CARBOHYDRATE COMPLEX OF THE FRUIT OF Chaenomeles maulei

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The carbohydrate complex of the fruit of Chaenomeles maulei contains more than 70% of water-soluble sugars, including sucrose, arabinose, galactose, fructose, and glucose. The bulk of the carbohydrate is represented by water-soluble pectin, and protopectin, hemicellulose, and cellulose. The monomeric compositions of all the fractions of carbohydrates and the ratio between the reducing and inverted sugars have been studied.

Until recently, Japanese flowering quince (Chaenomeles maulei, Chaenomeles japonica) has been grown predominantly as a decorative plant. However, thanks to successful selection-genetic work carried out in the Latvian SSR it has been possible to derive and propagate comparatively widely new varieties of this plant giving a good yield (up to 20 tons/ha) with larger fruit (20-50 g) having peculiar biochemical and biological properties. For example, the amount of ascorbic acid is of the order of 100 mg/100 g of dry weight [1], which gives grounds for calling the Japanese flowering quince the "northern lemon" since this index is even somewhat higher than in citrus fruits [2].

According to the experimental results, of the carbohydrate fractions isolated on alcohol-soluble fraction is present in the greatest amount (up to 73.83% of the dry matter of the Ch. maulei fruit), and contains, according to chromatographic determinations, free sugars (Table 1) – glucose, fructose, sucrose, arabinose, and galactose. In fresh technically ripe Ch. maulei fruit the total sugar content averages 9-12%.

In a solution of the alcohol-soluble substances before and after inversion, the qualitative composition of the monosaccharides was determined chromatographically, and the quantitative ratios by the Hagedorn–Jensen method [4].

Hydrolysis of an aliquot of the water-soluble residue with subsequent determination of the reducing substances (RSs) by Bertrand's method [3] showed that their proportion in this fraction of carbohydrates was 44.41%. The amount of reducing sugars determined in the protopectin residue was 42.49%.

When the alkali-soluble fractions were hydrolyzed with a solution of HCl, it was found that the proportion of RSs was 41.67 and 25.79%, respectively, for hemicellulose A and hemicellulose B. The hydrolysates of the hemicelluloses (HMCs) A and B had fairly close qualitative compositions of the monoses. An acid hydrolysate of the residue after the extraction of the HMCs was found to contain mainly glucose, the high level of which showed the presence of a glucan of the cellulose type.

It is known that the main structural component of the cell walls of plants responsible for their rigidity is cellulose. The cellulose fibers of the cells are cemented by a matrix consisting of three polymeric materials – hemicelluloses, pectin, and extensin. In order to determine the amount of extensin (a protein bound to the cellulose fibers) we determined the total nitrogen in an acid hydrolysate (72% H_2SO_4). The result obtained (0.19%) showed a low extensin content.

The residues after H_2SO_4 hydrolysis and fractionation were investigated with the aid of IR and UV spectroscopies. The capacity of the chromophoric groups of the residue for absorbing UV radiation in the 280 and 310-320 nm regions and also distinct bands in the 1600 and 1500 cm^{-1} regions (stretching vibrations of aromatic rings) permitted the identification in the residues of a lignin similar to the lignins of other plants materials. The amount of lignin in the residue after the hydrolysis of the cellulose was 0.11%. The ash content of the residue was 1.51% on the dry weight of the Ch. maulei fruit.

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TABLE 1. Carbohydrate Composition of the Fruit of Chaenomeles maulei

Fraction of hydrocarbons	Hydrocarbons, %		Monomeric composition of the carbohydrate, % on the sum of the oligo- and polysaccharides							
	in the fruit	on the dry weight	glucose	fructose	sucrose	arabinose	xylose	galactose	rhamnose	uronic acids
Alcohol-soluble (70% ethanol)	8,85	73,83	24,56	21,33	13,23	24,19	—	16,69	—	—
Reducing alcohol-soluble	1,32	11,04	17,91	14,32	26,45	24,79	—	16,53	—	—
Inverted alcohol-soluble	1,8	15,1	31,22	28,32	—	23,60	—	16,85	—	—
Water-soluble pectin	0,32	2,65	17,71	—	—	21,89	Tr.	23,65	—	36,75
Protopectin	0,44	3,68	10,88	—	—	15,85	1,54	15,46	Tr.	56,25
Hemicellulose A	0,33	2,79	34,91	—	—	16,81	34,00	11,15	3,11	Tr.
Hemicellulose B	0,87	7,28	35,72	—	—	24,99	28,27	11,01	Tr.	Tr.
Cellulose	0,16	1,32	98,50	—	—	—	Tr.	—	—	Tr.
Lignin	0,01	0,11	—	—	—	—	—	—	—	—
Sum of the readily hydrolyzable polysaccharides	0,69	5,78	7,34	—	—	35,97	33,63	23,06	—	Tr.
Hydrolyzable polysaccharides.	1,11	9,25	20,44	—	—	17,78	0,84	13,86	—	47,08

EXPERIMENTAL

The fruit of Chaenomeles maulei was collected in experimental plots of the Latvian Agricultural Academy (at Elgava) in 1988 in the stage of technical ripeness. The fruit was comminuted, the seeds were removed, and defatting was carried out with ether in a Soxhlet apparatus.

The chromatography of the carbohydrates on Whatman No. 1 paper was performed in the descending one-dimensional variant using the solvent system ethyl acetate—acetic acid—pyridine—water (10:3:3:2) which ensures satisfactory separation of the neutral monosaccharides and uronic acids [6].

As the revealing agent for aldoses we used aniline phthalate [7], and for ketoses we used urea [3].

The TLC of the sugars was carried out on 10 × 10 cm plates impregnated with boric acid, using the solvent system methyl ethyl ketone—acetic acid—methanol (3:1:1), which ensures the separation of glucose and galactose [8].

In the performance of paper chromtography and thin-layer chromatography, the monosaccharides were identified on the basis of a comparison of the mobilities of the compounds under investigation and standard substances, and also from literature information on R_f values in the systems used.

Quantitative determination was performed after the elution of the compounds from the sorbent by performing the reaction with a 0.2% solution of benzidine in acetic acid followed by the recording of the absorption at 350 nm [9].

IR spectra were taken in a UR-20 instrument in the wavenumber intervals of 4000-2000 (LiF prism) and 2000-700 cm^{-1} (NaCl prism).

The samples were prepared by molding the polysaccharide in KBr [10].

Fractionation of the Carbohydrates. After the elimination of the lipids and pigments from 100 g of raw material with a moisture content of 88%, it was treated with 70% ethanol at 70°C for 2 h.

The extraction of the alcohol-soluble substances was carried out three times, the total volume of the extract being 250 ml. The amount of reducing substances in the extract and the amount of inverted sugars (hydrolysis with 2% HCl) were determined by Bertrand's permanganate method. The solid phase was separated off by centrifugation, and the extract was evaporated in vacuum. The dry residue was extracted several times with water at 70°C for 2-3 h with periodic stirring. The total extracts were evaporated in vacuum to small volume

and were precipitated with ethanol. The precipitate of pectin was separated off by filtration through a Schott filter and was washed and dried to constant weight, after which the yield was determined gravimetrically. An aliquot of the precipitate was hydrolyzed and the amount of RSs was determined by Bertrand's permanganate method.

The residue after the removal of the water-soluble substances was extracted with a mixture of 0.25% ammonium oxalate and oxalic acid in a ratio of 1:1 at 65-75°C. The process was repeated three times with periodic stirring. The total extract was dialyzed against running water, was evaporated in vacuum to small volume and was dialyzed again, and the yield was determined. An aliquot part of the residue after hydrolysis was used for determining the RSs.

The residue after the elimination of the pectin substances was extracted several times with 6% KOH at room temperature. The extract was treated with acetic acid, and the hemicellulose A (HMC-A) was precipitated. The deposit was separated off and was washed and dried, and the yield was determined. An aliquot was hydrolyzed, and the RSs were determined.

Treatment with 24% KOH in the presence of 4% boric acid was carried out similarly, as a result of which the hemicellulose B (HMC-B) fraction was obtained. The content of RSs in the residue after hydrolysis was also determined.

The residue of cellulose after all the extractions was washed with water to neutrality and dried, and the yield was determined. Then it was treated with 72% H_2SO_4 at room temperature for 2 h, water was added (1:15), and hydrolysis was carried out in the boiling water bath for five hours. The residue after the hydrolysis of the cellulose was washed with water to neutrality and was dried, and the yield of lignin was determined.

The mineral composition was determined in the ash after the mineralization of the lignin [5].

The isolation of the polysaccharides from the water-soluble, the ammonium-oxalate, and the two alkali-soluble extracts was achieved by mixing the corresponding extracts with ethanol in a ratio of 1:3. The alkali-soluble fractions were first acidified with acetic acid to pH 5.0. The polysaccharides of each fraction were purified by two reprecipitations, for which the deposit of polysaccharides was dissolved in a threefold amount of NaOH and was precipitated with ethanol, washed with ethanol and with ethyl ether, and dried to constant weight over P_2O_5, after which the yield was determined gravimetrically.

Hydrolysis of the Polysaccharides and Identification of the Monosaccharides. The pectin and protopectin were hydrolyzed with 10% H_2SO_4 for 16 h, and the other fractions with 2% HCl at 100°C for 16 h.

To determine the proportion of readily hydrolyzable polysaccharides, a weighed sample after the evaporation of the alcohol-soluble substances was hydrolyzed with 2% HCl.

To analyze the difficulty hydrolyzable polysaccharides, the residue after the elimination of the readily hydrolyzable carbohydrates was washed with water to pH 5.0 and was hydrolyzed with 72% H_2SO_4 as described above.

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QUANTITATIVE DETERMINATION OF THE SUM OF THE ACIDS OF THE ABIETIC TYPE IN ROSIN

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A method has been developed for the quantitative determination of the sum of acids of the abietic type from the intrinsic absorption of abietic acid, which simplifies the performance and decreases the random error.

One of the important directions of the use of rosin is the synthesis from it of rosin-maleic resins. The latter are obtained as the result of the Diels–Alder reaction between maleic anhydride and levopimaric acid. In an acid medium, a dynamic equilibrium arises between the levopimaric, abietic, palustric, and neoabietic acids (acids of the abietic type) [1]. At the same time, the equilibrium mixture contains abietic acid in overwhelming amount. The content of levopimaric acid amounts to a fraction of a percentage part, but this amount is sufficient for the whole of the acids to be converted into the maleic anhydride adduct of levopimaric acid. The quality of the rosin as a starting material before the synthesis of rosin-maleic resins is therefore determined by its content of the sum of the above-mentioned acids [1, 2].

The basic scheme of the simplest method for the quantitative determination of the sum of the acids of the abietic type in rosin includes their isomerization to abietic acid and the quantitative determination of the latter in the equilibrium mixture. A method has been described in the literature [3, 4] which is based on the isomerization of these acids to abietic acid and its quantitative photometric determination with the preliminary performance of a color reaction with diazonium salt of 1-amino-2-naphthol-4-sulfonic acid or with Diazole Rose O.

We have developed a method for the quantitative determination of the sum of the acids of the abietic type from the intrinsic absorption of abietic acid which simplifies the performance of the analysis and decreases the random error of the determination. The method developed is based on the isomerization of the sum of the acids mentioned to abietic acid and the direct photometric determination of it by measuring the optical density of the solution at three wavelengths (the Beins–Ebi method) [5]. The only condition for the applicability of this approach to a particular "substance to be determined–impurity" system is the linear nature of the absorption of the impurity in the wavelength interval used.

The UV spectrum of abietic acid in ethanol has absorption maxima at 234 and 241.5 nm and a shoulder at 249-250 nm. The symmetrical arrangement of a short-wave maximum and the long-wave shoulder relative to the maximum at 241.5 nm is extremely convenient for quantitative measurements at three wavelengths, since it permits the determination of the density of a solution in horizontal sections of the spectrum. To check the applicability of the three-point method to the mixture to be analyzed (i.e., to check the hypothesis of the linearity of the absorption of the impurity in the working section of the spectrum) we studied the absorption of solutions of isomerized rosin at four different concentrations. As the analytical wavelength we selected two sets of three equidistant wavelengths – 234, 241.5, and 249 nm, and 237, 241.5, and 246 nm. The results are summarized in Table 1.

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