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Kinetics and mechanism of oxidation of apple pectin by Cr^{VI} in aqueous acid medium†

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Selective oxidation of galacturonic acid residues of apple pectin by Cr^{VI} affords CO_2/HCO_2H, oxidized pectin, and Cr^{III} as final redox products. The reaction shows first-order kinetics in [pectin], $[Cr^{VI}]$, and $[H^+]$, at fixed ionic strength and temperature. Kinetic studies show that the redox reaction proceeds through a mechanism combining $Cr^{VI} \rightarrow Cr^{IV} \rightarrow Cr^{II}$ and $Cr^{VI} \rightarrow Cr^{IV} \rightarrow Cr^{III}$ pathways. The mechanism is supported by the observation of free radicals, CrO_2^{2+} (the formation of which implies involvement of Cr^{II} and Cr^{IV}) and Cr^{V} (formed in monoelectronic redox processes) as reaction intermediates. The reduction of Cr^{IV} and Cr^{V} by pectin was independently studied and found to occur more than 10^3 times faster than pectin + Cr^{VI} reaction, in acid medium. At pH 3–5, apple pectin + Cr^{VI} redox reaction is slow, oxo-chromate(V)–pectin species stabilize and remain in solution during several hours. The present results show that these abundant and ubiquitous components of the cell walls of all land plants are able to reduce $Cr^{IV–VI}$ or stabilize high-valent chromium depending on pH. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: pectin; chromium; redox chemistry; kinetics; mechanism

INTRODUCTION

Cr^{VI} is a well-established carcinogen and mutagen,[1–2] and can be reduced to lower states by a wide variety of biological and chemical reductants.[3] Considerably interest has been shown in the chemistry of the intermediate oxidation states, Cr^{V} and Cr^{IV}, due to their observation in the oxidation of organic substrates by Cr^{VI} and implication in the mechanism of Cr-induced cancers.[4–6] Polyoxygenated compounds, such as polyalcohols and hydroxycarboxylic acids, are effective as non-enzymatic reductants (at low pH) and can stabilize the labile oxidation states of chromium.[3,7–9] Because of their potential biological and ecological relevance, the reduction and stabilization of hypervalent chromium by naturally occurring mono- and polysaccharides can provide useful information on the role that these polyoxygenated compounds play in the uptake and transport of chromium.

Chromium enters into the food chain through consumption of plant material. Several reports suggest a relationship between chromium concentration in soils and its accumulation in plants growing in them.[10] Pectins are a family of polysaccharides rich in 1,4-linked α-D-galactosyluronic acid (GalA) residues – at least 65% – that are present in all plant primary cell walls.[11] Three major pectic saccharides are recognized, all containing galacturonic acid: homogalacturonan (a linear chain of GalA residues in which some of the carboxylic groups are methyl esterified), substituted galacturonans (polysaccharides that contain a backbone of linear GalA residues with side chains attached to C-2 or C-3 of some of the backbone residues) and rhamnongalacturonan (a polymer consisting of the repeating disaccharide [→4)-α-D-GalA- (1→2)-α-L-Rha-(1→] to which different glycan chains are attached to the rhamnose residues).[12]

The presence of free carboxylic groups and *vic*-diols moieties makes these naturally occurring acid polysaccharides suitable for binding and reducing high-valent chromium. Although the reduction of hypervalent chromium by low molecular weight compounds is well documented,[3,13–16] little is known on the reaction of polysaccharides with Cr^{VI}. Pectins are abundant, ubiquitous, and multifunctional components of the cell walls of all land plants. The determination of the ability of pectins to reduce or stabilize hypervalent chromium will contribute to understand the potential role of these biopolymers in the biochemistry of this metal. In this work, we report the study of the redox reaction of apple pectin with Cr^{VI} and provide information on the relative reactivity of apple pectin toward Cr^{VI}, Cr^{V}, and Cr^{IV}, the influence of pH on the redox reactions, and the formation of long-lived Cr^{V}–pectin species.

EXPERIMENTAL

Materials

Apple pectin (Sigma, USP), glutathione (Sigma, 98–100%, reduced form), 2-ethyl-2-hydroxybutanoic acid (ehba, Aldrich grade), potassium dichromate (Mallinckrodt), perchloric acid (A.C.S. Baker), acrylonitrile (Aldrich, 99%), sulfuric acid (Cicarelli, p.a.), sodium hydroxide (Cicarelli, p. a.), hydrochloric acid (Cicarelli, p.a.), sodium thiosulfate (Cicarelli, p.a.), and potassium iodide (Cicarelli, puris.) were used without further purification. Aqueous solutions were prepared in milliQ deionized water (HPLC quality). For experiments performed at constant ionic

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strength ($\mu = 0.5$ mol/L) and different [H^+], mixtures of $NaClO_4$ and $HClO_4$ solutions were prepared. The concentration of stock solutions of $HClO_4$ was determined by titration using standard analytical methods. The stability of the polymer under conditions used in the kinetic studies was tested by HPLC. Oxygen concentration in O_2-saturated solutions of pectin was determined using a Clark-type oxygen electrode with an oxygen-monitoring system (Yellow Springs Instruments Co., Inc.).

Physical methods

Specific viscosity was measured using an Ostwald-type capillary viscosimeter immersed in a thermostated water bath at 25°C. Flow times were recorded with a stopwatch with reproducibility ±0.2 s. Density of solutions was measured by pycnometry. HPLC analyses were performed on a KNK-500 A chromatograph provided with a 7125 HPLC pump. The separation was carried out on an Aminex HPX-87X ($300 \times 7.8\,mm^2$, Bio-Rad Lab) HPLC column, using H_2SO_4 as eluent (pH = 1.5) and a flow rate of 0.6 ml/min, at 30°C. The samples were filtered through a 0.2 μm membrane prior to the injection into the chromatographic system. The eluent was monitored with a 115 UV Gilson ($\lambda = 220$ nm) detector. Spectrophotometric measurements were performed on a Jasco V-550 UV–Vis spectrophotometer with fully thermostated cell compartments (±0.2°C). X-band EPR spectra were carried out at room temperature on a Brucker ESP-300 E spectrometer, using flat cells. The microwave frequency was generated with a Brucker 04-ER (≈9.7 GHz) and measured with a Racal–Dana frequency-meter. The magnetic field was measured with a Brucker NMR-probe gaussmeter. Spectra were recorded as first derivatives of the microwave absorption in 1024 points at ambient temperature (20 ± 1°C) using 10 mW microwave power, 100 kHz modulation frequency and 3.9 G modulation amplitude. *g*-Values were determined by reference to diphenylpicrylhydrazyl ($g = 2.0036$) as an external standard.

Viscosity measurements

Measurements of specific viscosity were carried out on solutions of pectin (0.006–0.02 g/L) in 0.10 mol/L NaCl, at 25°C. The calculated value for intrinsic viscosity was 2.74.[17–19] Molecular weight (M_W) of apple pectin was estimated by applying the Mark Houwink–Sakurada equation[20] and average $M_W = 54\,500 \pm 100$ g/mol was obtained.

Measurement of free carboxylic groups of pectin

Titration of a solution containing 10 g/L pectin with 0.0969 mol/L NaOH afforded 0.0080 mol/L of free carboxylic groups. On the basis of $M_W = 54\,500$ g/mol, the employed pectin contains an average of 44 free carboxylic groups per mole of polymer.

In situ generation of (oxo)chromium(IV) (CrO^{2+}) and reaction with pectin

CrO^{2+} was generated by rapid oxygen-oxidation of Cr^{2+} employing the following procedure. Zn/Hg amalgam, prepared by stirring a mixture of Zn (10.0 g, washed with 3.0 mol/L HCl) and $HgCl_2$ (0.3 mol/L in 0.1 mol/L HCl) for about 30 min, was added to a solution of 0.006 mol/L $Cr(ClO_4)_3$ in 0.2 mol/L $HClO_4$ (100 ml) and left to stir with H_2 bubbling. After 3 h, $Cr(ClO_4)_3$ was quantitatively reduced to yield 0.006 mol/L Cr^{2+}. The Cr^{2+} concentration was determined by treating a reaction aliquot with an aqueous solution of $[Co^{III}(NH_3)_5Cl]Cl_2$, under Ar; the mixture was then poured into concentrated HCl and the Co^{II} content was analyzed by measuring the absorbance of $CoCl_4^{2-}$ at 692 nm.[21] For the *in situ* generation of CrO^{2+}, a deoxygenated solution of Cr^{2+} was injected into an acidic aqueous solution of pectin saturated with oxygen (1.26×10^{-3} mol/L). In a typical experiment, 100 μl of 4.97×10^{-3} mol/L Cr^{2+} were injected into a septum-capped spectrophotometric cell, with path length of 1 cm, filled with 2.3 ml of an O_2-saturated solution containing 1.24–3.29 g/L pectin and appropriate concentration of $HClO_4$ and $NaClO_4$. Under these conditions, CrO^{2+} immediately formed and then reacted with pectin. At very low Cr^{II}/O_2 ratios (≤0.05), CrO_2^{2+} is quantitatively formed, while at intermediate Cr^{II}/O_2 ratios (≈0.15), the reaction affords mixtures of CrO^{2+} and CrO_2^{2+}.[15] Under our conditions, the reaction between Cr^{2+} and O_2 rapidly produced 6.24×10^{-5} mol/L CrO^{2+} (30% average based on total Cr^{2+}), which then reacted with the organic substrate to render Cr^{2+} and oxidized organic products. Under these experimental conditions, Cr^{2+} formed by reaction of pectin with CrO^{2+}, quantitatively transformed into CrO_2^{2+} (Cr^{2+}/O_2 ratio ≤ 0.05) and no autocatalytic consumption of CrO_2^{2+} by Cr^{2+} occurred.[15,22] The concentration of CrO^{2+} generated by reaction of Cr^{2+} with O_2 was determined by injection of 100 μl of 5×10^{-3} mol/L Cr^{2+} to 2.3 ml of an O_2-saturated solution of 0.1 mol/L ehba buffer (pH 3.0), at 15°C. Immediately, the solution turned pink and the absorbance of $[Cr(O)(ehba)_2]^{2-}$ at 512 nm (ε 2380 L mol^{-1} cm^{-1}) was measured.[23]

Detection of superoxoCr(III) ion (CrO_2^{2+}) during the reaction of pectin with Cr^{VI}

The presence of CrO_2^{2+} in mixtures of pectin/Cr^{VI} was investigated by UV–Vis spectrophotometry in the 230–400 nm region, at 25°C. The instrument was zeroed to an arrangement of the reference and sample beams passing through matched cells, both containing pectin in $HClO_4$. Then, the solution in the sample cell was replaced by the reaction solution containing 2.25 g/L pectin, 1.26×10^{-3} mol/L O_2, and 4.35×10^{-5} mol/L Cr^{VI} in 2.0 mol/L $HClO_4$. Periodic scanning of the reaction mixture showed that the Cr^{VI} band at 350 nm decreased in intensity, while new peaks at 290 and 247 nm, characteristic of CrO_2^{2+}, grew in.

Generation of long-lived (oxo)chromium(V)–pectin species

Long-lived oxo-chromium(V)–pectin complexes were generated by reaction of $K_2Cr_2O_7$ (1 ml, 0.30 mol/L) with 56.5 ml of an aqueous solution containing glutathione (5.3×10^{-3} mol/L) as a reductant and apple pectin (9.95 g/L) as a complexant, at 25°C.[9,13,24–27] The pH of this mixture was 3.0. The formation of oxo-Cr^V–pectin species was monitored by EPR spectroscopy. Only a signal at $g = 1.9785$, characteristic of oxo-Cr^V-O-donor ligand complexes, was observed during several hours. Alternatively, a mixture of 10.7×10^{-3} mol/L Cr^{VI} + 10.7×10^{-3} mol/L glutathione + 4.5 g/L pectin was reacted in buffer acetate (pH = 4.66). Also in this case, a stable oxo-Cr^V–pectin species could be observed by EPR spectroscopy for at least 6 h. The same EPR spectral pattern was observed in reaction mixtures of pectin (4.5 g/L) and Cr^{VI} (10.7×10^{-3} mol/L) in buffer acetate (pH 4.66).

Kinetic measurements

Cr^{VI} + apple pectin reaction

The kinetics of the Cr^{VI} + pectin reaction was studied under pseudo-first-order conditions, at 60°C, using excess of pectin over Cr^{VI} and different [$HClO_4$]. Reactant solutions were thermally equilibrated at 60°C prior to the experiment and $NaClO_4$ was used to maintain a constant ionic strength (μ). The Cr^{VI} consumption was followed by iodometric titration until at least 80% conversion. In the kinetic measurements, the concentration of Cr^{VI} and μ were kept constant at 1.20×10^{-3} mol/L and 0.5 mol/L, respectively, while pectin concentration was varied from 9.78 to 22.17 g/L, at various [$HClO_4$]. The experimental pseudo-first-order rate constants ($k_{6\mathrm{exp}}$), obtained from nonlinear least-square fits of kinetic data, were averages of at least three determinations and were within ±5% of each other. The first-order dependence of the rate upon [Cr^{VI}] was verified in a set of experiments where the $[Cr^{VI}]_0$ was varied between 8.05×10^{-4} and 4.00×10^{-3} mol/L, but temperature, $[\mathrm{pectin}]_0$, [H^+], and μ were kept constant.

Cr^{IV} + apple pectin reaction

Spectrophotometric monitoring of the oxidation of pectin by CrO^{2+} showed an increase of the two intense absorption bands at 290 and 247 nm, with relative intensity ($Abs_{247}/Abs_{290} = 2.3$), characteristic of CrO_2^{2+}.[15,28] CrO_2^{2+} was additionally confirmed by the disappearance of its characteristic absorption at 290 nm when Fe^{2+} was added to convert CrO_2^{2+} to Cr^{3+}_{aq}.[29] The difference absorption spectra between the final solution and those after addition of Fe^{2+}, showed a negative difference at 290 nm, consistent with the presence of CrO_2^{2+}. The kinetic data were collected spectrophotometrically by following the formation of CrO_2^{2+} at 290 nm ($\varepsilon = 3100\ \mathrm{L\,mol^{-1}\,cm^{-1}}$),[30] at 25°C. At this wavelength, neither pectin nor the oxidized products absorb. In the kinetic measurements, the concentration of Cr^{IV} and μ were kept constant at 6.24×10^{-5} and 0.5 mol/L, respectively, while pectin concentration was varied from 1.24 to 3.29 g/L, at various [$HClO_4$]. The experimental pseudo-first-order rate constants ($k_{4\mathrm{exp}}$), obtained from nonlinear least-square fits of absorbance data at 290 nm were averages of at least five determinations and were within ±10% of each other. The first-order dependence of the rate upon [Cr^{IV}] was verified in a set of experiments where the $[Cr^{IV}]_0$ was varied between 1.86×10^{-5} and 6.24×10^{-5} mol/L, but T, $[\mathrm{pectin}]_0$, [H^+], and μ were kept constant.

Cr^{V} + apple pectin reaction

The pseudo-first-order rate constant $k_{5\mathrm{exp}}$ was estimated on a mixture of apple pectin (9.78 g/L), glutathione (1.207×10^{-3} mol/L) and Cr^{VI} (1.207×10^{-3} mol/L) that was allowed to react for 30 min and then acidified with $HClO_4$ (0.12 mol/L), at 25°C and $\mu = 0.5$ mol/L. The reaction was quenched at different times in a glass-bath and unreacted Cr^{V} was measured iodometrically. The value of $k_{5\mathrm{exp}}$ was the average of three determinations that were within ±5% of each other. Under these conditions, the reaction rapidly yielded Cr^{III}, HCO_2H, and oxidized pectin as final redox products.

Free-radical test

Cr^{VI}–pectin reaction

Acrylonitrile (0.5 ml) was added to a reaction mixture containing 10 g/L pectin and 1.2×10^{-3} mol/L Cr^{VI} in 10 ml of 0.5 mol/L $HClO_4$. The mixture was left 2 h at 60°C. After this time, a white precipitate appeared. ^{13}C NMR spectrum of a D_6-DMSO solution of the white solid and FT-IR spectrum of the solid showed the pattern characteristic of polyacrylonitrile. Blank experiments with either Cr^{VI} or pectin gave no detectable white precipitates. The possible reaction of Cr^{V} with acrylonitrile was excluded based on the absence of a precipitate after mixing $[Cr^{V}O(\mathrm{ehba})_2]K$[31] and acrylonitrile under the same reaction conditions as before.

Cr^{IV}–pectin reaction

A mixture of 0.5 ml acrylonitrile + 5 ml of 9 g/L pectin in 0.5 mol/L $HClO_4$ was saturated with O_2. CrO^{2+} was generated *in situ* by injecting 50 µl of 0.03 mol/L Cr^{2+} ($[Cr^{2+}]_{\mathrm{final}} = 0.27 \times 10^{-3}$ mol/L). The mixture was left 1 h at 25°C. After this time, a white precipitate of polyacrylonitrile appeared. Blank experiments with either Cr^{IV} or pectin gave no detectable white precipitates.

Product analysis

Cr^{VI}–pectin reaction

Evolved carbon dioxide was measured from a mixture of 19.3 g/L pectin + 0.01 mol/L Cr^{VI} in 0.5 mol/L $HClO_4$. The temperature was kept constant at 60°C and the mixture was continuously stirred and flushed with pure nitrogen. The gaseous products were passed through three flasks containing 0.0218 mol/L NaOH solution. After reaction completion, the NaOH solutions were titrated with standard 0.0267 mol/L HCl to determine the carbon dioxide yielded. Aliquots of the reaction mixture were analyzed by HPLC, and HCO_2H was identified as a reaction product by comparing the retention time (t_R) of the product against a standard sample of formic acid in 0.5 mol/L $HClO_4$ ($t_R = 13'20''$). Co-chromatography of the reaction mixture and formic acid resulted in the increase of the peak of the reaction product appearing at $t_R = 13'20''$. Additionally, the presence of HCO_2H acid was confirmed through the $HgCl_2$ reaction.[32] Peak-area *versus* [HCO_2H] curves were obtained by HPLC and used for quantitation purposes.

Cr^{IV}–pectin reaction

Cr^{2+} (3.2 ml of 0.0625 mol/L) was injected to a 5.0 g/L pectin solution in 0.5 mol/L $HClO_4$ saturated with O_2 (1 L). The temperature was kept at 25°C and the mixture was continuously stirred and flushed with pure oxygen. The gaseous products were passed through three flasks, each containing 50 ml of 0.0103 mol/L NaOH solution. After the completion of the reaction, the NaOH solutions were titrated with standard 0.0199 mol/L HCl to determine the carbon dioxide yielded, and the solution in the reaction vessel was analyzed for its HCO_2H content by HPLC, as described above for the Cr^{VI} + pectin reaction.

Cr^{V}–pectin reaction

Cr^{VI} (1.0 ml of 0.30 mol/L), glutathione (1.0 ml of 0.30 mol/L), and pectin solution (55.5 ml of 5g/L) were mixed and left to stir for 30 min. Then 2.5 ml of 11.6 mol/L $HClO_4$ were added. Temperature was kept at 25°C and the mixture was continuously stirred and flushed with pure nitrogen. The gaseous products were passed through three flasks containing 0.0200 mol/L NaOH solutions. After the completion of the reaction, the NaOH solutions were titrated with standard 0.0248 mol/L HCl to determine the carbon dioxide yielded, and the solution in the

Table 1. Observed pseudo-first-order rate constants (k_{6exp})^a for different concentrations of $HClO_4$ and pectin

	$10^4\ k_{6exp}$ (s^{-1}) for [$HClO_4$] (mol/L)				
[Pectin] (g/L)	0.072	0.12	0.24	0.36	0.48
9.78	0.61(2)	0.971(9)	1.92(4)	3.11(2)	4.28(9)
11.75	0.756(5)	1.21(1)	2.38(5)	3.72(2)	5.2(1)
13.29	0.849(6)	1.29(3)	2.66(7)	4.20(2)	5.96(9)
17.52	1.10(7)	1.78(3)	3.60(9)	5.68(4)	8.1(1)
22.17	1.40(1)	2.21(2)	4.66(4)	7.3(1)	10.5(1)
$10^3\ k_{6p}$ ($L\ mol^{-1}\ s^{-1}$)	1.17(1)	1.84(1)	3.79(4)	5.98(6)	8.5(1)

$T = 60°C$; $[Cr^{VI}]_0 = 1.20 \times 10^{-3}$ mol/L; $\mu = 0.50$ mol/L.

[a] Mean values from multiple determinations. Uncertainty in the last figure is given in parentheses.

reaction vessel was analyzed for its HCO_2H content by HPLC, as described above for the Cr^{VI} + pectin reaction.

RESULTS AND DISCUSSION

Oxidation of pectin by Cr^{VI}

Rate studies

The kinetics of the reaction of pectin with Cr^{VI} was examined by iodometric determination of [Cr^{VI}] consumption under conditions of excess of pectin over Cr^{VI}, in the 0.07–0.48 mol/L $HClO_4$ range. Under these conditions, Cr^{IV} and Cr^{V} do not interfere with the measurements of [Cr^{VI}] because their decay rates are much higher than that of Cr^{VI} (as shown below). A monophasic decrease of [Cr^{VI}] with time was observed, and the kinetic profiles could be adequately described by a single exponential decay from which Cr^{VI} pseudo-first-order rate constants (k_{6exp}) were calculated. Table 1 summarizes values of k_{6exp} for various concentrations of pectin and $HClO_4$. The value of k_{6exp} did not vary with different $[Cr^{VI}]_0$ at fixed *T*, μ, [pectin], and [$HClO_4$], confirming the first-order dependence of rate on [Cr^{VI}]. Plots of k_{6exp} *versus* [pectin] gave good straight lines (Fig. 1) from which values of k_{6p} were determined (Table 1, bottom). Values of k_{6p} showed linear dependence on [$HClO_4$] (inset of Fig. 1), and the rate constant k_6, calculated from the slope of the k_{6p} *versus* [H^+] plot, was found to be $(1.70 \pm 0.04) \times 10^{-2}\ L^2\ mol^{-2}\ s^{-1}$. Consequently, the complete rate law for the Cr^{VI} consumption can be expressed as in the equation:

$$\frac{-d[Cr^{VI}]}{dt} = k_6[H^+][\text{pectin}][Cr^{VI}] \qquad (1)$$

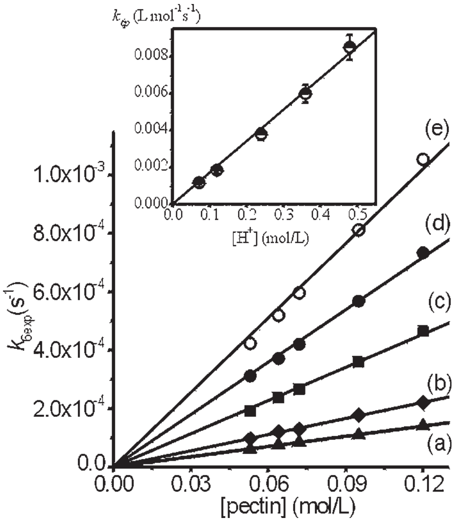
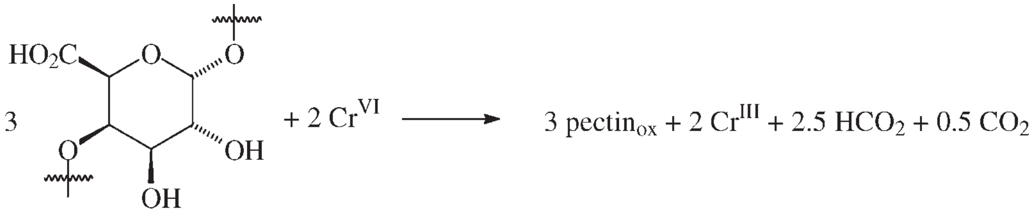


Figure 1. Effect of [pectin] on k_{6exp} at 60°C, $\mu = 0.50$ mol/L, and (a) 0.072, (b) 0.12, (c) 0.24, (d) 0.36, (e) 0.48 mol/L [$HClO_4$]. Inset: linear dependence of k_{6p} on [H^+]

Under conditions used in the kinetic studies, HCO_2H and CO_2 were identified as reaction products in the way described in the experimental part. Quantitative analysis of these products showed that the reaction yielded 1.25 ± 0.05 mol of HCO_2H and 0.25 ± 0.05 mol of CO_2 per mole of Cr^{VI}. The site in pectin being oxidized can be rationalized taking into account the relative reactivity of functional groups in saccharides toward Cr^{VI}: —(H)C(OR)$OH_{hemiacetal}$[33–35] (or aldehyde) > —CO_2H[13,36–37] > —$H_2COH_{primary}$[38] >> —(H)$COH_{secondary}$,[39] —(H)$COR_{glycoside}$,[27,40] RCO_2R_{ester},[41] and the proportion of these groups in pectin. In the polymer, terminal hemiacetalic groups and free carboxylic groups of GalA residues are the most reactive sites toward Cr^{VI}. However, excess of —CO_2H in the polymer compared to —(H)C(OR)$OH_{hemiacetal}$ favors the reaction of Cr^{VI} with —(H)C(OR)—CO_2H moieties of GalA residues. Therefore, C—C bond break in —(H)C(OR)—CO_2H moieties prevails over C—H cleavage of terminal hemiacetals, to yield HCO_2H/CO_2 and oxidized pectin. In this reaction, chromic oxidation of HCO_2H to yield CO_2 can be ruled out based on the relative reactivity of pectin and HCO_2H toward Cr^{VI}. The value of k_6 for the oxidation of HCO_2H by Cr^{VI} was found to be two-order of magnitude lower than for pectin.[42] Therefore, the oxidation of HCO_2H by Cr^{VI} competes unfavorably with the reaction of Cr^{VI} with free carboxylic groups of GalA residues, which can either yield HCO_2H or CO_2 (as shown in mechanism below). Stoichiometry of the oxidation of pectin by Cr^{VI} is shown in Scheme 1.



Scheme 1. Stoichiometry of the oxidation of apple pectin by Cr^{VI}. $Pectin_{ox}$ = oxidized pectin

Intermediacy of Cr^V

The most common means of detecting Cr^V complexes in solution is EPR spectroscopy, where strong isotropic signals are observed at room temperature in X-band spectra. Typical Cr^V EPR spectra exhibit a single narrow signal (1–5 G) centered at $g_{iso} \sim 1.98$. The Cr^{VI} + pectin reaction at $[H^+] > 0.01$ mol/L and $T = 60°C$, did not result in any EPR signal. However, when the reaction was performed in the pH range 3–5 and 20°C, the EPR spectra were dominated by a signal at $g_{iso} = 1.9785$ (Fig. 2), typical of five-coordinate oxochromate(V) complexes formed with *O*-donor ligands.[3,9,43] The same EPR signal was observed when Cr^{VI} was reacted with a mixture of glutathione as a 1-electron reductant (1:1 glutathione/Cr^{VI}) and excess of pectin as a complexant,[44] at pH 3.0–4.7 and 20°C. Under these conditions, Cr^V – formed by rapid reaction between glutathione and Cr^{VI} – was efficiently stabilized by pectin. The formed oxo-Cr^V–pectin species remained in solution during several hours, such as observed for other oxo-Cr^V species with *O*-donor ligands.[3,9] The 10 G half-width of the EPR signal was broader than expected for single oxo-Cr^V species, and could not be resolved even with low modulation amplitude. The broadness of the signal can result from overlap of signals belonging to oxo-Cr^V species with Cr^V bound to different galacturonate residues of pectin. It must be noted that the average g_{iso} value is in the range of that expected for five-coordinated oxochromate(V) complexes with ligand bound to the metal through two carboxilato and two alcoholato donor sites.[43] This means that Cr^V binds at least two GalA units, which may belong to the same or different polymeric chain, thus affording the unresolved EPR signal.

Intermediacy of Cr^{II}

Involvement of Cr^{II} in the mechanism of the oxidation of a number of alcohols, saccharides, and hydroxyacids by Cr^{IV} and Cr^{VI} in $HClO_4$ has been demonstrated by conversion into CrO_2^{2+} upon reaction with molecular oxygen [14–15,30,45]. At high [O_2] and low [Cr^{VI}], the reaction of Cr^{II} with O_2 can compete successfully with the reaction of Cr^{II} with Cr^{VI} and the autocatalytic consumption of CrO_2^{2+} by Cr^{II}, and if formed, Cr^{II} should yield the CrO_2^{2+} product.[14–15,30,36] We examined the presence of intermediate Cr^{II} in the reaction of pectin with Cr^{VI}, by monitoring the formation of CrO_2^{2+}, using $[Cr^{VI}]_0$ low enough to avoid the Cr^{VI} + Cr^{II} competitive reaction, and 2.0 mol/L $HClO_4$ to accelerate the Cr^{VI} + pectin reaction at a temperature lower than used in the kinetics experiments. A periodic scanning of the O_2-saturated solution (1.26×10^{-3} mol/L O_2) of a Cr^{VI} + pectin reaction mixture in 2.0 mol/L $HClO_4$ over a period of 6 h, showed that the band at 350 nm, characteristic of Cr^{VI}, decreased in intensity while two absorption bands at $\lambda_{max} = 247$ and 290 nm appeared (Fig. 3). These two bands are characteristic of CrO_2^{2+} formed as a long-lived intermediate,[45] that then slowly transforms into the final Cr^{III} (as shown below). Since CrO_2^{2+} can be exclusively formed by reaction of Cr^{II} with O_2, and, in turn, Cr^{II} had previously been demonstrated to form exclusively through two-electron reduction of Cr^{IV},[15,30] our spectroscopic results provide evidence that Cr^{II} and Cr^{IV} are involved in the redox mechanism of the reaction between Cr^{VI} and pectin.

Reduction of Cr^V by pectin

Rapid chill quenching and iodometric titration of unreacted Cr^V in a mixture of Cr^V + pectin in 0.12 mol/L $HClO_4$, at 25°C, showed a monophasic decrease of [Cr^V] with time. The kinetic profile could be adequately fitted to a single exponential decay from which Cr^V pseudo-first-order rate constant (k_{5exp}) was calculated. The value of k_{5exp} estimated in this way resulted to be $0.17 \pm 0.01\ s^{-1}$.

Acidified solutions containing excess of pectin over equimolar Cr^{VI}glutathione mixtures, were analyzed for CO_2 and HCO_2H as reaction products. Only HCO_2H was detected. It was not possible to determine the quantity of HCO_2H yielded per mole of Cr^V based on $[Cr^V]_0$ because, although all Cr^V present in the starting solution (before acidification of the solution) exists as oxo-Cr^V–pectin, a fraction of Cr^V can disproportionate or yield ligand oxidation before pectin traps it. EPR and electronic spectroscopy show that Cr^{III} is the ultimate fate of chromium in these reactions.

Reaction of CrO^{2+} with pectin

The addition of variable quantities of pectin to O_2-saturated solutions containing 6.24×10^{-5} mol/L of CrO^{2+} resulted in the increase of the CrO_2^{2+} spectrum intensity. The formation of CrO_2^{2+} was followed at 290 nm and the monotonic increase of absorbance was found to follow first-order kinetics. The experimental rate constants k_{4exp} were calculated by nonlinear

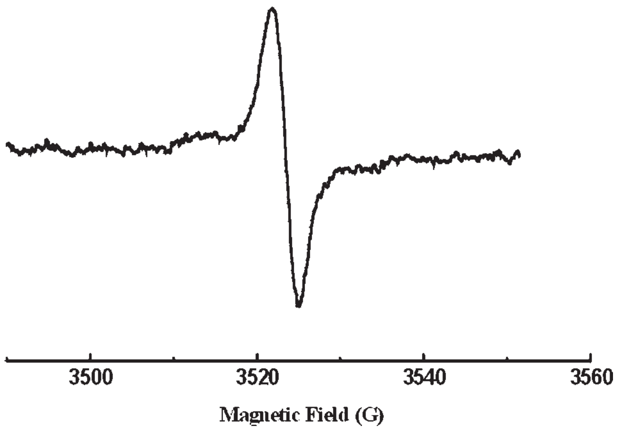


Figure 2. X-band EPR spectrum from a mixture of 0.0107 mol/L Cr^{VI} and 4.5 g/L pectin, at $T = 20°C$. $\mu = 0.50$ mol/L; pH = 4.66; mod. ampl. = 3.9 G; $\nu = 9.75893$ GHz

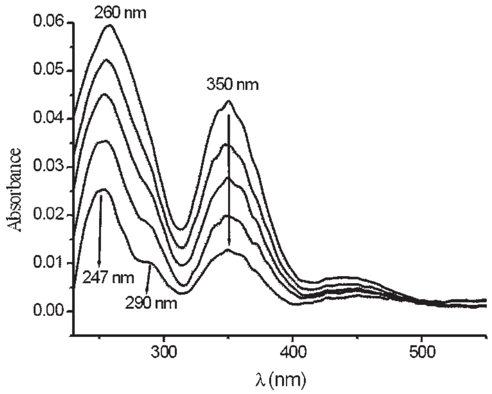


Figure 3. Formation of CrO_2^{2+} from the reaction between 2.27 g/L pectin, 1.26×10^{-3} mol/L O_2, and 4.35×10^{-5} mol/L Cr^{VI} in 2.0 mol/L $HClO_4$, at 25°C

Table 2. Experimental pseudo-first-order rate constants ($k_{4\exp}$) for different concentrations of $HClO_4$ and pectin

$[HClO_4]$ (mol/L)	$10^2\ k_{4\exp}{}^{a}$ (s^{-1})	[Pectin] (g/L)	$10^2\ k_{4\exp}{}^{b}$ (s^{-1})
0.21	7.9(4)	1.24	7.0(1)
0.30	7.8(3)	1.65	9.0(1)
0.40	8.0(1)	2.00	10(2)
0.50	8.3(1)	2.49	13(2)
		3.29	17(3)

$T = 25°C$; $[Cr^{IV}]_0 = 6.24 \times 10^{-5}$ mol/L; $\mu = 0.50$ mol/L.
[a] [pectin] = 1.28 g/L.
[b] $[HClO_4] = 0.20$ mol/L.

least-square fit of absorbance-time data to the equation:

$$\mathrm{Abs}_t = \mathrm{Abs}_\infty + (\mathrm{Abs}_0 - \mathrm{Abs}_\infty)\, e^{(-k_{4\exp} t)} \quad (2)$$

The value of $k_{4\exp}$ did not vary with different $[Cr^{IV}]_0$, but fixed T, μ, [pectin], and $[HClO_4]$, confirming the first-order dependence of rate on $[Cr^{IV}]$. Table 2 summarizes values of $k_{4\exp}$ for various concentrations of pectin and $HClO_4$. Experimental conditions were chosen so that the Cr^{IV} + pectin reaction was much faster than Cr^{IV} disproportionation into Cr^{VI} and Cr^{III}. In the absence of pectin, or when the pectin concentration was too low, disproportionation of Cr^{IV} was evidenced by the grow-up of absorbance at 350 nm due to formation of Cr^{VI}. Using reactant concentrations of Table 2, Cr^{VI} was not detected. Values of $k_{4\exp}$ are independent of $[H^+]$ in the range 0.2–0.5 mol/L, but vary linearly with [pectin], as shown in Fig. 4. The slope of the plot gives $k_4 = 9.7\ \mathrm{L\ mol^{-1}\ s^{-1}} \pm 0.1\ \mathrm{L\ mol^{-1}\ s^{-1}}$ as the second-order-rate constant for the reaction of CrO^{2+} and pectin, at $\mu = 0.5$ mol/L and $T = 25°C$. Consequently, the complete rate law for the Cr^{IV} consumption can be expressed as in the equation:

$$\frac{d[CrO_2^{2+}]}{dt} = \frac{-d[Cr^{IV}]}{dt} = k_4[\mathrm{pectin}][Cr^{IV}] \quad (3)$$

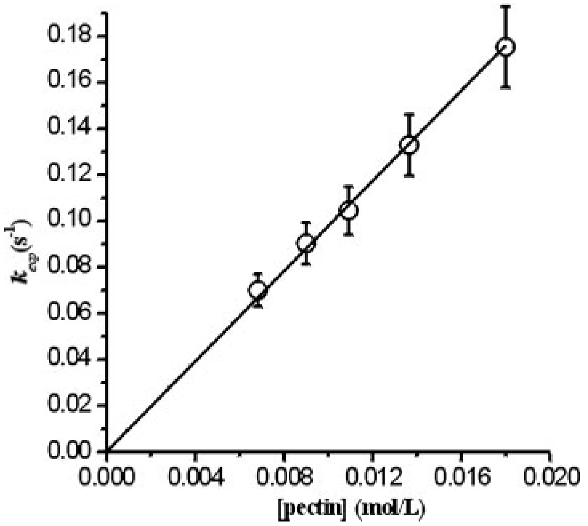


Figure 4. Effect of [pectin] on $k_{4\exp}$, at 25°C. $[HClO_4] = 0.20$ mol/L, $[Cr^{IV}]_0 = 6.24 \times 10^{-5}$ mol/L; $\mu = 0.50$ mol/L

CrO_2^{2+}, formed in this reaction, slowly decays to final Cr^{III} through a pectin-independent pathway (Fig. 5). The rate of disappearance of CrO_2^{2+} depends on $[H^+]$.[46] In 0.2 mol/L $HClO_4$, $[CrO_2^{2+}]$ decay rate is 100–250 times slower than that of the CrO^{2+} + pectin reaction ([pectin] = 7–18 × 10^{-3} mol/L). An independently prepared solution of CrO_2^{2+} in 0.2 mol/L $HClO_4$,[46] decomposed at the same rate as CrO_2^{2+} formed in the Cr^{IV} + pectin reaction, thus confirming that pectin does not react with CrO_2^{2+}.

Reaction mixtures containing excess of pectin over CrO^{2+} in 0.5 mol/L $HClO_4$ were analyzed for CO_2 and HCO_2H as reaction products. Only CO_2 was detected. Quantitative analysis of CO_2 showed that the reaction yielded 1.0 ± 0.2 mol of CO_2 per mole of Cr^{IV}. In a separate experiment, a mixture containing $HCO_2H + CrO_2^{2+}$ in O_2-saturated 0.5 mol/L $HClO_4$ yielded CO_2 quantitatively. Therefore, CO_2 found in the Cr^{IV} + pectin reaction can either come from oxidation of pectin by Cr^{IV} or from further CrO_2^{2+}-induced oxidation of HCO_2H (formed by reaction of pectin with Cr^{IV}) by O_2.[15]

Reaction mechanism

In the range of substrate and acid concentration used in the kinetic measurements, the oxidation of pectin by Cr^{VI} is a complex reaction that yields Cr^{III}, CO_2/HCO_2H, and oxidized pectin as final redox products. The fact that CrO_2^{2+} is detected in the reaction of pectin with Cr^{VI} together with the observation of Cr^{V} species at pH higher than used in the kinetic measurements, indicate that both Cr^{IV} and Cr^{V} intermediate species are formed in this reaction. However, under conditions used in the kinetic measurements, Cr^{IV} and Cr^{V} react with pectin much faster than Cr^{VI} and do not accumulate in the reaction mixture. Thus, the concentration-time profiles obtained by iodometric titration of the Cr^{VI} + pectin mixtures reflect the $[Cr^{VI}]$ monotonic decay without interference of $[Cr^{IV}]$ or $[Cr^{V}]$. In Scheme 2, we propose a mechanism that combines $Cr^{VI} \rightarrow Cr^{IV} \rightarrow Cr^{II}$ and $Cr^{VI} \rightarrow Cr^{IV} \rightarrow Cr^{III}$ pathways, and takes into account: (a) kinetic results, (b) the polymerization

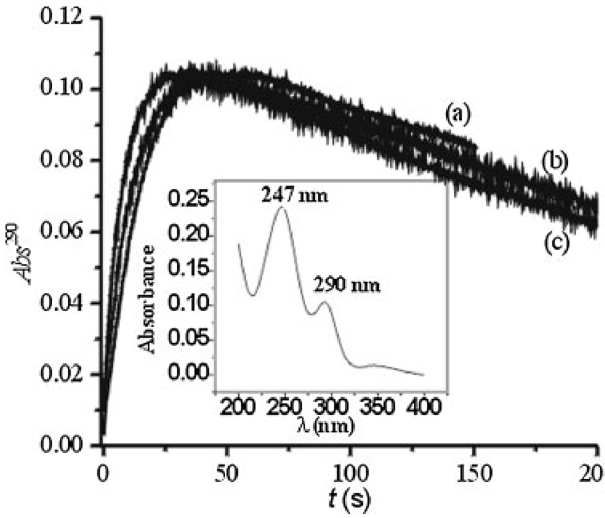


Figure 5. Curves showing absorbance *versus* time for the oxidation of (a) 0.63 g/L, (b) 1.26 g/L, and (c) 2.59 g/L apple pectin by Cr^{IV} (6.24×10^{-5} mol/L) in O_2-saturated 0.20 mol/L $HClO_4$. $\mu = 0.50$ mol/L; $T = 25°C$. Inset: UV–Vis spectrum of CrO_2^{2+} taken at $t = 1$ min, under experimental conditions of curve (a)

$$\text{pectin} + \text{Cr}^{VI} \xrightarrow{k_6,\ H^+} \text{Cr}^{IV} + \text{HCO}_2\text{H} + \text{pectin}_{ox} \quad (4)$$

$$\text{Cr}^{IV} + \text{pectin} \xrightarrow{\text{fast}} \text{Cr}^{III} + \text{CO}_2 + \text{pectin}^{\bullet} \quad (5)$$

$$\text{Cr}^{IV} + \text{pectin} \xrightarrow{\text{fast, } (k_4)} \text{Cr}^{II} + \text{HCO}_2\text{H} + \text{pectin}_{ox} \quad (6)$$

$$\text{Cr}^{II} + \text{Cr}^{VI} \xrightarrow{\text{fast}} \text{Cr}^{V} + \text{Cr}^{III} \quad (7)$$

$$\text{Cr}^{VI} + \text{pectin}^{\bullet} \xrightarrow{\text{fast}} \text{Cr}^{V} + \text{pectin}_{ox} \quad (8)$$

$$\text{pectin} + \text{Cr}^{V} \xrightarrow{\text{fast } (k_5)} \text{Cr}^{III} + \text{HCO}_2\text{H} + \text{pectin}_{ox} \quad (9)$$

$$\text{Cr}^{II} + \text{O}_2 \xrightarrow{\text{fast}} \text{CrO}_2{}^{2+} \rightrightarrows \text{Cr}^{III} \quad (10)$$

$$\text{O}_2 + \text{pectin}^{\bullet} \xrightarrow{\text{fast}} \text{pectin}'_{ox} \quad (11)$$

Scheme 2. Mechanism of the oxidation of apple pectin by Cr^{VI}

of acrylonitrile added to the reaction mixture, (c) detection of intermediate of oxochromate(V) species, (d) observation of CrO_2^{2+}, and (e) formation of CO_2/HCO_2H and Cr^{III} as final reaction products.

The reduction of Cr^{VI} by pectin requires H^+ to occur. In contrast, reduction of Cr^{IV} by pectin is pH independent and in the 0.2–0.5 mol/L [H^+] range and 25°C, pectin + Cr^{IV} reaction is 7×10^5–7×10^4 times faster than pectin + Cr^{VI} at 60°C. Cr^{V} also oxidizes pectin faster than Cr^{VI} does. In 0.12 mol/L HClO_4 and 25°C, Cr^{V} oxidizes pectin 2×10^3 times faster than Cr^{VI} at 60°C. Therefore, Cr^{IV} and Cr^{V}, although formed in the Cr^{VI} + pectin reaction, should be involved in fast steps of the reaction pathway. The slow redox step proposed in Scheme 2 involves C—C bond cleavage through a two-electron redox process to yield Cr^{IV}, HCO_2H, and oxidized pectin (eqn (4)). The initial two-electron reduction of Cr^{VI} by pectin is in agreement with previous reports on various acid saccharides that were selectively oxidized by Cr^{VI} to the lower homolog.[13,36–37] In the mechanism, we have included two competitive one- and two-electron reductions of Cr^{IV} by pectin. Thus, Cr^{IV} is proposed to react with excess pectin to yield Cr^{III}, CO_2, and pectin radical, or Cr^{II}, HCO_2H, and oxidized pectin, through two alternate fast steps (eqns (5) and (6)). The first is supported by the observed polymerization of acrylonitrile when it is added to the Cr^{IV} and Cr^{VI}/pectin reaction mixtures, while the second, by the observation of CrO_2^{2+} (the product of the reaction of Cr^{II} with O_2). Cr^{V} can form by fast reaction of Cr^{II} with Cr^{VI} (eqn (7)) and, alternatively, by rapid reaction of the pectin radical with Cr^{VI} (eqn (8)). Cr^{V} can further oxidize pectin to yield Cr^{III}, HCO_2H, and oxidized pectin as final redox products (eqn (9)).

In O_2-saturated solutions (1.26×10^{-3} mol/L) and $[\text{Cr}^{VI}]_0 < 0.1 \times 10^{-3}$ mol/L, reactions (7) and (8) can be neglected because pectin$^{\bullet}$ and Cr^{II} intermediates formed in reactions (5) and (6) should be rapidly trapped by O_2 (reactions 10 and 11).[47] The proposed mechanism is in accordance with the observation that O_2 has no kinetic effect on this reaction, because when $[\text{Cr}^{VI}]_0 \geq 0.5 \times 10^{-3}$ mol/L (as employed in the kinetic measurements), both Cr^{II} and pectin$^{\bullet}$ react with Cr^{VI} faster than they do with O_2, and reactions (10) and (11) can be neglected.[15,29,48]

Detection of free radicals and CrO_2^{2+} yield < 100% in the Cr^{IV} + pectin reaction in O_2-saturated solutions, provided information supporting that the reduction of Cr^{IV} by pectin takes place through reactions (5) and (6). If the reaction took place exclusively through the Cr^{IV} → Cr^{II} pathway (reaction (6)), the yield of CrO_2^{2+} had to approach 100%.[15] However, in all the tested Cr^{IV} + pectin redox reactions, the yield of CrO_2^{2+} reached a limiting value of ≈50%, independently of [pectin], while CO_2 yield was 100% (HCO_2H, if formed, was further oxidized to CO_2 by O_2 in the presence of CrO_2^{2+}). These results suggest that part of the CrO^{2+} reacted through a pathway that did not involve Cr^{II}. The observed polymerization of acrylonitrile in the reaction of Cr^{IV} + pectin suggests that this competitive reaction could involve one-electron $Cr^{IV} \rightarrow Cr^{III}$ reduction concomitantly with the formation of pectin radical.[14] To account for the observed 50% yield of CrO_2^{2+}, both competitive reactions (5) and (6) should occur with similar rate.

The reaction of Cr^V with pectin is also much faster than Cr^{VI} + pectin. Only a few seconds are required to reduce Cr^V in acid solution, at 25°C. The redox reaction involves two-electrons and yields HCO_2H as the only low molecular weight organic product. At higher pH, the redox reaction is slower, and Cr^V–pectin species remain in solution for several hours. At pH 3–5, pectin is a good scavenger of Cr^V generated by one-electron reduction of Cr^{VI}, and the EPR *g*-parameter suggests that the donor sites of pectin involved in coordination to the metal are carboxylate and hydroxyl groups.

The fact that in the Cr^{VI} + pectin reaction mechanism steps 5 and 6 are much faster than reaction 4, implies that Cr^{II} and pectin$^\bullet$ are produced at the same rate to afford $[Cr^{II}]/[pectin^\bullet] = [CrO_2^{2+}]/([Cr^{VI}]_0 - [CrO_2^{2+}]) = 1$. Detection of CO_2 in the Cr^{VI} + pectin reaction under anaerobic conditions means that, in this reaction, CO_2 could not be formed by oxidation of HCO_2H with O_2, but through oxidation of pectin by high-valent Cr. To account for the observed products and stoichiometry, in Scheme 2 it is proposed that reaction (5) affords CO_2 while reaction (6) yields HCO_2H. The overall reaction stoichiometry deduced from reactions (4) to (9) of Scheme 2 agrees with the experimental 1.25:0.25:1 $HCO_2H/CO_2/Cr^{VI}$ ratio found in mixtures of Cr^{VI} and apple pectin, under anaerobic conditions.

The present results suggest that Cr^{VI} (as well as Cr^{IV} and Cr^V) selectively oxidizes the free carboxylic groups of GalA units through C—C bond break of the —(H)C(OR)—CO_2H moieties. This pattern of selectivity distinguishes chromic oxidation of polyuronic acids from that observed for $NaVO_3$ that oxidizes the terminal unit of the polymeric chain,[49] and MnO_4^- that yields ketoderivatives upon oxidation of vicinal hydroxyl groups.[50]

CONCLUSIONS

The reaction of pectin with Cr^{VI} strongly depends on pH. In acid medium, redox reaction occurs and reactive Cr^V, Cr^{II}, and Cr^{IV} intermediate species are generated in the redox process, together with CO_2/HCO_2H, free radicals, and oxidized forms of pectin. At $pH > 3$, apple pectin slowly reduces Cr^{VI}, and stabilizes Cr^V yielding long-lived oxo-Cr^V–pectin species, that, if the medium becomes acid, rapidly affords redox processes. The Cr-binding sites of apple pectin are mainly the free carboxylic groups that can undergo redox processes with Cr^{VI-IV} in acid medium or stabilize Cr^V together with hydroxo donor groups of the polymer. The present results evidence that pectin can act as reductant of chromate (specially at low pH), with formation of highly toxic free radicals, CrO^{2+} and Cr^V intermediate species, or can be involved in the transport of high-valent chromium at $pH > 3$.

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