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Effect of cations on heat degradation of chelator-soluble carrot pectin

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The effects of monovalent salts, NaCl, KCl, and NH_4Cl, and divalent salts, $ZnCl_2$, $CaCl_2$, $SrCl_2$, $CdCl_2$, and $MgCl_2$, on heat degradation of pectin at pH 6·1 were examined. The β-eliminative degradation of extracted carrot pectin increased as cation concentration in the pectin solution was raised. At the same level of pectin methoxyl content, divalent cations promoted more depolymerization during the heating of pectin than did monovalent cations. The enhancement effect of cations on pectin degradation was greater for low-methoxyl pectin than for high-methoxyl pectin. The enhancement effect of cations increased in the order $Zn^{++} > Ca^{++} > Cd^{++} \approx Sr^{++} > Mg^{++} \approx Na^{+} \approx K^{+} > NH_4^{+}$.

INTRODUCTION

The preservation and preparation of pectin-containing foods frequently involves heating. Lineweaver (1945) noted a greater stability of pectin in neutral or slightly acidic solutions when the salt concentration was kept to a minimum and multivalent cations were avoided. Under alkaline conditions pectin degrades by way of a β-elimination mechanism (Albersheim *et al.*, 1960). Increases in the absorption at 235 nm of heated apple pectin resulted from the inclusion of additional cations in the heating buffer. This suggested an enhancing effect of ions on the β-elimination depolymerization of pectin (Keijbets & Pilnik, 1974). Later, Keijbets *et al.* (1976) showed that heat solubilization of pectin from potato cell wall was increased by cations (K^+, Ca^{++}). The promoting effects, however, were small. This low level of influence could be due partly to the ability of cations, especially calcium, to retard solubilization of cell wall pectin by ionic-pectin-gel interaction.

Ions affect firmness changes of plant tissue during heating. Van Buren (1981, 1983) showed a softening effect of K^+ and Na^+ on the firmness of canned beans. Removal of endogenous salts from bean pods before cooking slowed their softening (Van Buren, 1986). Fleming *et al.* (1987) showed that the firmness of brined cucumbers was maintained by decreasing the NaCl concentration from that traditionally used in the pickle process. Recently, McFeeters *et al.* (1989) reported an increase in softening rate of cucumber mesocarp with higher concentration of NaCl. The softening effect was also found for Li^+, K^+, Rb^+, and Cs^+ ions. A first-order rate for the tissue softening was shown.

Thus, the nature and quantity of ions and salts in plant tissues affect the heat degradation of native pectin and the firmness of tissues. The complexity of plant tissues and cell walls has made it difficult to identify unambiguously the effect of ions upon particular degradation reactions. For this reason, it is important to study the effect of cations on pectin in a known environment to gain information which would be useful in evaluating pectin changes in cooked plant tissues. This study examined the effect of monovalent and divalent cations on heat degradation in a model system of purified carrot pectin at high and low levels of methoxylation. The formation of uronide reducing groups and unsaturated bonds was followed.

MATERIALS AND METHODS

Pectin preparation

Alcohol-insoluble cell walls were prepared from carrots (var. PY-60 Peter Edwards) by homogenizing

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and washing with cold distilled water, chloroform-methanol (1:1 (v/v)), and 80% acetone according to Sajjaanantakul *et al.* (1989). Water-soluble pectin was removed by washing exhaustively with distilled water. Chelator-soluble pectin preparation (pectin) was extracted at pH 7 from the cell-wall residue with 0·1 M Na_2EDTA/0·1 M TRIS. This step was repeated twice and the pectin fractions were pooled. A Romicon Hollow fiber ultrafiltration membrane (30 000 molecular weight cut off) was used to concentrate pectin and remove low molecular weight carbohydrates and salts from the pectin preparation. This was the pectin used in this study.

The ultrafiltrated pectin had a degree of esterification (DE) of 46% (Sajjaanantakul, 1989). Some of the ultrafiltrated pectin was freeze-dried and then esterified using 2 N methanolic sulfuric acid at 1°C for 14 days according to Morris *et al.* (1980). The esterified pectin was washed with methanol, 80% acetone, then air-dried, redissolved in distilled water and dialyzed against distilled water. The esterified pectin had a DE of 97%. It should be noted that the DE 46% native pectin may be blockwise esterified (Anger & Dongowski, 1985) while the DE 97% pectin was randomly re-esterified.

Heat treatment

A 0·15% pectin solution in 0·05 M MES-NaOH of pH 6·1 (MES = 2 [*N*-morpholino] ethanesulfonic acid) was used. MES has a pKa of 6·15 and a small binding constant for Mg^{++} and Ca^{++}, and does not bind Cu^{++} (Good *et al.*, 1966; Good & Izawa, 1972). In addition, at 0·05 M it absorbs very little light at 230 nm and negligible amounts above 240 nm. Low molarity of the buffer minimized the effect of counter ion (Na^+) and buffer on pectin degradation.

Monovalent cations (Na^+, K^+, and NH_4^+) were added in a solid chloride form to give a specified concentration. Concentrated chloride solutions of divalent cations (Ca^{++}, Mg^{++}, Zn^{++}, Cd^{++}, and Sr^{++}) were added dropwise with vigorous vortex stirring to get a particular concentration without gelation of pectin. The salt-pectin solutions were pipetted into 16 mm × 100 mm screw-cap test tubes and placed in a boiling-water bath. The caps were closed tightly after 5 min. Total heating time was 1 h.

ANALYTICAL METHODS

Galacturonic acid content was quantified by the *m*-phenylphenol assay according to Blumenkrantz and Asboe-Hanssen (1973). Changes in reducing end groups were determined by the acid copper reagent method at pH 4·6–5·0 (Keijbets, 1974) as described by Sajjaanantakul *et al.* (1989). Galacturonic acid was used as a standard for the absorbance reading at 750 nm. The assay is about 100 times more sensitive to the reducing end groups of uronic acids than to those of aldoses (Milner & Avigad, 1967; Sajjaanantakul, 1989). The degree of polymerization was calculated from the amount of reducing end-groups, based on the assumption that the reducing groups came from polygalacturonic acids. Ultrafiltrated pectin contained about 20% neutral sugar based on glucose. Analysis by anion exchange chromatography has shown that the neutral sugar co-elutes with galacturonic acid, and that there are no other free neutral polysaccharides in the pectin preparation (Sajjaanantakul *et al.*, 1989).

Methyl ester content of the pectin was measured spectrophotometrically at 412 nm after conversion of saponified pectin methanol to formaldehyde by an alcohol oxidase (EC 1.1.3.13) (Klavons & Bennett, 1986). Unsaponified pectin samples were assayed at the same time for the free methanol correction.

Concentrations of 4,5-unsaturated galacturonides were measured in diluted samples by UV absorbance at 235 nm (Varian-Cary 219 Spectrophotometer, Sugar Land, TX, USA). An average molar extinction coefficient (ε) of 5 412 M^{-1} cm^{-1} was used to calculate the amount of unsaturated uronides from the UV absorbance (Nagel & Wilson, 1969; Voragen, 1972). The amounts of calculated unsaturated uronides were expressed as μmoles per μmole of galacturonic acid. The periodate thiobarbituric acid (TBA) test (Weissbach & Hurwitz, 1959) was modified by using a periodate oxidation time of 20 min at 70°C to increase the sensitivity for the 4,5-unsaturated uronides (Okamoto *et al.*, 1964). An ε of 35 000 M^{-1} cm^{-1} (Keijbets, 1974) was chosen for the calculation of the amount of unsaturated uronides from the absorbance at 550 nm.

Results in the graphs are expressed relative to the values found for pectins heated for 1 h at 100°C with no salt addition. During this period, with the 97 DE pectin, there was an increase of 10·3 mmoles of reducing groups, 8·2 mmoles of double bonds (UV), and 6·5 mmoles of double bonds (TBA) per mole of galacturonic acid. With 46 DE pectin the increase was 5·1 mmoles of reducing groups, 3·0 mmoles of double bonds (UV), and 3·6 mmoles of double bonds (TBA) per mole of galacturonic acid. Statistical analyses were performed by the MINITAB computer package (Ryan *et al.*, 1980).

RESULTS

Depolymerization of the heated pectin

The relative effects of Na^+, K^+, and NH_4^+ in reducing the degree of polymerization (DP) during heating at pH 6·1 are shown in Fig. 1. Increased salt concentration in the pectin solutions caused greater cleavage of the polymer chains. At both high and low DE, Na^+ and K^+ affected the degradation to about the same extent.

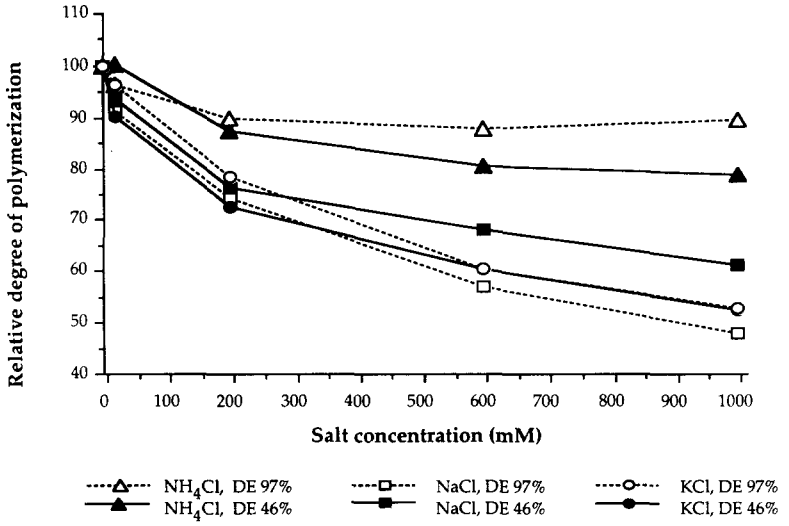

Fig. 1. Effect of monovalent cations on the relative degree of polymerization of chelator-soluble carrot pectin of different degrees of esterification (DE) heated 1 h at 100°C in 0·05 M MES, pH 6·1.

NH_4^+ showed the least degradation effect. Figures 2 and 3 illustrate the effect of divalent cations on the chain cleavage of pectin with DEs of 46% and 97%, pH 6·1. The enhancing effect of Zn^{++} was seen with both high- and low-DE pectins. Calcium ion (Ca^{++}) exhibited less enhancing than that of Zn^{++}, while Cd^{++} and Sr^{++} had about the same effect. Mg^{++} had the least effect. Depolymerization was greater as the cation content increased.

Although the absolute number of chain cleavages was higher with the DE 97% pectin than with the DE 46% pectin (Sajjaanantakul *et al.*, 1989), the percent increase in rate due to the addition of divalent cations was greater with the DE 46% pectin than the DE 97% pectin (Figs 2 and 3). This suggests that an interaction of the divalent cations with free carboxylic groups on the pectin molecules contributed to the enhancing effect of the cations. The greater effect on the low-methoxyl pectin than the high-methoxyl pectin was not as clearly seen with monovalent cations (Fig. 1) as with the divalent cations. This might be due to less interaction between monovalent cations and the carboxylic groups on the pectin compared to that of divalent cations. Divalent cations had a greater enhancing effect on depolymerization of the pectin than did monovalent cations of the same concentration.

Occurrence of the 4,5-unsaturated uronides

Absorbance in the UV range is a measure of double bonds. Changes in the absorption at 235 nm after heating pectin in the presence of different monovalent

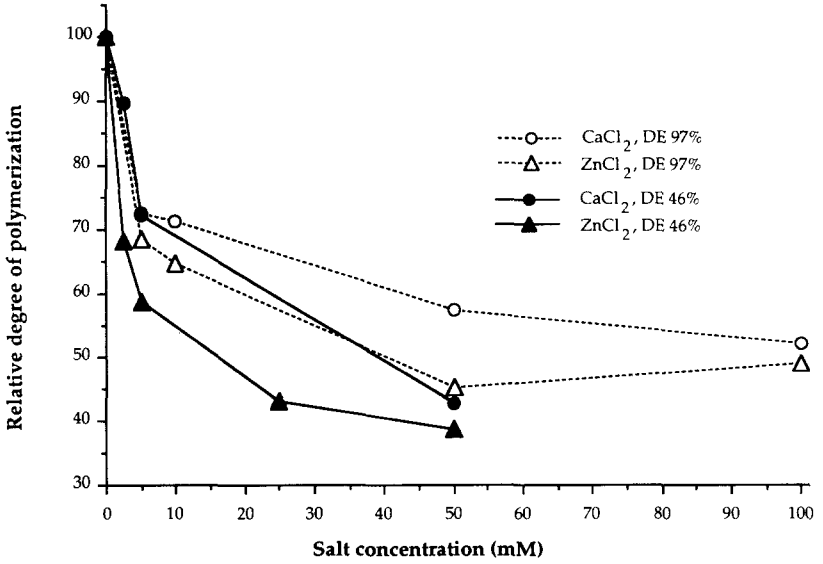

Fig. 2. Effect of Zn and Ca on the relative degree of polymerization of chelator-soluble carrot pectin of low (46%) and high (97%) degree of esterification heated 1 h at 100°C in 0·05 M MES, pH 6·1.

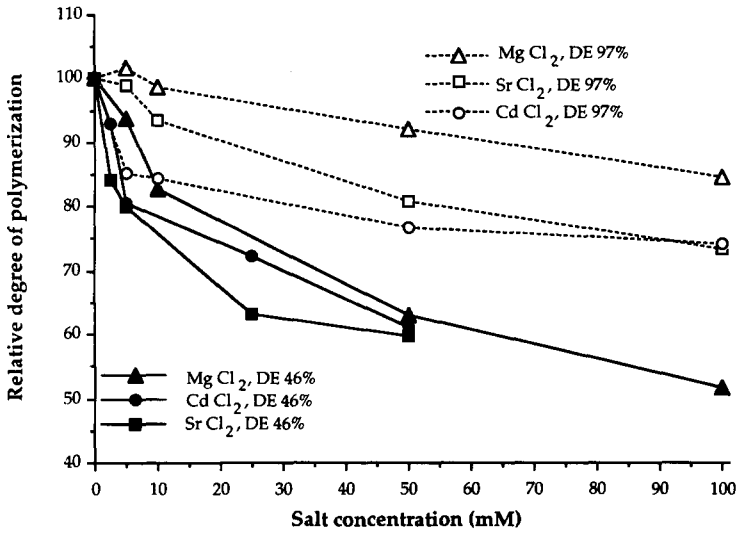

Fig. 3. Effect of Cd, Sr, and Mg on the relative degree of polymerization of carrot chelator soluble pectin of low (46%) and high (97%) degree of esterification heated 1 h at 100°C in 0·05 M MES, pH 6·1.

cations are shown in Fig. 4. The heated pectin showed increases in UV absorbance with increasing salt concentration. Formation of 4,5-unsaturated uronides was also shown by increases in periodate TBA values (Fig. 5). Ammonium ion (NH_4^+) resulted in the least formation of unsaturated uronides (UV and periodate TBA values), similar to the low effect of this salt on depolymerization.

Increases in the absorption at 235 nm were also seen with divalent cations. Figures 6 and 7 show the changes in UV absorbance of heated pectin (DE 46% and DE 97%, respectively) in the presence of various divalent cations. The formation of 4,5-unsaturated uronides increased as the levels of cations were raised. Mg^{++} resulted in the least changes. The unexpected low values of the Zn^{++} sample with the DE 46% pectin may have resulted from difficulties in the UV measurement related to weak gel formation in the pectin solution. The effect of Zn^{++} on the relative changes in the UV absorption at 235 nm was better seen with the DE 97% pectin. For each divalent cation, the relative effect on the formation of the unsaturated uronides was more pronounced for the DE 46% pectin than for the DE 97% pectin. This agrees with the effect on depolymerization as determined by reducing end-group analysis.

The enhancing effect of divalent cations on the formation of unsaturated uronides was supported by the increases in the periodate TBA values. Relative changes in periodate TBA values for DE 46% pectin and DE 97% pectin are shown in Fig. 8. The values for Zn^{++} samples were not included due to a precipitation which interfered with chromagen formation. The effect

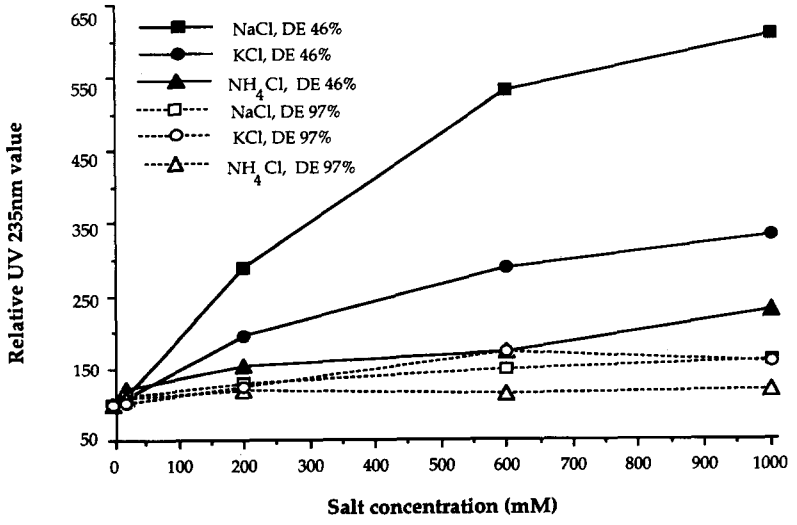

Fig. 4. Effect of monovalent cations on the relative changes in UV absorption at 235 nm of chelator-soluble carrot pectin of different degrees of esterification (DE) heated 1 h at 100°C in 0·05 M MES, pH 6·1.

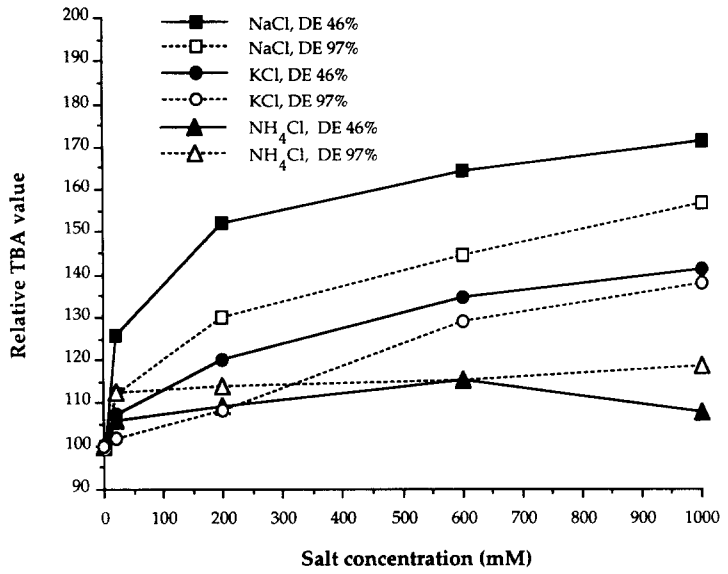

Fig. 5. Effect of monovalent cations on the relative changes in the periodate TBA value (moles unsaturated uronides/mole galacturonic acid) of chelator-soluble carrot pectin of different degrees of esterification (DE) heated 1 h at 100°C in 0·05 M MES, pH 6·1.

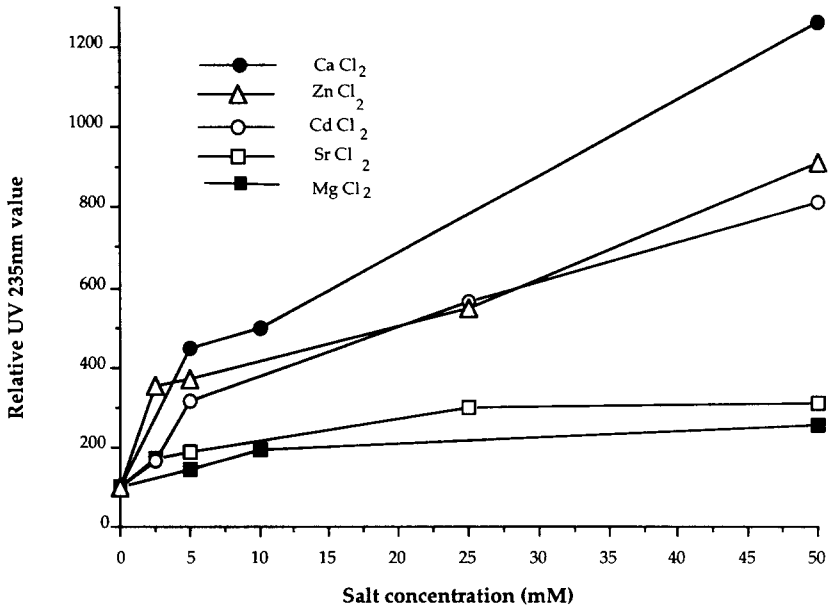

Fig. 6. Effect of divalent cations on the relative changes in UV absorption at 235 nm of chelator-soluble carrot pectin, 46% degree of esterification, heated 1 h at 100°C in 0·05 M MES, pH 6·1.

of divalent cations on the periodate TBA values was similar to that on the UV values. Both measurements had greater increases due to cations with low methoxyl pectin than with high methoxyl pectin. This again showed that the interaction of divalent cations with carboxylic acid groups of the pectin enhanced eliminative cleavage in the pectin chain, corresponding to the observed reduction in polymer size.

There were good correlations between the changes in reducing end-groups and the formation of unsaturated uronides as calculated from UV absorbance values, Table 1. There were no significant differences for the mean correlations between monovalent and divalent cations or between pectins differing in DE.

DISCUSSION

Various cations gave different enhancements of pectin β-elimination, the enhancement increasing as the salt level was increased. The monovalent cations, Na^+ and K^+, promoted more depolymerization than NH_4^+,

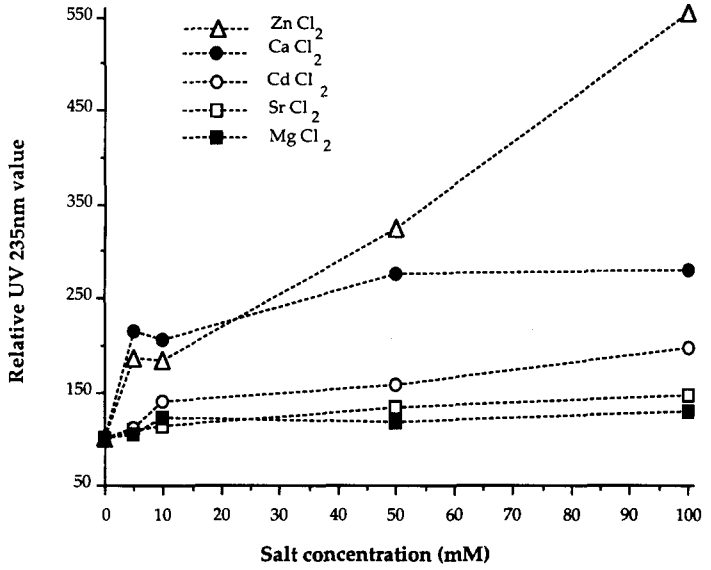

Fig. 7. Effect of divalent cations on the relative changes in UV absorption at 235 nm of chelator-soluble carrot pectin, 97% degree of esterification, heated 1 h at 100°C in 0·05 M MES, pH 6·1.

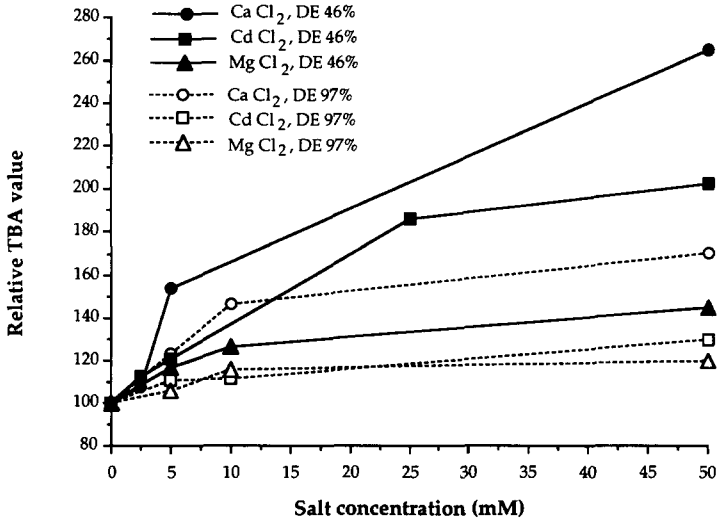

Fig. 8. Effect of divalent cations on the relative changes in periodate TBA values (moles unsaturated uronides/mole galacturonic acid) of chelator-soluble carrot pectin of low (46%) and high (97%) degree of esterification, heated 1 h at 100°C in 0·05 M MES, pH 6·1.

which had the least effect among all cations tested. Kiss (1970) demonstrated that the amidated derivatives of (1 → 4) linked dihexopyranosiduronates were less susceptible to β-elimination than methoxylated derivatives. Had the *trans*-amidation of the pectin methoxyl groups taken place during our heating in NH_4Cl solution, this might, at least in part, be responsible for the slight effect of NH_4^+ seen in the current study.

Zn^{++} and Ca^{++} resulted in much greater breakdown of pectin polymers than did Cd^{++} and Sr^{++}, while Mg^{++} had the least effect among the divalent cations. Similar results were found with the periodate TBA and UV values.

Divalent cations enhanced degradation to a greater extent than did monovalent cations, except for Mg^{++}, which had about the same effect as Na^+ and K^+. Differences in binding ability between cations and pectin carboxyl groups possibly contribute to the enhancement differences between monovalent and divalent cations. Differences in binding strength, however, are probably not the only factors responsible for differences in enhancement. The order of oligouronide binding strength for the divalent cations, Ca ≈ Sr ≈ Zn < Cd < Cu < Pb, (Kohn *et al.*, 1976; Kohn, 1987) does not coincide with the enhancing effect of these cations on β-eliminative pectin cleavage. An influence of ion solvation on the complex binding of cations with carbohydrates was emphasized by

Table 1. Mean correlations (*r*) between pectin end-group values (moles reducing end-group/mole galacturonic acid) and 4,5-unsaturated uronide values (moles unsaturated uronide/mole galacturonic acid) as measured with UV or TBA

Cation type	% DE of pectin	UV(*r*)	TBA(*r*)
Monovalent	46	0·96	0·90
Monovalent	97	0·93	0·94
Divalent	46	0·98	0·98
Divalent	97	0·91	0·79
Least significant difference		0·10	0·24

Samples heated one hour at pH 6·1.

Rendleman (1966). The ineffectiveness of Mg^{++} ions in complexing with and promoting electrophoretic migration of carbohydrates results from the tightly bound sphere of solvation surrounding these ions (Rendleman, 1966). This solvation effect may restrict interaction of ions with pectin (Paoletti *et al.*, 1986), and therefore contribute to the ineffectiveness of Mg^{++} or NH_4^+ in enhancing β-elimination.

It should be emphasized that, because of the dominant effect of methyl ester groups in enhancing β-elimination (Sajjaanantakul *et al.*, 1989), highly esterified pectin (DE 97%) was degraded to a smaller polymer size than was low esterified pectin (DE 46%) when heating was done at low salt levels. The percent increase in pectin depolymerization due to divalent cations was more pronounced for the low-esterified pectin (DE 46%) than for the high-esterified pectin. Keijbets and Pilnik (1974) suggested that the association of cations with acid groups of pectin favors the approach of hydroxyl ions. The interaction of cations with carboxylic acid groups of uronide residues creates an electron deficit at the C5 of the uronides. This would then allow the C5-proton to leave more easily. The electron-withdrawing effect due to interaction of cations with uronide carboxyl groups was less for monovalent cations than for divalent cations. Since there were more available carboxylic acid groups in DE 46% pectin than DE 97% pectin, the effect of adding cations was more pronounced for low esterified pectin than for highly esterified pectin.

Enhancements of β-elimination are not the sole function of either the charge, group, valency, electronegativity, ionic radius, atomic radius, or hydration radius of the cation, as can be seen from the properties of cations listed in Table 2. The importance of ionic electronegativity and hydration radii upon electrostatic interactions and desolvation of ions interacting with carboxylic acid groups are discussed by Paoletti *et al.* (1986). Those authors pointed out that the enhancing effect of cations could be due only in part to valency. The interaction of cations with pectin involves complex formation and electronic coordination with carboxylic acid groups, the uronide ring oxygens, and the glycosidic oxygen (Anthonsen *et al.*, 1972). The interaction can be influenced by other functional groups on galacturonide residues as well as intermolecular association of pectin chains, thus further complicating the picture.

CONCLUSIONS

This study showed that cations promote β-elimination in pectin. There was an increased β-eliminative degradation as cation concentration was increased. The magnitude of enhancement increased in the order $Zn^{++} > Ca^{++} > Cd^{++} \approx Sr^{++} > Mg^{++} \approx Na^+ \approx K^+ > NH_4^+$. Divalent cations, except for Mg^{++}, exhibited a greater effect than monovalent cations at the same level of salt concentration (molar basis). In addition, the percent promoting effect was more pronounced for pectins with low methoxyl content than for those of high methoxyl content. The accelerating effect of cations on β-elimination appeared to include consequences of interactions between cations and free carboxylic acid groups on the pectin. This enhancement effect, however, was not a simple function of the

Table 2. Physicochemical properties of some cations

Ion	Electronegativity structure[a] of atom	Ionic radii[b] (Å)	Atomic radii[a] (Å)	Hydration radii[b] (Å)	Crystal
NH_4^+	—	1·48 (+1)	—	3·31	—
Na^+	0·9	0·95 (+1)	1·90	3·58	Cubic body centered
K^+	0·8	1·33 (+1)	2·35	3·31	Cubic body centered
Mg^{2+}	1·2	0·65 (+2)	1·60	4·28	Hexagonal
Sr^{2+}	1·0	1·13 (+2)	2·15	4·12	Cubic face centered
Cd^{2+}	1·7	0·97 (+2)	1·54	4·26	Hexagonal
Ca^{2+}	1·0	0·99 (+2)	1·97	4·12	Cubic face centered
Zn^{2+}	1·6	0·74 (+2)	1·38	4·30	Hexagonal
Cu^{2+}	1·9	0·69 (+2)	1·28	4·19	Cubic face centered

[a]From periodic table of elements, Sargent-Welch Scientific Co., Skokie, Illinois, USA, 1968.
[b]From Nightingale (1959).
Note: Numbers in parentheses represent ionic charge of ion in first column.

charges, electronegativities, ionic radii, or hydration radii of the cations.

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