MONOSACCHARIDE COMPOSITION AND HYDRODYNAMIC PROPERTIES OF INDUSTRIAL PECTIN SUBSTANCES

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A study of the monosaccharide composition and hydrodynamic characteristics of pectin substances has made it possible to judge their structure and evaluate the quality of the desired product obtained in industry. The results of the investigation show that, depending on the method of extraction, it is possible to obtain both a homogalacturonan and also a graft copolymer consisting of residues of galacturonic acid and rhamnose with branches formed through the neutral sugars.

The monosaccharide composition and molecular mass are the main parameters of pectin substances (PCs) and practically determine the possibility of their use in the food industry as gelling agents. The composition and structure of pectin substances from apples has been studied by many authors [1-8]. It has been found that the method for their isolation has a substantial influence on both indices. To improve the quality of the pectin substances it is necessary to study their structural and molecular parametes as functions of the technology of their production. With this aim, we have used two types of domestic apple pectins obtained by acid hydrolysis in the Bendery factory of the Moldavian SSR (PSB) and in the experimental shop of the Shakhrinau preserves factory of the Tadzhik SSR (PSS). The technology for obtaining the PSS differs from that for obtaining the PSB by the fact that the plant raw material is first frozen. Both pectin substances were light yellow and were subjected to additional purification. The quantitative characteristics of the purified pectins obtained by methods [9] and [10] are given below, %:

Pectin	K_f	K_e	λ	OCH_3
PSS-1	4,06	8,6	68,0	9,0
PSS-2	3,92	13,4	77,4	10,2
PSB-2	4,18	14,0	77,0	12,0

The monosaccharide compositions of the purified pectins were determined by the GLC method. Quantitative analysis was carried out by the internal-standard method [11]. The concentration of each component (C_i, mass %) was determined from the formula

$$C_i = \frac{S_i \cdot f_i \cdot M_{st}}{S_{st} \cdot M_m} 100,$$

where S_i is the area of the peak of component i of the mixture being analyzed; f_i is the relative pairwise coefficient in relation to the standard; S_{st} is the area of the peak of the standard; M_{st} is the mass of internal standard added; and M_m is the mass sample of mixture to be analyzed to which the definite amount of internal standard was added. The relative error of the coefficient f_i was determined on a model mixture (rhamnose, arabinose, xylose, mannose, glucose, galactose) containing 2-deoxy-D-glucose as internal standard:

$$f_i = a_i \cdot S_{st}/a_{st} \cdot S_i,$$

where $f_{st} = 1$, a_i is the amount of component i in the mixture, mass %; a_{st} is the amount of the component taken as standard in the mixture, mass %; S_{st} is the area of the peak taken as the standard; and S_i is the area of the peak of component i.

Information on the monosaccharide compositions of samples of PSS and PSB before and after purification are given in Table 1. It can be seen that both samples of the initial pec-

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TABLE 1. Amounts and Monosaccharide Compositions of the Pectins

Pectin	Yield, %	Monosaccharide composition, mass %						Galacturonic acid, mass %*
		Rha	Ara	Xyl	Man	Gal	Glc	
PSS		2,9	7,5	1,5	2,61	2,28	9,0	45,5
PSS-1	49,6	3,3	2,05	2,5	0,74	1,76	7,16	74,3
Alcoholic extract	50,4	3,8	41,0	0,82	1,13	4,7	46,0	—
PSS-2	51,0	2,02	7,5	0,80	0,82	4,32	12,68	70,10
PSS-3	80,4	3,1	5,13	1,6	0,54	2,68	8,25	78,2
PSB		8,4	5,4	5,83	2,9	4,37	6,28	50,6
PSB	68,9	4,59	0,24	1,31	1,84	4,64	0,53	86,0

*The fact that the sum does not amount to 100% is connected with the presence of unidentified substances.

tins were characterized by relatively low contents of galacturonic acid residues, which had arisen appreciably after purification. However, this led to a considerable fall in the yield of desired product. It can also be seen from Table 1 that, in spite of the identity of the method of purification and the close values of the monosaccharide compositions of the initial samples, these indices differed considerably for the corresponding samples of PSS and PSB. For example, after purification by both methods the amount of galacturonic acid in the PSB sample had risen considerably in comparison with the PSS.

The results obtained can be explained on the basis of model ideas on the macromolecule of apple pectin given in [6]. According to this model, the peptin macromolecule consists of a so-called "hairy region" and is represented by sections of a rhamnogalacturonan chain with branches of neutral sugar residues. Together with this, the chain of the pectin macromolecule contains "smooth regions" of galacturonan. In the "hairy region" the side chains are attached to the main chain through the rhamnose residues, and, in some cases, the xylose residues, present in it. It is known that the acid extraction of pectin substances at pH values of 1.0-1.5 and a temperature of 85°C leads to a considerable destruction of the covalent bonds between the rhamnose and arabinose residues [12]. It has been reported [13] that on treatment with alcoholic hydrochloric acid an increase in the amount of anhydrogalactouranan is observed.

On the basis of these ideas and the facts presented, it is possible to draw certain conclusions concerning the structure of the pectin substances investigated. The fact that after purification by the second method the total amount of galacturonic acid and rhamnose residues in the PSB samples had risen by more than 90% indicates the presence of oligosaccharides as impurities in the initial pectin. After their elimination, it was mainly linear macromolecules of homogalacturonan that remained in the pectin. At the same time, with an analogous method of purification the amount of these components in the PSS-2 rose by only 72%. This gives grounds for considering that the macromolecules of the purified PSS consist predominantly of a graft copolymer (hairy region). This was also shown by the composition of this pectin after purification by alcoholic hydrochloric acid. It can be seen that in the sample PSS-1 the total amount of galacturonic acid and rhamnose (~80%) was greater than in the sample PSS-2. Finally, the greater content of xylose in PSS-1 as compared with PSS-2 also falls within the framework of the hypothesis expressed above.

While in the PSS-1 samples the increase in the amount of galacturonic acid was connected with the elimination of ions of polyvalent metals (Ca^{2+}; Mg^{2+}), on prolonged treatment of PSS-2 with alcoholic hydrochloric acid there was an increase in the amount of uronide component in PSS-3, which was also connected with the splitting out of the side chains of the pectin macromolecule, and this is an additional argument in favor of the existence of a "hairy region."

Attention is attracted by the high content of glucose in the PSS pectin, particularly in its alcoholic extract. This may be connected with the degradation of cellulose, since according to the technology of obtaining the PSS the initial raw material is first frozen. It must be mentioned that the presence of glucose in the pectin may be connected with the presence of a glucan of the starch type in the pectin. To determine the amount of glucan we made qualitative (reaction with iodine) and quantitative determinations of starch in the samples of PSS and PSB pectins by the procedure described in [14].

TABLE 2. Intrinsic Viscosities, Sedimentation Coefficients, and Molecular Masses of Pectins

Pectin	[η], deg	S_w, svedbergs	M_W, daltons	MSn
PSS-1	1,44	2,0	24552	44000
PSS-2	0,82	2,5	—	47100
PSB-2	1,6	2,94	28100	83900

When iodine was added to a solution of PSS pectin, the color of the solution became red-violet, which may indicate the presence of amylopectin. At the same time, under analogous conditions a solution of the PSB pectin did not change. The amount of starch in the PSS pectin was 1-2%, depending on the conditions of purification, which cannot explain the high glucose content in the pectin samples.

Thus, in spite of the milder conditions of extraction of the PSS pectin (freezing of the raw material and acid hydrolysis at 60°C), the structure of its molecule, unlike that of the PSB, was not homogeneous, which explains its relatively low quality.

Another parameter determining the quality of pectins is the molecular mass. In the general case, investigations of the hydrodynamic properties of pectins enable not only the molecular mass to be determined but also information to be obtained on the structure of the macromolecule. We therefore determined the values of the intrinsic viscosity [η] and the sedimentation coefficient S_0, which were used to estimate the molecular masses of the PSS and PSB. The corresponding results are given in Table 2.

The values of $M_{S\eta}$ were calculated from the Flory–Mandelkern equation [15], and those of M_W by using the equation $[\eta] = 4.9 \cdot 10^{-4} \cdot M^{0.79}$, found for apple pectin [16]. It follows from Table 2 that the molecular mass of the PSB was greater than that of the PSS, which, together with the high content of galacturonic acid residues, is responsible for the high quality of the purified pectin. Attention is attracted by the high intrinsic viscosity of PSS-1 in comparison with PSS-2, although in the first case, the molecular mass should be lower than in the second. As already mentioned, on treatment with alcoholic hydrochloric acid (PSS-1), the side chains of the pectin break down and its macromolecule becomes linear, this process leading to a fall in molecular mass. If the change in molecular mass does not involve a change in the structure of the macromolecules, simultaneously with the fall in the molecular mass there should be a decrease in [η]. It can be seen from Table 2 that no such process took place. It is known that, at the same value of the molecular mass, [η] is smaller for a branched polymer than for a linear polymer, and the ratio $[\eta]_{br}/[\eta]_{lin}$ is the smaller the greater the degree of the branching. On the basis of these facts, a natural explanation of the increase in [η] for PSS-1 as compared with PSS-2 may be a decrease in the degree of branching of the macromolecules. These facts correlate fairly well with the results obtained in a study of the monosaccharide compositions of the corresponding pectins.

Thus, the freezing of the raw material and subsequent hydrolysis under mild conditions leads to the production of a more native pectin with a branched structure, while under more severe conditions of hydrolysis a pectin with a linear structure is obtained. In the latter case, in spite of the fairly high content of galacturonic acid, the low molecular mass impairs the quality of the pectin to some degree. The results obtained place on the agenda the question of the development of new technologies enabling a homogalacturonan with a high molecular mass to be obtained.

EXPERIMENTAL

Industrial pectins from the Bendery and Shakhrinau preserves factories the characteristics of which are given in the TU [Technical Conditions] OST [Sector Standard] 111.3.82 and TU-10 TazhSSR OST 5-38-88, respectively, were used. The brown industrial apple pectins [for the PSS, SÉ = 63.7 and for PSB (type B), SÉ = 67.0] were purified by two methods:

Method A. PSS was treated for 30 min with 72% isopropanol that had previously been acidified with concentrated HCl in pH 2.0. The sample was washed on a Schott No. 2 filter first with 72% isopropanol until the reaction for the Cl^- ion was negative and then with 96% isopropanol. It was dried in vacuum. The yield of PSS-1 was 49.6%.

Method B. Mechanical contamination was eliminated by centrifugation at 5000g for 15 min and then the microgel was eliminated in the ultracentrifuge at 40,000 rpm for 1 h. The supernatant was precipitated with alcohol (1:3), and after a day the precipitate was decanted with 96% alcohol and with acetone and was dried in vacuum and stored at 4°C. The yields of PSS-2 and PSB-2 amounted to 51.0 and 68.9%, respectively.

Chromatographic Analysis. Sugars were first detected on type M paper (Leningrad Paper Mill No. 2) in the butan-1-ol—water—pyridine (6:3:4) and butan-1-ol—pyridine—water—benzene (5:3:3:1) systems. The spots were identified with acid aniline phthalate. The GLC of the neutral sugars was performed in a Chrom-5 chromatograph (Czechoslovakia) with a flame-ionization detector in a glass column (3.0 × 2500 mm) containing 5% of Silicone XE-60 on Chromaton M-AW-DMCS (0.20-0.25 mm). The monosaccharides were analyzed in the form of the corresponding polyol acetates under the following conditions: temperature of the evaporator and the detector 250°C; temperature of the thermostat 200°C; carrier gases helium (60 ml/min), hydrogen (30 ml/min), and air (400 ml/min).

The amount of anhydrogalacturonic acid was determined by the carbazole method [19] and the amounts of methoxy and free carboxy groups by the methods described in [9] and [10], respectively.

Determination of the Hydrodynamic Parameters. The pectin was dissolved in a 1% solution of KCl, and the solution was filtered through a Schott No. 2 filter. The intrinsic viscosity ([η], dl/g) was obtained at 20°C by means of an Ubbelohde viscometer (time of flow of the solvent 94 sec). Sedimentation coefficients were determined on a MOM-3180 ultracentrifuge with Schlieren optics by the velocity sedimentation method at 20°C and a speed of the rotor of 5000 rpm. The buoyancy factor ($1 - \delta\rho$), determined pycnometrically [20], was 0.345. Molecular masses were determined by the two methods described in [15] and [16].

Acid Hydrolysis. The hydrolysis of 100 mg of PS was carried out in 5 ml of 2 N H_2SO_4 at 100°C for 7 h; the reaction mixture was then neutralized with 0.025 M $Ba(OH)_2$ to pH 6 and was filtered, and the resulting sugars were reduced and acetylated by the procedure of [18].

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