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Analysis of Mixtures of Pectins and Amidated Pectins

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ABSTRACT

Amidated pectins and non-amidated pectins are transformed by saponification into amidated pectic acid and pectic acid. By heat treatment under alkaline conditions only amidated pectic acid is depolymerized and can be separated from the high-molecular pectic acid by gel-filtration. On this basis the composition of mixtures can be analysed.

INTRODUCTION

Low methoxyl (LM) pectins and amidated pectins are increasingly used in the food industry, mainly for the manufacture of gelled low-sugar fruit products. Whereas mixtures of pectins of different degree of esterification (DE) can be separated by ion-exchange chromatography (van Deventer-Schriemer & Pilnik, 1976), this is not the case for amidated pectins and non-amidated pectins because the elution behaviour on ion-exchange columns depends mainly on the free carboxyl groups. We have therefore studied the selective degradation of amidated pectin and the use of gel-filtration to separate the fractions in mixtures of amidated and non-amidated pectins.

MATERIALS AND METHODS

Samples of apple pectin and amidated apple pectin were obtained from Obipektin Ltd (Switzerland). They were respectively an LM pectin, DE 35%, an amidated pectin, DE 40% and DA (degree of amidation) 10% and an amidated pectin, DE 35% and DA 20%. Tomato pectin-esterase

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(PE) was from Sigma Chemical Comp., No. P6763. Sodium borohydride was from Merck, No. 806372. *m*-Hydroxydiphenyl (mhdp) reagent was from Eastman Kodak Co., No. 1161322. For gel-filtration Sephacryl S-200 from Pharmacia was used.

The amidated pectins and the non-amidated pectin are de-esterified to obtain amidated pectic acid and non-amidated pectic acid respectively. At high temperature under alkaline conditions the amidated pectic acids are depolymerized by β-eliminative splitting of glycosidic linkages next to amidated groups whereas non-amidated pectic acid, in contrast to pectin with ester groups, is not attacked. The fractions are separated by gel-filtration and anhydrogalacturonic acid (AUA) is determined by the mhdp method.

For de-esterification 1 g of each pectin sample (amidated and non-amidated) was dissolved in 250 ml of distilled water, complete dissolution was ensured by boiling briefly. After cooling, 150 ml Tris–succinate buffer 0·1M pH 7·9 was added in conjunction with 3 mg PE dissolved in 1 ml of the same buffer. After incubation for 16 h at 30°C the solution was cooled to 5°C and pre-cooled 2N sodium hydroxide was added to bring the pH to 11. In order to complete the de-esterification an incubation period of 16 h at 5°C was maintained. This combined method was chosen after preliminary studies had shown that in this way almost complete de-esterification without significant depolymerization could be achieved. The pectic acids were then quantitatively precipitated by adding 2·5 volumes of 96% isopropanol containing 10% by volume of concentrated hydrochloric acid. After standing for 1 h the precipitate was collected on a G3 glass filter and washed on the filter consecutively once with the acid alcohol, with 70% v/v isopropanol to a negative chloride reaction, once with 96% isopropanol and once with acetone. The precipitate was air-dried and finally ground in a coffee grinder. AUA, DE and DA of these samples were determined by titration (Food Chemicals Codex II or III).

For depolymerization 25 mg of the pectic acids was weighed into a Kimax tube and mixed with 12·5 mg sodium borohydride. Then 1·25 ml 0·1N sodium hydroxide was added and after careful mixing left standing at room temperature for 1 h. The reduction of end-groups protects polysaccharides against degradation by alkali (Aspinall, 1977). We also noticed that the separation on the gel-filtration column is improved by the reduction treatment. Water (3·75 ml) was then added and the mixture brought to boiling. The tubes were tightly closed and kept for 1 h in a boiling water bath. After air-cooling the solution was diluted with distilled water to 10 ml, mixed well and filtered through paper to protect the column against the eventual build-up of particles or fibres.

Gel-filtration was performed with 1 ml of each filtered solution over a column (2·6 × 60 cm) filled with Sephacryl S-200. Elution was carried out with a Tris–succinate buffer 0·1M pH 6 at 20 ml/h. Fractions of 2·85 ml were collected. AUA was determined in the fractions using an automated mhdp method (Thibault, 1979). For the examination of mixtures 1 ml of filtered solutions of the amidated and the non-amidated heat-treated pectic acids were chromatographed together. The saponified untreated samples were directly dissolved in the elution buffer (25 mg/10 ml) and 1 ml was applied to the column. To check recovery from the column AUA in each sample solution was directly determined by mhdp after appropriate dilution. It should be noted that amide groups depress colour development with mhdp (Reitsma *et al.*, 1986) so that recovery of amidated samples is really a comparison of colour development.

RESULTS AND DISCUSSION

Table 1 gives the analysis of the saponified samples. Figure 1(a) shows that the heat treatment has not significantly affected the non-amidated pectic acid. Figure 1(b) clearly shows the degradation of the two amidated pectic acids in comparison to an untreated sample. A small fraction of obviously more resistant material elutes in the void volume. As expected, more degradation occurred with the more highly amidated sample. Figure 1(c) shows the elution pattern of the heat-treated 1:1 mixtures (Table 1). The peaks of the degradation-resistant fraction of the amidated pectic acids are hidden in the large void peak of the non-amidated pectic acid. In Fig. 1(d) two samples (Table 1) are compared in which the same DA was found by titration analysis. The elution behaviour after heat treatment shows the difference. Again a heat-resistant fraction of amidated pectic acid is noted where amidated pectic acid alone is chromatographed. Figures 1(b), (c) and (d) show a certain overlap of the peaks from the high molecular weight and the degraded fractions.

Calculation: colour development by mhdp is depressed by amide groups. This means that after elution of a mixture of high molecular weight non-amidated pectic acid and degraded amidated pectic acid calculations must be based on the non-amidated pectic acid peak (Fig. 1(c)). Fractions were considered up to an elution volume at which the decrease of concentration levelled off because of overlap with degraded fractions. If AUA and DA of a mixed sample is known by titration analysis, the determination of the amount of non-amidated pectic acid

TABLE 1
Analysis of De-esterified Pectins

	%DE	%DA	%AUA
Pectic acids (PA)	4·4	—	76·4
Amidated pectic acids with 10% DA (APA 10%)	3·0	10·6	76·0
Amidated pectic acids with 20% DA (APA 20%)	2·6	22·1	74·8
PA and APA 10% mixed 1:1	3·7	5·3	76·2
PA and APA 20% mixed 1:1	3·5	11·1	75·6

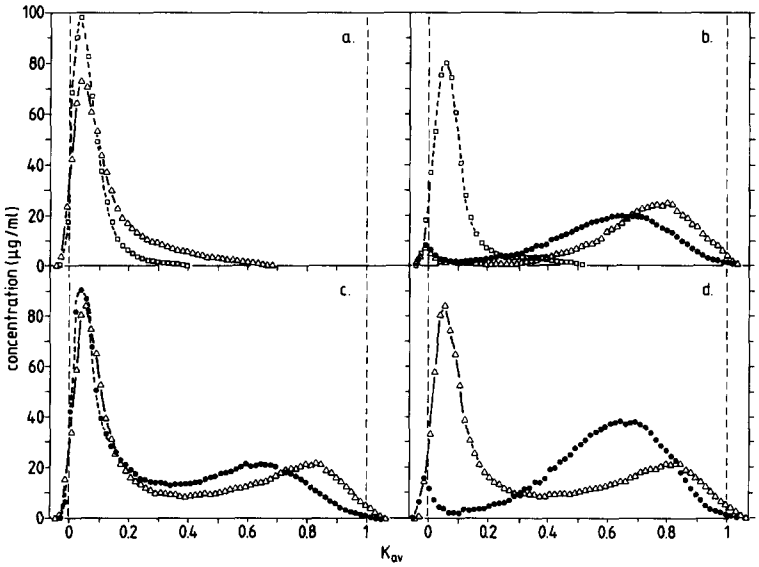

Fig. 1. Elution patterns from S-200 column. (a) □ PA before treatment, △ PA after treatment; (b) □ APA 20% before treatment, ● APA 10% after treatment, △ APA 20% after treatment; (c) ● mixed PA and APA 10% after treatment, △ mixed PA and APA 20% after treatment; (d) ● APA 10% after treatment, △ mixed PA and APA 20% after treatment.

allows us to calculate the amount and DA of the amidated pectic acid. Results are best based on AUA as the pectins in a mixed sample must be expected to have different AUA contents. The calculation is given in Table 2.

Potential sources of error are the colour developed by the small, not degraded fraction of the amidated pectic acids, the choice of the cut-off

TABLE 2
Calculation of Mixed PA and APA (Fig. 1(c))

	PA and APA 10%	*PA and APA 20%*
μg AUA brought on column (titration, Table 1)	3810	3780
μg AUA recovered to K_{av} 0·24/0·28 (mhdp)	1935 (50·8%)	1941 (51·3%)
μg AUA in amidated fractions (difference)	1875 (49·2%)	1839 (48·7%)
DA of amidated fractions[a]	10·8%	22·8%

[a] (DA of mixed sample by titration/% amidated fractions) × 100%.

K_{av} with an inevitable small loss of non-amidated pectic acid but also a small content of degraded amidated pectic acid and an eventual disagreement of AUA determinations by titration and mhdp. The good agreement between the results in Table 2 and the analytical values of Table 1 shows that these sources of error need not strongly influence the results. Further analyses of 1:1 mixtures were made and the content of the amidated fraction obtained varied between 48 and 51%.

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