Acta Bot. Neerl. 43(2), June 1994, p. 205–207

A rapid method for quantitative determination of pectic substances

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SUMMARY

A rapid and quantitative method has been developed to determine the amount of soluble and insoluble pectins. The method does not require extraction or purification, even plant homogenates may be used, and it can be applied to pectins of various origin. The determination of pectic substances by previous methods can be performed only on pectins in solution. The present rapid method is based on the titration of de-esterified pectins after acid precipitation. The speed and simplicity also makes this quantitative method highly useful for the determination of pectins during food processing.

Key-words: anhydro-galacturonic acid, pectin, plant cell wall composition, food processing.

INTRODUCTION

Pectic substances are designated as a group of colloidal substances, with a high proportion of anhydro-galacturonic acid. Their composition is variable and very complex. D-galacturonic acid and its methyl ester are (1→4)-linked as poly-(α-D-galactopyranosyl)-uronic acid in the backbone of pectin. Blocks of galacturonic acid are interspaced by (1→2) linked α-L-rhamnopyranosyl units. Also non-rhamnose sugars like galactose, arabinose, glucose, mannose and xylose occur. Acetyl ester groups are supposedly linked to galacturonate residues in the backbone, whereas feruloyl ester groups are thought to be linked to the neutral sugar side chains, at least in sugar beet pectin (Fishman 1988).

Pectin molecules consist of 'hairy regions', the rhamnogalacturonan segments, carrying the neutral sugar side-chains, and 'smooth regions', the homogalacturonan segments (Vries *et al.* 1983).

The compositional complexity of pectin has resulted in a wide range of methods for its analysis. Methods that have been used include: weight of alcohol precipitates, titration of acid caboxyls plus saponification of methyl esters, weight of calcium pectate, decarboxylation by heating in concentrated mineral acids, optical rotation, colorimetric determination by carbazole-sulphuric acid or m-hydroxy-diphenylsulphuric acid procedures (Kertesz 1951; Kintner & Van Buren 1982; McComb & McCready 1952). These methods are complex and time-consuming.

Pectins determine the texture of fruits and vegetables during processing and storage, and are highly effective in the removal of heavy metals from their environment, even

This paper was presented at the 6th Cell Wall Meeting in Nijmegen, The Netherlands, 1992. Other contributions from the Cell Wall Meeting have been published in Vol. 42, Nos 2 and 3, 1993.

after ingestion by humans (unpublished data). Therefore, a rapid and quantitative method for the determination in food and drugs was developed. This method allows the calculation of pectin (as polyuronides) from a titrimetrical determination of alkali absorption by the pectic acids of materials under test (USSR patent 1651184).

MATERIALS AND METHODS

Reagents

HCl and NaOH were reagent grade. Hinton's indicator was freshly made (mixed indicator: 1 volume each of Bromothymol blue (0·4%) and Phenol red (0·4%), 3 volumes Cresol red (0·4%) and 1 volume distilled water. All chemicals were obtained from a local supplier.

Sample preparation

Pectin powders were ground to particles smaller than 0·2 mm. Solutions or extracts containing pectin were adjusted to pH 5–6. Solutions with low concentrations (less than 50 mg ml^{-1}) of pectin were concentrated by evaporation at 50–60°C. Dry fruits, vegetables, apples and sugar beet pulp were ground to fragments less than 0·2 mm. Fresh berries and vegetables, as well as canned fruits and salads were homogenized in a blender, or grated, or pounded. In this study we used an isolated apple pectin fraction, apple and citrus peel and cherry and apricot jams from local suppliers.

Determination of pectic contents

The carbazole test was carried out essentially as described by Filipov & Vlasyeva (1973). The rapid procedure was carried out as follows. Sample sizes were: 0·2–0·5 g dried pectin, 0·5–1·0 g dry rough material or 20–25 g fresh weight of fruits, vegetables, berries or canned fruits. From solutes, neutralized pectic hydrolysates or juices, 20–50 ml was used.

Before use, the samples of the dried material were weighed in vials, moistened with 1–2 ml ethanol to prevent clotting, and subsequently dissolved or suspended in 10–20 ml distilled water each. Per 10 ml of solution or suspension, 1–2 ml of 1 N NaOH was added and thoroughly mixed. De-esterification occurred for 20 min at 20–25°C. Subsequently, the suspension was acidified by addition of 1 N HCl, 1·5 × the volume of 1 N NaOH added before, and mixed thoroughly. To precipitate the pectin, 50 ml of 0·1 N HCl was added. The suspension was stirred and kept for 5 min at room temperature to equilibrate the concentrations in medium and pectin flakes. The final volume or weight of each sample was measured. The mixture was then filtered through a wide pore filter (Whatman 1). From the filtrate 10–20 ml were pipetted into a 250 ml flask. The residue of the filter was pooled with the remaining filtrate. Funnel and vial were washed twice with distilled water and the wash solutions were added to the residue-filtrate mixture and thoroughly stirred.

The filtrate in the flask and the mixture were separately titrated with 0·1 N NaOH using Hinton's indicator. From the result of the titration of the 10–20 ml filtrate, the HCl content of the original volume was calculated. Together with the results of the titration of the pooled filtrate, residue and washings, the total amount of all pectic acids of the original sample can be estimated.

Table 1. Determination of pectic contents of fruit products (in % of dry or fresh weight) (± SD)

Materials	% of poly-galacturonic acid (n=11) Rapid method	Carbazole test
Dry weight		
Apple peel	15·12 (±0·78)	14·85 (±0·80)
Apple pectin	51·26 (±2·49)	50·99 (±2·04)
Citrus peel	21·42 (±1·19)	20·85 (±0·99)
Fresh weight		
Cherry jam	0·37 (±0·03)	0·39 (±0·03)
Apricot jam	0·76 (±0·06)	0·83 (±0·05)

The amount of poly-galacturonic acid (pectin) was calculated as follows:

$$P\,\% = \frac{(V_2 - V_1) \cdot 176 \cdot 0{\cdot}1 \cdot K}{1000\,W} \times 100$$

where V_1 is the calculated volume (ml) of 0·1 N NaOH for the titration of HCl in the entire reaction mixture; V_2 is the volume (ml) of 0·1 N NaOH for the titration of the entire reaction mixture; 176 is the gram-equivalent of pectic acid; 0·1 is the concentration of NaOH, used for titration; K is the coefficient to the NaOH concentration; and W is the weight of the sample (g).

RESULTS AND DISCUSSION

Using the described procedure, the titration curve of pure pectin and pectic acid was linear in the range of 5–30 mg ml^{-1} (data not shown). The results of the determinations of the pectic contents of a partly purified apple pectin sample and of several fruit homogenates using the rapid and colorimetric carbazole test are shown in Table 1. Addition of neutral sugars did not influence the results (data not shown). Also, soluble acids did not influence the results as they are separately titrated in the filtrate. The only bias that could occur is the occurrence of non-soluble acids. However, such non-pectic components are hardly present in any cell wall (John & Dey 1986), and they will not significantly influence the results. The results of the rapid and the colorimetric method agree well, and no significant differences were observed (Table 1).

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