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Note

β-Elimination of pectin in the presence of anions and cations

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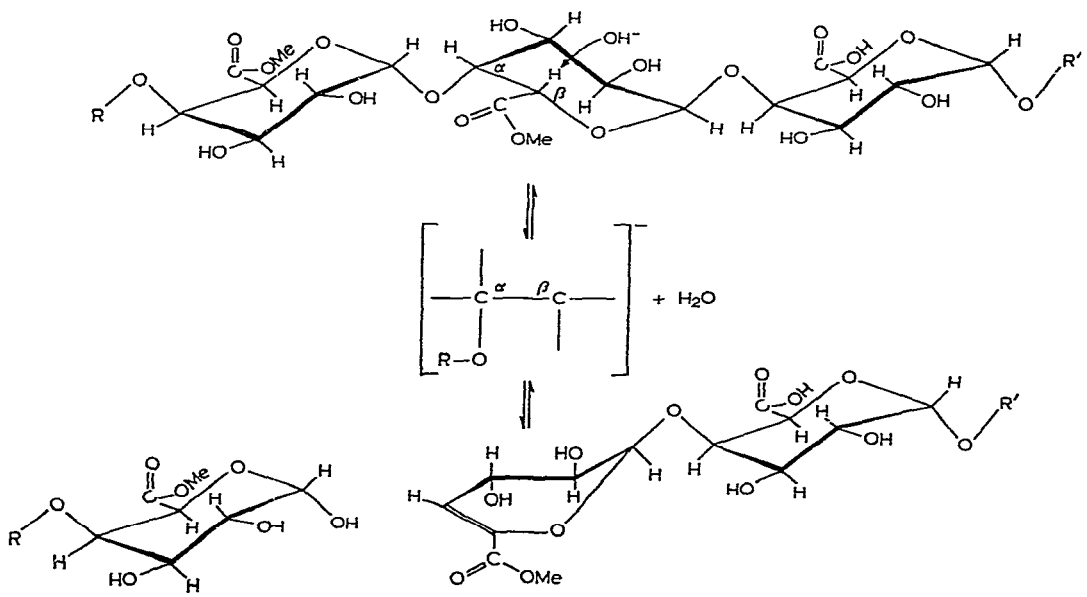
An outstanding finding in pectin chemistry during the last fifteen years has been the elucidation of the mechanism of pectin degradation in alkaline environments Neukom and Deuel[1] suggested that the splitting of pectin chains, which accompanies de-esterification on treatment with alkali, results from a base-catalyzed, β-elimination reaction The same reaction proceeds when pectin is heated at neutral[2] or weakly acidic pH[3,4] Proof of the β-elimination character has been given by Heim and Neukom[5].

The mechanism of β-elimination of such glycuronans as pectin was thought to be an E2 mechanism[6], but recently some evidence for an E1cB mechanism has been



Scheme 1

obtained[7], in which the intermediate carbanion, formed by removal[6] of H-5 by hydroxyl ions, is stabilised by elimination at C-4 as shown in Scheme 1 A prerequisite[2,8] is the presence of a methyl ester group at C-6, which renders H-5 sufficiently acidic

When pectin is degraded at pH 6 8, a relatively high Q_{10} is found at 50–95°, thereby demonstrating the temperature dependence of β-elimination[2]

Doesburg[9] indicated the possibility of depolymerization of native pectin by β-elimination during cooking of plant tissues at a weakly acidic pH The implications of pectin degradation for the texture of cooked-plant tissue prompted us to investigate the effect of some cations (Ca, K, Mg) and anions (citrate, malate, phytate, chloride) on the β-elimination reaction The ions were chosen because of their occurrence in potato tissue, but they are also major constituents of various other plant tissues

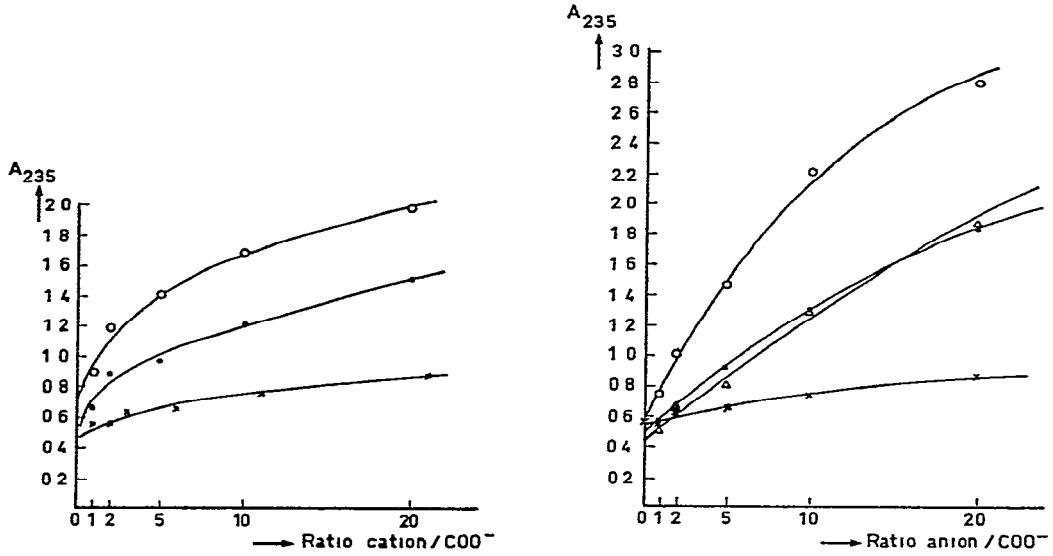


Fig 1 Effect of cations on the β-elimination of pectin in 0 02M Tris–pipes buffer (pH 6 1) and in the presence of Cl^- pectin concentration 0 29%, equiv Cl^- = equiv cations − equiv COO^-, COO^- free pectic acid groups in equiv Cations Ca (○), Mg (●), K (×)

Fig 2 Effect of anions on the β-elimination of pectin in 0 02M Tris–pipes buffer (pH 6 1) and in the presence of K^+ pectin concentration 0 29%, equiv K^+ = equiv COO^- + equiv anions, COO^- free pectic acid groups in equiv Anions citrate (○), malate (●), phytate (△), chloride (×)

The cations, Ca, Mg, and K, and the anions, citrate, malate, phytate, and chloride, stimulate β-eliminative breakdown of 60% esterified pectin at pH 6 1 and boiling temperature (Figs 1 and 2) The extent of β-elimination is due only in part to the concentration of the ions, the nature of the ions being a more important factor. The association of cations with the pectin polyanion, which has a comparatively close accumulation of ionised acid groups[10], will decrease the overall, negative charge and

facilitate the approach of the hydroxyl ions needed to initiate β-elimination The different, enhancing action of Ca, Mg, and K ions can be due only in part to differences in valency (*cf.* the effect of Ca and Mg)

No explanation is available at present for the stimulation of β-elimination by the organic anions A decreased dissociation of the weak organic acids, together with a possible function as proton acceptors at boiling temperature, may be contributing factors

The results of the experiments described herein are particularly interesting in view of the texture of heat-processed plant tissues During heating of plant tissues above pH 4–5, the pectin molecules will be degraded by β-elimination The extent to which this takes place is important for intercellular cohesion, which more or less determines texture[11] From this point of view, the nature and quantity of ions present in plant tissues may contribute to the texture of heat-processed food by their effects on the β-elimination of pectin

EXPERIMENTAL

Green ribbon pectin (60% esterified apple pectin, Obipectin Ltd , Bischofszell, Switzerland) was used during all experiments The free-acid form was obtained[12] by percolating a ~1% aqueous solution sequentially through columns of Amberlite IR-120(H^+) and IR-45(OH^-) resins

Degradation of pectin — A 0 29% solution of pectin in 0 02M Tris–piperazine-*N,N'*-bis-2-ethanesulphonic acid (Tris–pipes) buffer (pH 6 1) was used This buffer does not chelate Ca^{2+} and Mg^{2+} ions The solutions containing the appropriate ion were boiled for 30 min and then cooled For cations, the free-acid groups of pectin were neutralized with 0 02M calcium hydroxide or 0 1M potassium hydroxide On account of the insolubility of magnesium hydroxide, potassium hydroxide was used for neutralization in the magnesium experiments. Additional cations were added as their chlorides Neutralization is necessary because of the limited buffering capacity of the diluted buffer, which is used to minimize the influence of the buffer ions on β-elimination The results are shown in Fig 1

For the anions (citrate, malate, and phytate[13]), the pectin was neutralized with 0 1M potassium hydroxide, the anions were then added as free acids and neutralized with potassium hydroxide. The pH of these solutions was adjusted to 6 1 with dilute hydrochloric acid because of the limited buffering capacity The results are shown in Fig 2 Chloride ion causes very little degradation, and the effect of its presence with other anions is not important

Degradation was assessed by the increase in absorption[2] at 234–235 nm, and expressed as A_{235} A Beckman DU spectrophotometer was used with the unboiled solution as the blank. Some boiled, test solutions containing Ca^{2+} were slightly turbid due to calcium pectinate[14] These samples were diluted with EDTA solution before spectrophotometry

The degree of esterification and glycuronan content of the pectin solution

before, and of some test solutions after, boiling was determined by the copper precipitation method[15,16].

Boiling of the test solutions slightly lowers the pH The lowest pH measured was 5 89 The degree of esterification decreases simultaneously

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