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Features of Pectin Biodegradation in the Presence of Sodium Ethylenediaminetetraacetate

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Abstract—We have studied the effect of sodium ethylenediaminetetraacetate on enzyme-induced degradation of apple pectin. Capillary viscometry and IR spectroscopy data have shown that the ligand binds Ca^{2+} ions (present in pectin) thus accelerating its biodegradation 1.2–1.6 times at relatively low concentration of the complexone. At the same time, sodium ethylenediaminetetraacetate reduces enzymatic activity of lytic enzymes thus slowing down pectin hydrolysis as higher concentration of the complexone.

Keywords: pectin, enzyme-induced degradation, enzymatic activity, ethylenediaminetetraacetate

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Pectins (complex heteropolysaccharides of the glycogalacturonates class) contain units of galacturonic acid (GA–H), methoxylated derivatives of galacturonic acid, and its calcium salts (GA–Ca); the latter units form the bonds linkages between macromolecular chains. Biocatalyzed degradation of pectins can be induced by enzymes of the hydrolases or lyases classes. Hydrolases cleave the 1→4-glycosidec linkage between pairs of non-methoxylated units. Pectin lyases are active towards highly methoxylated macromolecules, whereas pectate lyases act on GA–H [1]. The presence of GA–Ca units in the molecule prevents degradation of pectins with all the listed enzymes [2]. Natural pectins contain rather much calcium galacturates, for example, 18–24% in flax raw material [3]. As the enzymes catalyzing transformation of GA–Ca into hydrolysable forms are unknown, other processes should be used to remove calcium ions from pectins.

Enzymatic degradation of macromolecular substrates can be accelerated in the presence of chelating agents. Both in the stages of pectin isolation from raw materials and modification of cellulose-containing substrates, sodium ethylenediaminetetraacetate EDTA is often used [4, 5]. In complex systems, the chelating agent can affect the substrate as well as the enzyme. In this work we aimed to study the effect of EDTA on the processes in the course of the biochemical degradation of the natural polymer.

As Ca^{2+} ions link pectin macromolecules together, changes of pectin solutions viscosity could be anticipated under action of EDTA. We measured viscosity of 1 wt % solution of pectins in pH range from 5.0 to the polymer isoionic point and at different temperatures (Fig. 1).

As seen from Fig. 1, the introduction of the chelating agent reduced the kinematic viscosity of pectin solutions. Furthermore, the viscosity decreased with heating and increased with pH. The effect of pH was due to changed ionization state of carboxy groups of pectin macromolecules [6–8]. In the acid medium the carboxy groups were not dissociated, and the polysaccharide behaved as uncharged macromolecule. With increasing pH, the macromolecules swelled due to the electrostatic repulsion of carboxylate groups, and therefore the viscosity increased. Noteworthily, within the studied pH range the ionic cross-links through calcium ions were retained. In the presence of EDTA, the calcium bridges were cleaved, and individual pectin macromolecules appeared in the solution; therefore, the viscosity decreased. The most prominent effect of EDTA was observed at pH 5.

Calcium ions extraction from pectins and its complex formation with EDTA was due to the lower stability of calcium complexes with galacturonic acid (log K 1.84 [9]) by 9 orders of magnitude as compared with the complexes with EDTA (log K 10.56 [10]).

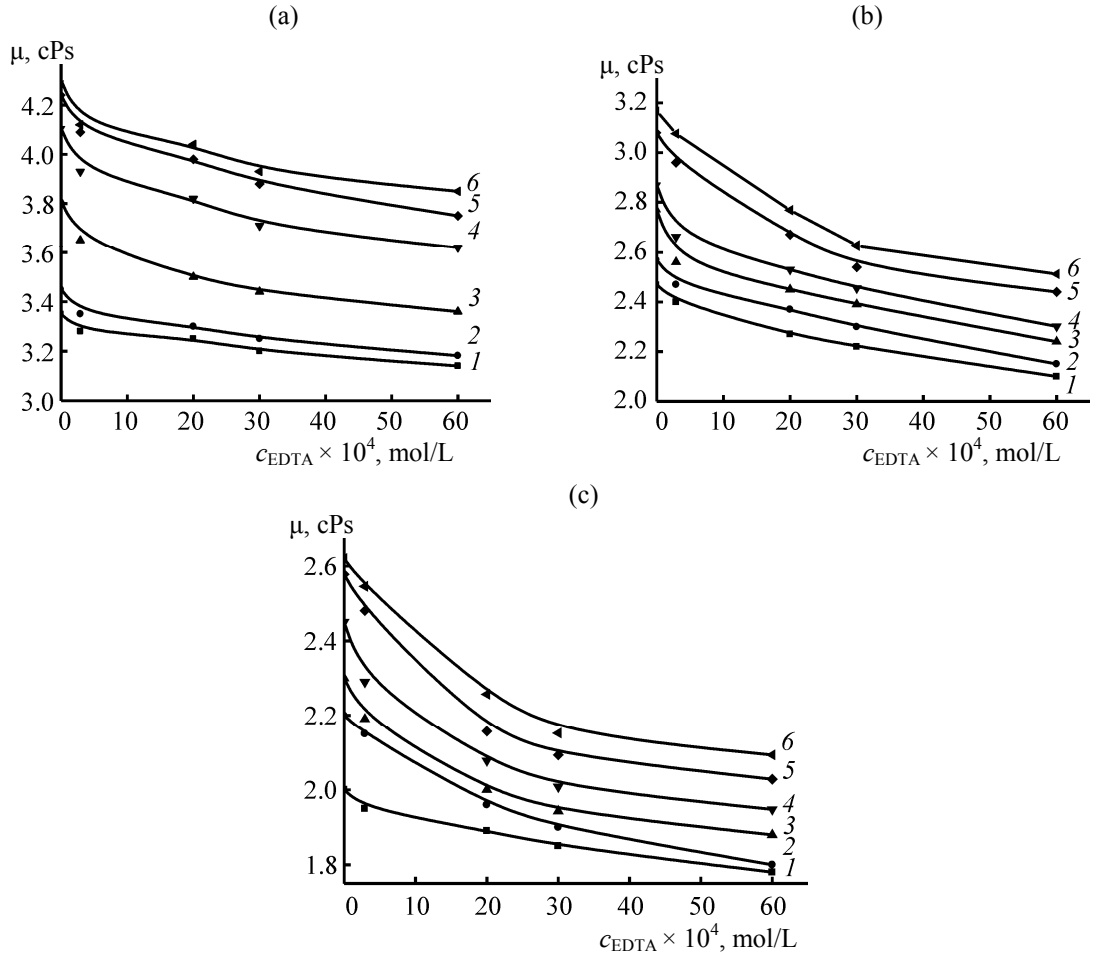

Fig. 1. Viscosity of pectin solutions (1 wt %) as a function of EDTA concentration at 30°C (a), 40°C (b) and 50°C (c); pH 3.1 (*1*), 3.4 (*2*), 3.6 (*3*), 4.0 (*4*), 4.5 (*5*), and 5.0 (*6*).

The changes in the pectin state in the presence of EDTA was further studied by means of IR spectroscopy (Fig. 2). In particular, the content of GA–Ca units in pectin was estimated from the intensity of characteristic band of the stretching vibrations of metal-bound ionized carboxyl C=O [$\nu_{as}(COO^-)$, 1615 ± 15 cm^{-1}] [11]. In order to eliminate the effect of inhomogeneous film thickness, the bands intensity was related to that of the internal standard (the strongest band at ~1020 cm^{-1} assigned to the stretching vibrations of C–C and C–O bonds in pyranose ring). The bands intensity was corrected for baseline as well, the baseline was held through the absorption minima at 1900 and 900 cm^{-1}. The analysis of the spectra in Fig. 2 showed that the introduction of EDTA reduced the content of GA–Ca units in the polymer 1.3 times.

Figure 3 illustrates the kinetic studies of enzyme-induced hydrolysis of pectin (endopolygalacturonase in the presence of EDTA).

The reaction acceleration at relatively low EDTA concentration was observed at both temperatures studied, due to cleavage of ionic linkages between the macromolecules making the substrate chains more accessible to the enzymatic attack. The hydrolysis rate was the highest at EDTA concentration of 1.2–1.5 mmol/L; at higher concentration of the complexone the degradation of the polysaccharide was slowed down. Likely, EDTA could act upon the enzyme active site leading to its inactivation.

The necessary condition for endopolygalacturonase to be active is the presence of inorganic cofactor that stabilizes the macromolecule spatial structure including that of the active site involving the doubly-charged metal cations.

As seen from Fig. 4 the enzymatic activity of endopolygalacturonase with respect to polygalacturonic acid was reduced in the presence of EDTA over the whole studied range of concentrations.

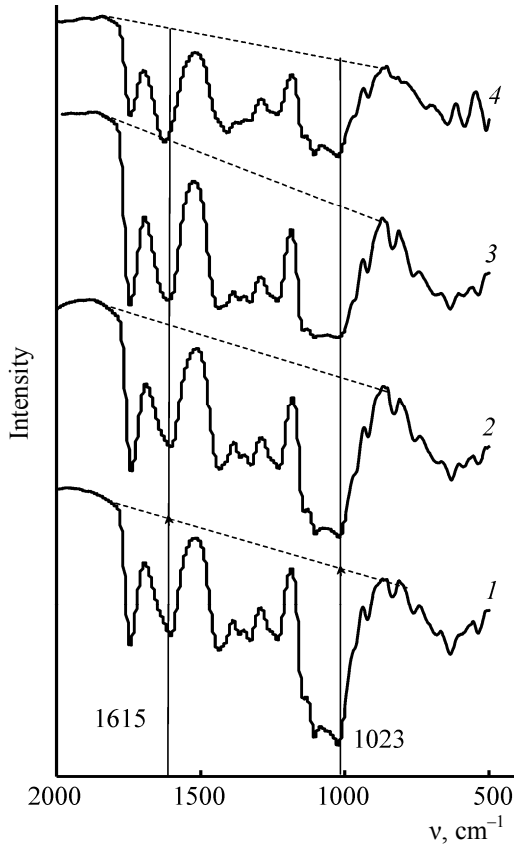

Fig. 2. IR spectra of pectin before (*1*) and after (*2–4*) treatment with EDTA. c_{EDTA} 0.29 mmol/L (*2*), 0.61 mmol/L (*3*), and 2.4 mmol/L (*4*).

We have determined the equilibrium composition of the studied system at pH 5 taking into account the following equilibriums (Y is ethylenediaminetetraacetate ion, GA is ionized monomer unit of galacturonic acid).

$$Ca^{2+} + GA^{-} \rightleftarrows GA\text{–}Ca^{+},\ \log K = 1.84,$$

$$Ca^{2+} + Y^{4-} \rightleftarrows CaY^{2-},\ \log K = 10.56,$$

$$H^{+} + Y^{4-} \rightleftarrows HY^{3-},\ \log K = 10.26,$$

$$2H^{+} + Y^{4-} \rightleftarrows H_2Y^{2-},\ \log K = 16.38,$$

$$H^{+} + GA^{-} \rightleftarrows GA\text{–}H,\ \log K = 2.96,$$

$$H_2O \rightleftarrows H^{+} + OH^{-},\ \log K = -14.$$

According to the calculations, with increasing EDTA concentration the fraction of its complex with calcium increased from 3 to 70% (with respect to the total concentration of calcium). At EDTA concentration above 1.5 mmol/L, the HY^{3-} and H_2Y^{2-} species were accumulated in the enzyme–pectin–EDTA system, able to form stable complexes with metal ions of the enzyme active site thus reducing the enzyme activity.

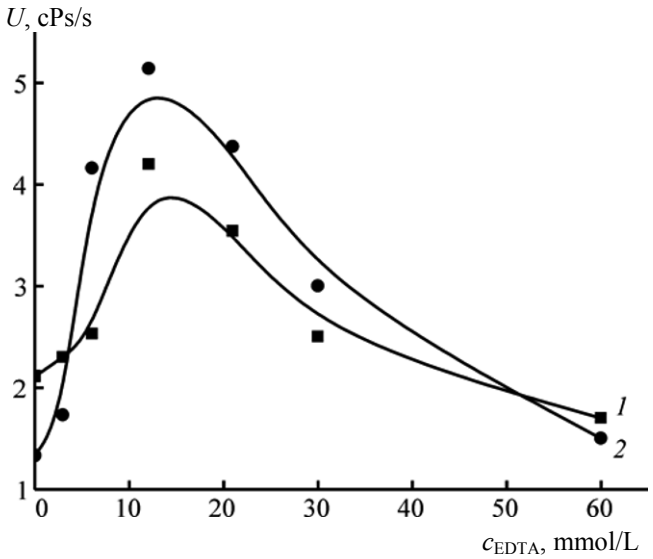

Fig. 3. Rate of enzyme-catalyzed degradation of pectin as a function of EDTA concentration at pH 5 and 40°C (*1*) or 50°C (*2*).

To conclude, the introduction of EDTA influenced significantly the hydrolytic degradation of pectin. At relatively low concentration, EDTA accelerated pectin degradation 1.2–1.6 times. At EDTA concentration above 1.5 mmol/L, the polysaccharide degradation was slowed down due to the enzyme inactivation.

EXPERIMENTAL

Apple pectin with esterification degree of 58% was used. The major components of enzymatic specimen pectofoetidin were pectin esterase and endopolygalacturonase.

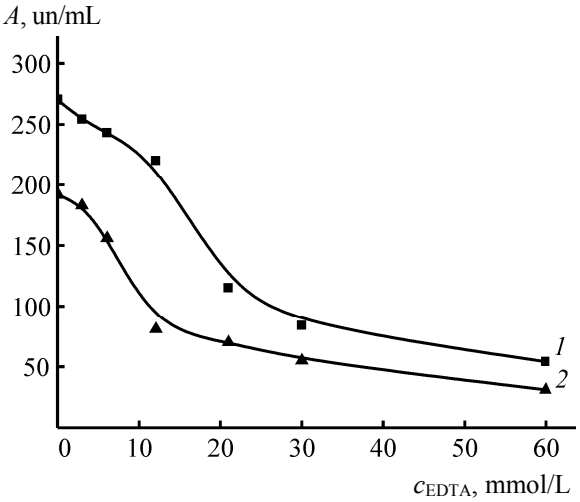

Fig. 4. Activity of endopolygalacturonase as a function of EDTA concentration at pH 5 and 40°C (*1*) and 50°C (*2*).

pH 3.0 was isoionic point of 1 wt % pectin solution. The experiments at other pH values were carried out after addition of NaOH (0.1 mol/L).

EDTA concentration was determined using the zinc sulfate reference solution.

Kinematic viscosity of the solutions were measured using the capillary viscometer equipped with ultra-thermostat and automated system of the flow time measurement.

IR spectra of pectin films were recorded using the AVATAR-360 spectrophotometer at 500–4000 cm^{-1}. The films were prepared by casting of 10 mL of aqueous polymer solution (6 g/L) onto the Teflon mold (60 cm^2) and air-drying.

The effect of EDTA on the enzyme specimen activity was studied in the model system endopolygalacturonase–polygalacturonic acid. The solution of pectofoetidine was prepared by dissolving the weighed sample in water. 2 mL of the solution were transferred into the 50 mL volumetric flask, the necessary amount of EDTA was added, and the volume was adjusted to precisely 50 mL. EDTA concentration ranged between 0 and 6 mmol/L. Then, 10 mL of solution of polygalacturonic acid was put into the viscometer, 5 mL of the enzyme–EDTA solution was added, and activity of the enzyme was determined as described in [12].

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