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MODIFICATION OF THE TITRIMETRIC METHOD OF ANALYZING PECTIN SUBSTANCES

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A potentiometric variant of the titrimetric method of analyzing pectin substances has been developed which is distinguished by adequate accuracy and good reproducibility. The modified method has been tested on methyl (methyl 2,3-di-O-methyl-α,β-galactopyranosid)uronate and has been used for determining the free (K_f) and esterified (K_e) carboxy groups of the pectin substances of mint. The methods of isolating, purifying, and fractionating the pectin substances and determining their K_f and K_e values are given. Formulas are presented for calculating the percentage values of K_f and K_e.

The development of methods for the complex utilization of the wastes of a number of processing sectors of the national economy is widely based on biotechnological processes at the present time [1-3]. Acidic polysaccharides that are valuable for the food industry and for medicine can be obtained both from wastes of a food nature (apple pomace, sugar-beet pulp, citrus rind) and also from nonfood wastes [4-6].

To evaluate the practical suitability of the acidic polysaccharides obtained, rapid methods of determining the quality and, in particular, the amount of free carboxy groups, and also the degree of their esterification, are important. The methods described in the literature [7-10], and also the widely used titrimetric method [11], have a number of disadvantages and require certain corrections. The disadvantages include, in the first place, the large size of the sample of substance to be determined (of the order of 1 g) and, in the second place, the fact that in the determination of the amount of esterified carboxy groups the excess of HCl (0.5 N) is back-titrated with dilute NaOH solution (0.1 N) in the presence of Hinton's mixed indicator, the color of which changes slowly; this interferes with the strict fixation of the equivalence point.

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TABLE 1. Results of the Potentiometric Determination of the Free (K_f) and Esterified (K_e) Carboxy Groups in the Pectin Substances (PSs) of Mint (budding phase) ($\alpha = 0.95$, $n = 8$)

PSs of mint		Mol. wt., c.u.	K_f, %			K_e, %			K_{tot}, %
variety	organ		$\bar{x}$	s	$K_f \pm \varepsilon$	$\bar{x}$	s	$K_e \pm \varepsilon$	
Zarya	Leaves	28900	8,41	0,151	8,41±0,16	5,77	0,079	5,77±0,07	14,18
Prilukskaya-6	Leaves	24600	5,52	0,093	5,52±0,09	6,64	0,178	6,64±0,15	12,16
Oktyabr'skaya	Leaves	21700	7,98	0,104	7,98±0,10	6,38	0,080	6,38±0,08	14,36
Zarya	Stems	18500	9,04	0,165	9,04±0,15	5,57	0,092	5,57±0,09	14,61
Prilukskaya-6	Stems	20900	14,53	0,120	14,53±0,11	4,43	0,100	4,43±0,08	18,96

We have modified the method of determining the amounts of free and esterified carboxy groups in acid polysaccharides (pectin substances). The fixation of the equivalence point by a potentiometric method permits more reliable results to be obtained and is applicable to turbid solutions of pectin substances (PSs).

As a standard sample we used methyl (methyl 2,3-di-O-methyl-α-D-galactopyranosid)uronate synthesized by one of the authors [12]. Titration was performed on a EV-74 pH-meter with a glass electrode and a silver-chloride comparison electrode. Comparative titration gave the following results for the amount of esterified carboxy groups (K_e, %):

titrimetric method with Hinton's indicator — 15.45 ± 0.25;

titrimetric method with potentiometric determination of the equivalence point — 17.93 ± 0.18;

K_e, calculated for the standard sample, 17.99%.

A comparison of the results of titration obtained by the two methods shows that the potentiometric determination of carboxy groups in acidic polysaccharides (pectin substances) is more accurate than ordinary titration and is characterized by adequate reproducibility.

It has been established experimentally that the optimum conditions for esterification in the determination of K_e are: temperature 20-50°C, and time 1.5-2.0 h.

EXPERIMENTAL

Isolation of the Acidic Polysaccharides [13-15]. The air-dry raw material was treated successively with ethanol—benzene (1:4) and with water at 50-65°C. The pectin substances (PSs) were extracted with a pH 2.5 buffer (0.25% of ammonium oxalate in a 0.25% solution of oxalic acid) at 65-70°C for 6-8 h at a liquor ratio of 1:10-1:15. The extracts were concentrated by evaporation in a rotary evaporator.

Purification of the PSs. The PSs were precipitated with ethanol that had been acidified with HCl to pH 3.0-3.5; the precipitate was separated off by centrifugation and was dissolved in the buffer solution and the precipitation operation was repeated three times, after which the product was dialyzed.

Fractionation [16]. The mono- and oligosaccharides were separated on Sephadex F-50. Elution was performed with a pH 4.5 buffer (10 ml of CH_3COOH and 4 ml of pyridine in 1000 ml of H_2O).

On DEAE-cellulose (DE-32) by successive elution we isolated neutral polysaccharides (0.05 M NaH_2PO_4 solution) and acidic polysaccharides (0.5 M solution of NaCl in 0.05 M NaH_2PO_4). The fractions were analyzed on a Technicon carbohydrate analyzer.

Determination of Free Carboxy Groups. A sample of about 0.1 g of dried material was weighed out on an analytical balance and it was wetted with 96% ethanol (≈ 5 ml), and then 100 ml of H_2O was added and the mixture was stirred with a magnetic stirrer until the sample had dissolved completely (1.5-2.0 h). Then an aliquot (10-20 ml) was titrated with a 0.01 N solution of sodium hydroxide potentiometrically to pH 7.5. The amount of free carboxy groups (K_f) was calculated from the formula

$$K_f,\ \% = \frac{N_{NaOH} \cdot V_{NaOH} \cdot 0.0045}{0.1 \cdot q} \cdot 100,$$

where q is the weight of purified PSs, g, contained in the aliquot (1 ml of 0.1 N NaOH solution corresponds to 0.0045 g of carboxy groups).

Determination of Esterified Carboxy Groups. To the back-titrated solution (I) was added exactly 5 ml of 0.1 N sodium hydroxide solution and the vessel was tightly closed and was left at 20-40°C for 2 h for complete saponification. The excess of alkali was back-titrated with 0.1 N HCl solution, also potentiometrically, to a sharp pH jump. A blank experiment was performed in parallel. The amount of esterified carboxy groups (K_e) was calculated from the formula

$$K_e,\ \% = \frac{N_{HCl} \cdot (V'_{HCl} - V''_{HCl}) \cdot 0.0045}{0.1 \cdot q} \cdot 100,$$

where V'_{HCl} is the volume of HCl solution having normality N consumed in titration in the blank experiment, and V''_{HCl} is the volume of HCl solution with normality N consumed in the titration of the sample; q is the weight of PSs, g, present in the aliquot.

The results obtained are given in Table 1.

SUMMARY

1. A modification of titrimetric method of analyzing pectin substances which is distinguished by high accuracy and good reproducibility has been proposed.

2. The modified method permits the analysis both of acidic polysaccharides and of partially or completely methylated derivatives of glucuronic and galacturonic acids to be performed.

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