase and (D) mixture of two enzymes.

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