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PECTIN SUBSTANCES OF THE MULBERRY

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Several variants of the isolation of pectin substances from mulberry boughs are considered.

In the present paper we consider several variants of the isolation of pectin substances from mulberry (Morus) boughs – wastes from silkworm rearing – which can be used as a source of gel-forming polymers.

In order to ensure the maximum yield of acidic polysaccharides, the raw material was hydrolyzed by the freezing method described in [3]. Such factors as the pH of the medium, the time and temperature of hydrolysis were varied.

In the first stage, two-hour hydrolysis of the plant raw material was carried out by the method generally adopted [1, 2] at 90°C. The yield of pectin material amounted to 1.38%. Analysis of its functional composition showed (Table 1) that the product obtained consisted of a weakly methoxylated pectin (16.2% of –COOH groups and 1.1% of $-OCH_3$ groups) containing 64.8% of D-galacturonic acid (GA) according to the carbazole method [6].

Experiments on the isolation of the pectin by the freezing and hydrolysis of the plant raw material showed that the temperature parameters exerted an influence on the yield of pectin, which reached its maximum values at 80-90°C and pH 1.2 (see Table 1).

The pectin obtained by precipitation with acetone contained 3.5-3.8% of ash, which affected the absorption band of the stretching vibrations of the carboxylic C=O groups. The amount of D-galacturonic acid determined by a titrimetric method [5, 6] was between 57 and 58% (see Table 1).

When the pH of the reaction mixture was changed to 1.2, the temperature dependence of the yields of pectin likewise changed and its maximum was found at a temperature of about 90°C (see Table 1). Functional-group analysis showed that the pectin obtained was a weakly methoxylated product. With a rise in the temperature of hydrolysis the amount of D-galacturonic acid increased, which was possibly due to the saponification of the L-arabinofuranosyl residues of the main chain of the rhamnogalacturonan. With a further rise in the temperature of hydrolysis the degradation of the polygalacturonan macromolecule itself increased still more.

An investigation of the influence of the pH of the hydrolyzing reagent on the yield and functional composition of the pectin from mulberry boughs showed that the highest yield pectin (4.2-4.7%) was observed under the following experimental parameters: time of freezing at –20°C 30 min; hydrolysis-extraction of the pectin at 80-90°C for 60 min; pH of the medium 1.2. The amount of polyuronide fraction and the level of D-galacturonic acid in the preparation fell, in the main, with a rise in the pH of the medium.

In view of the absence of literature information on the carbohydrate composition of the pectin isolated from mulberry boughs, we first investigated the carbohydrate composition of

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TABLE 1. Results of the Analysis of Pectin Obtained by the Hydrolysis of Mulberry Boughs (bath ratio 1:10)

Experiment No.	Sample weight, g	pH of the medium	Freezing		Hydrolysis		Yield %	Functional composition			
			τ, min	t, °C	τ, min	t, °C		–COOH groups	$-OCH_3$ groups	PU*	GA**
1	36,0	0,8	30	—20	60	70	2,20	7,9	3,1	49,0	56,0
2	36,0	"	30	—20	60	80	2,70	8,8	3,0	49,5	58,6
3	36,0	"	30	—20	60	90	2,38	9,2	1,8	42,0	57,6
4	36,0	1,2	30	—20	60	55	1,8	5,8	5,6	46,0	33,2
5	36,0	"	30	—20	60	60	2,4	5,6	3,7	37,4	43,2
6	35,0	"	30	—20	60	70	3,2	7,4	4,3	47,0	43,6
7	36,0	"	30	—20	60	80	4,2	6,3	4,9	46,0	47,0
8	35,0	"	30	—20	60	90	4,7	6,2	5,1	46,3	86,4
9	36,0	"	30	—20	60	96	4,0	7,0	1,1	42,1	77,4
10	36,0	0,4	30	—20	60	90	1,70	8,3	2,2	41,8	36,3
11	36,0	0,8	30	—20	60	90	2,70	9,2	3,1	49,5	57,6
12	36,0	1,2	30	—20	60	90	4,20	5,9	6,8	52,3	86,4
13	36,0	1,3	30	—20	60	90	3,50	6,20	2,2	33,8	82,1
14	36,0	1,6	30	—20	60	90	1,60	1,1	4,2	22,5	43,6
15	36,0	2,0	30	—20	60	90	1,37	1,5	0,7	8,7	39,4

*PU – polyuronide fraction of the material.
†GA – galacturonic acid.

the pectin obtained at pH 1.2 (Table 1, experiment 8), using paper chromatography. In hydrolysates we detected D-galacturonic acid, galactose, arabinose, rhamnose, xylose, and traces of mannose and glucose. The same carbohydrates are components of the pectins obtained from other plant raw material [8-11].

The IR spectra of all the preparations obtained at pH 0.8 and 1.2 (Table 1, experiments 7 and 11) showed absorption band specific for pectin substances in the 1020-1100 cm^{-1} region characterizing the vibration of a pyranose ring. The absorption band of the stretching vibrations of carboxylic C=O groups unsubstituted by metals is usually observed at 1720-1760 cm^{-1}. The high ash content of the preparations obtained caused a shift of theis band into the lower-frequency region characteristic for the stretching vibrations of an ionized carboxy group. Thus, in the preparation obtained at pH 1.2 the ash content was 3.6% and the IR spectrum contained absorption bands characteristic both for the stretching vibrations of a carboxy group (1730 cm^{-1}) and for its ionized form $\left(C\begin{matrix}O\\O\end{matrix}\right)^-$, which were shifted into the lower-frequency region (1640 cm^{-1}). Absorption bands of the stretching vibrations of CH groups in the 2930 cm^{-1} region and an intense band at 3420-3440 cm^{-1} specific for secondary hydroxy groups were also detected.

EXPERIMENTAL

Analysis of the Pectin. Its functional composition was determined by a known procedure: carboxy and methoxy groups by a titrimetric method [5, 6]; D-galacturonic acid by the carbazole method [6], and the ash content by combustion in a crucible furnace [7].

Isolation of the Pectin. The mulberry boughs were comminuted, washed with water, dried, and treated with hexane. The ratio of raw material to solvents was 1:6, the temperature 68-70°C, and the time 1 h. After evaporation of the solution, a fatty-waxy fraction amounting to 1.8% of the initial raw material was obtained. A weighed sample of the purified preparation was mixed with an aqueous solution of HCl (liquor ratio 1:10), the mixture was poured into a vessel which was then hermetically sealed and frozen for a predetermined time, and it was then thawed out in a steam bath and hydrolysis was performed. After the end of hydrolysis, the mixture was filtered. The filtrate was purified additionally in a centrifuge at 8000 rpm for 30 min. The pectin was precipitated with two volumes of acetone. The precipitate was washed with the same solvent until Cl^- ions had been eliminated and then with ether. It was dried in the air.

Complete Hydrolysis of Pectin and Paper Chromatography [8]. The pectin was hydrolyzed with 80% sulfuric acid, the hydrolysate was neutralized with barium hydroxide, and the precipitate of barium sulfate was filtered off and washed. The filtrate was treated with KU-2 cation-exchange resin in the H^+ form. The hydrolysate was separated from the resin by filtra-

tion and the filtrate was evaporated to small volume (V = 20 ml). Paper chromatography was conducted on FN-IV paper (GDR) at 20°C for 6 h in the acetic acid–butan-1-ol–water (1:4:5) system with aniline phthalate as the revealing agent.

SUMMARY

1. In mulberry boughs the extractable amount of acid polysaccharide was 4.7%. It consisted of a weakly methoxylated pectin composed of D-galacturonic acid and accompanying neutral carbohydrates.

2. The optimum conditions for obtaining pectin from mulberry boughs are freezing the plant raw material at −20°C for 30 min in an HCl solution at pH 1.2 and hydrolysis-extraction at 90°C for 60 min.

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