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PLANT BIOPOLYMERS

Quantification of Polysaccharides in Vegetable Raw Materials and Lignin Preparations

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Abstract—The phenol-sulfuric acid method has been studied for its capability to analyze vegetable raw materials. This method has made it possible to determine rather simply and with high accuracy polysaccharides in both vegetable raw materials (agricultural vegetables, softwood, and hardwood), and in various lignin preparations (the laboratory-scale and technical). The method is based on the color reaction of monosaccharides with phenol in the presence of concentrated sulfuric acid. The developed modified phenol-sulfuric acid method is universal because allows for the detection of polysaccharides in the samples with both high and low polysaccharide content, i.e., in vegetable raw materials and lignin preparations, respectively. The method is highly sensitive; it is possible to analyze monosaccharides in the mixture at the concentration of 1×10^{-4} mol L^{-1} on average. The hydrolysate volume of 0.25 mL that is ten-times diluted is enough for analysis. The duration of the hydrolysate analysis including the mix preparation, recording of the spectrum, and calculation by the formula does not exceed 30 min. The method can be used for the analysis of the chemical composition of renewable vegetable raw materials when developing technologies for obtaining alternative energy sources.

Keywords: phenol-sulfuric acid method, renewable vegetable raw materials, polysaccharides, monosaccharides, lignin

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INTRODUCTION

Because of depletion of fossil nonrenewable resources (oil, gas, and coal), there has been an intensive search for alternative sources of energy and chemicals for organic synthesis in recent years. The renewable vegetable raw materials including wood are considered as such an alternative. The concept of "biomass refinery" is proposed that provides for the full-integrated use of all components of the tree biomass [1].

Technologies for obtaining bioethanol [2] and biobutanol [3] as an alternative fuel are elaborated and successfully developed. Both processes are based on either acidic or enzymatic hydrolysis of vegetable raw materials followed by the biochemical treatment of hydrolysates. The "biomass refinery" concept includes the use of not only polysaccharides but also lignin, with polysaccharides being undesirable impurities in the technology of the processing and use of lignin [1]. The quantitative evaluation of polysaccharides in vegetable raw materials and technical lignins is, therefore, the actual problem.

To date, the content and composition of polysaccharides in wood and agricultural vegetables are mainly evaluated by chromatographic methods after a preliminary acidic hydrolysis of an analyzed sample [4]. The Theander method is the most widespread [5]; it includes the following stages: preliminary extraction of the sample, acidic hydrolysis, neutralization of the hydrolysate, reduction and acetylation of monosaccharides, extraction of alditol acetates, and gas chromatographic analysis. This method is quite complex and multistage; it requires calibration and the use of standard samples. The total content of polysaccharides is evaluated by summarizing the results of the monosaccharide analysis accounted for the individual polysaccharides.

The goal of this study was to develop the method for the quantitative evaluation of the total content of polysaccharides in vegetable raw materials and lignin preparations.

EXPERIMENTAL

The proposed method is based on the color reaction of monosaccharides with phenol in the presence of concentrated sulfuric acid [6]. The preliminary studies were performed to determine the optimal conditions of the hydrolysis of the analyzed samples and to quantitatively evaluate the reaction products, which resulted in the development of the following method.

A vegetable raw material sample (5 g) with a particle size of 0.25 mm was extracted with ethanol (95%)

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Table 1. Results of analysis of vegetable raw material [4]

Component	Component content, %			
	wood		wheat straw	bagasse
	Populus deltoides	*Pinus radiata*		
Ash	1.0	0.3	10.3	4.0
Extractive compounds	2.4	2.7	13.0	4.4
Lignin	25.6	25.9	15.7	23.1
Glucuronic acid	3.6	2.5	1.8	1.2
Arabinan	0.6	1.5	2.2	1.7
Xylan	13.4	5.9	18.7	20.4
Mannan	2.0	10.7	0.3	0.3
Galactan	0.6	2.4	0.7	0.6
Glucan	42.2	41.7	32.9	38.6
Sum of components	91.4	93.6	95.6	94.3
Sum of polysaccharides	**58.8**	**62.2**	**54.8**	**61.6**

in a Soxhlet extractor for six hours (30 overflows). The content of extractive compounds was evaluated after the removing the solvent on a rotor evaporator and drying the residue.

The mixture of the purified sample (1 g) and 72% H_2SO_4 (15 mL) was kept for 2.5 h at 25°C under periodic stirring, following by transfer to a 500-mL flask. After the addition of distilled water (200 mL), the mixture was refluxed for 1 h. The described method of the preparation of the lignin samples leads to the formation of very fine precipitates that pass through porous glass filters. Therefore, acid-nonsoluble lignin (Klason lignin) was analyzed in all samples using the known methodical technique [7].

The lignin suspension was filtered through stacked together two paper filters equilibrated on an analytical balance. The lignin precipitate and filters were washed with water until acid was completely removed. The filters along with lignin were dried at 103 ± 2°C to the constant weight and weighed by placing the upper filter (with lignin) and the lower filter on the left-hand and the right-hand cups of the analytical balance, respectively. It should be noted that a part of the filtrate was taken up before the washing step for the subsequent analysis of the content of acid-soluble lignin and monosaccharides. Acid-soluble lignin was determined by the method described in [8] at 205 nm.

To evaluate the polysaccharide content, 10-times diluted hydrolysate (filtrate) (0.25 mL) was placed in a vial, followed by the addition of 0.6 M freshly distilled phenol (5 mL) and conc. H_2SO_4 (5 mL). The hot solution was kept for 10 min and cooled then for 10 min at 25°C. The UV spectrum (Fig. 1) was recorded in 1-cm cuvettes on a UV-2400PC Series spectrophotometer (Shimadzu, Japan). The solution containing the same reagents at the same concentrations except for hydrolysate, which was replaced by distilled water (0.25 mL), was used as the reference solution.

The content of polysaccharides as the percentage to the dry initial (untreated) sample was calculated by the formula:

$$C = \frac{DK_d M_m V K_p}{\varepsilon \times 1000 g} K_e \times 100,$$

where D is the optical absorption; K_d is the dilution coefficient; M_m is the molar mass of monosaccharide, g mol^{-1} (hexoses, 180.16; pentoses, 150.13); V is the hydrolysate volume, mL; K_p is the coefficient for the conversion of monosaccharides to polysaccharides (0.9 for hexosans and 0.88 for pentosans); ε is molar absorption coefficient, L mol^{-1} cm^{-1}; g is the weight of absolutely dry sample, g; and K_e is the extraction coefficient.

DISCUSSION

Using the above method, we studied the samples of the vegetable raw material with known chemical composition (Table 1) to evaluate the molar absorption coefficients (ε). The table summarizes the results obtained under the international Round-Robin program on Whole Feedstock Analysis [4]. The author of the presented work was one of the participants of this program.

The data of Table 1 show that the hydrolysates of the studied samples contain hexoses (glucose, mannose, and galactose), pentoses (arabinose and xylose), and glucuronic acid in different ratios depending on the type of the raw material.

It is obvious that optical absorption of the hydrolysate depends on the monosaccharide content, their concentration, and molar absorption coefficients.

Table 2. Optical characteristics of monosaccharide and glucuronic acid solutions and their contribution to the total absorption of vegetable raw material hydrolysates

Monosaccharide	λ_{max}, nm	ε, L mol^{-1} cm^{-1}	Contribution to the total absorption, fraction of optical density			
			Populus deltoides	*Pinus radiata*	wheat straw	bagasse
Glucose	487.6	3734	0.38	0.36	0.27	0.29
Mannose	487.6	13683	0.07	0.34	0.01	0.01
Galactose	487.4	6729	0.01	0.04	0.01	0.01
Arabinose	476.8	11704	0.02	0.05	0.01	0.05
Xylose	476.6	12764	0.51	0.21	0.63	0.64
Glucuronic acid	479.8	1815	0.01	0.01	0.01	0.01

Therefore, we studied the optical characteristics of individual monosaccharides, i.e., D-glucose, D-mannose, D-galactose, L-arabinose, D-xylose, and D-glucuronic acid (analytical grade, twice recrystallized from ethanol), and determined the contribution of each monosaccharide to the total absorption of the vegetable raw material. The composition and concentrations of monosaccharides in this material were calculated taking the data of Table 1 and hydrolysis conditions into account. The results are presented in Table 2. It should be noted that the initial ε value for xylose was corrected for the absorption of furfural because xylose was converted into furfural under the analysis conditions [6].

The mean molar absorption coefficient value of hydrolysate (ε_c) was evaluated taking into account the contribution of hexose and pentose to the total absorption. Their contribution in the hydrolysate of hardwood (*Populus deltoides*) is the same; therefore, the ε values were averaged for all compounds in the solution, which was determined to be $\varepsilon_c = 8400$ L mol^{-1} cm^{-1} (Table 3). In the hydrolysate of softwood (*Pinus radiata*), the contribution of hexoses (0.74) into the total hydrolysate adsorption is significantly higher than that of pentoses (0.26); therefore, the ε values for hexoses were averaged, thus obtaining $\varepsilon_c = 8050$ L mol^{-1} cm^{-1}.

Although pentoses predominate in the wheat straw hydrolysate, the more real value for the total polysaccharide content has been obtained with the use of the ε_c value of 8400 L mol^{-1} cm^{-1}. This is probably because the contribution of individual monosaccharides to the total absorption is not completely additive because the absorption maxima of hexoses and pentoses do not match and differ by ~10 nm (Table 2).

The use of the $\varepsilon_c = 8400$ L mol^{-1} cm^{-1} value for the calculation of the total polysaccharide content in the bagasse hydrolysate gave a slightly increased result. The difference can be explained by the fact derived from the literature data that the bagasse hydrolysate contains rhamnose along with hexoses and pentoses in comparable amounts [9]. We evaluated the optical characteristics of rhamnose under the same conditions ($\lambda_{max} = 478.4$ nm, $\varepsilon = 15349$ L mol^{-1} cm^{-1}), used them when calculating the average ε values for all compounds in the solution, and obtained $\varepsilon_c = 9400$ L mol^{-1} cm^{-1} for the bagasse hydrolysate.

The results on the chemical composition of the vegetable raw materials (Table 4) show that the developed method for the analysis makes it possible to evaluate the polysaccharide content with a sufficiently high accuracy (the relative error is in the range of 0.3–3.2%). Table 4 includes the results of the determination of extractive compounds and the sum of all components to compare them with the literature data (see Table 1).

The additional verification of the developed method was carried out by studying the chemical composition of spruce wood (*Picea excelsa*). Like *Pinus radiata*, *Picea excelsa* is a coniferous tree; therefore, it is possible to use the $\varepsilon_c = 8050$ L mol^{-1} cm^{-1} value for the calculation of the polysaccharide content, which gave 66.2%. According to the literature data [10], the total yield of reducing substances under the quantitative hydrolysis of spruce wood is 72.5%. The recalculation of monosaccharides to polysaccharides (K_p = 0.89) gives 64.5% of the latter. In other words, the relative determination error is also low (2.6%) in this case.

The preparations of lignin from the spruce wood were studied by the same technique, except that they were analyzed without preextraction. After the evaluation of Klason lignin, the filtrate was not diluted, and the lignin sample weighed 0.3 g. The methods of lignin

Table 3. Optical characteristics of vegetable raw material hydrolysates

Hydrolysate	λ_{max}, nm	ε_c, L mol^{-1} cm^{-1}
Populus deltoides	479.6	8400
Pinus radiata	482.6	8050
Wheat straw	477.8	8400
Bagasse	478.2	9400

Table 4. Content of polysaccharides and other components in vegetable raw materials

Component	Component content, %			
	wood		wheat straw	Bagasse
	Populus deltoides	*Pinus radiata*		
Ash*	1.0	0.3	10.3	4.0
Extractive compounds	2.6	3.1	13.0	3.3
Lignin	24.9	25.6	16.8	23.8
Glucuronic acid*	3.6	2.5	1.8	1.2
Sum of polysaccharides	**58.6** (0.3)	**62.4** (0.3)	**55.7** (1.6)	**59.6** (3.2)
Sum of components	90.7	93.9	97.6	91.9

* Data from [4]. In brackets are the relative errors of determination, %.

Table 5. Optical characteristics of hydrolysates and chemical composition of lignin preparations from spruce wood

Preparation	λ_{max}, nm	ε_c, L mol^{-1} cm^{-1}	Klason lignin, %	ASL*, %	PS*, %	Sum, %
Freudenberg's lignin	486.8	8050	88.8	0.9	6.5	96.2
Björkman's lignin	485.6	8050	92.3	2.6	5.3	100.2
Pepper's lignin	484.2	8050	95.2	2.0	3.7	101.1
Pepper's lignin oxidized	482.8	8050	92.2	2.9	3.4	98.5
Dioxane lignin	482.8	8050	90.2	3.2	2.1	95.5
Hydrolysis lignin	487.2	8050	88.1	0.8	6.4	95.3
Oxidized hydrolysis lignins	486.4	8050	86.3	3.7	6.3	96.3
Soda lignin	478.4	8400	84.2	6.0	7.3	97.5
Kraft lignin	479.6	8400	86.4	5.7	7.1	99.2

* ASL, acid-soluble lignin; PS, polysaccharides.

isolation are described in the following works: [11] (Freudenberg's lignin), [12] (Björkman's lignin), [13] (Pepper's lignin), [14] (dioxane lignin), [15] (hydrolysis and oxidized hydrolysis lignins), and [16] (soda and kraft lignins). The results are presented in Table 5.

There are few literature data on the polysaccharide content in lignin. Among laboratory-scale preparations, Björkman's lignin from the spruce wood was studied in more detail. It can contain carbohydrates from 2 to 8% [17]. The additional purification of the sample including the treatment with diluted alkali may decrease the carbohydrate content to 0.14–1.6% [18]. In our opinion, the use of alkali for the lignin purification inevitably decreases its yield because of its good solubility in alkali. In this case, the isolation of lignin with the use of the Björkman method leads to the yield of only 25% of Klason lignin [17].

The content of hexosans is several times higher than that of pentosans in both spruce wood [10] and Björkman's lignin of spruce [18]. Therefore, we used the same value of $\varepsilon_c = 8050$ L mol^{-1} cm^{-1} for the evaluation of polysaccharides in all lignin samples (Table 5) except soda and kraft lignins because of the polysaccharide composition in these preparations.

Unlike other samples, soda and kraft lignins are isolated from the alkaline cooking liquor of wood. Under these conditions, hemicelluloses, mainly xylan, are partially dissolved [19] and coprecipitated with lignin during the subsequent isolation. It was found that kraft pine lignin contains 69% of pentosans and 32% of hexosans, and soda lignin contains 73% of pentosans and 27% of hexosans [20]. The predominance of pentosans in alkaline lignins is also confirmed by the proximity of the absorption maxima of their hydrolysates (478.4 and 479.6 nm, respectively) to the maxima of arabinose (476.8 nm) and xylose (476.6 nm) (Table 2). Therefore, we used for them the value of $\varepsilon_c = 8400$ L mol^{-1} cm^{-1}. On the other hand, the λ_{max} values for the hydrolysates of the rest lignin preparations are characteristic for hexoses.

The analysis of the data of Table 5 shows that the total content of Klason lignin, acid-soluble lignin, and polysaccharides in all samples are close to 100%. This is another argument in favor of the correctness of the developed method for the polysaccharide determination.

It should be noted in conclusion that the developed method of the analysis allows for the relatively simple and highly accurate determination of polysaccharides

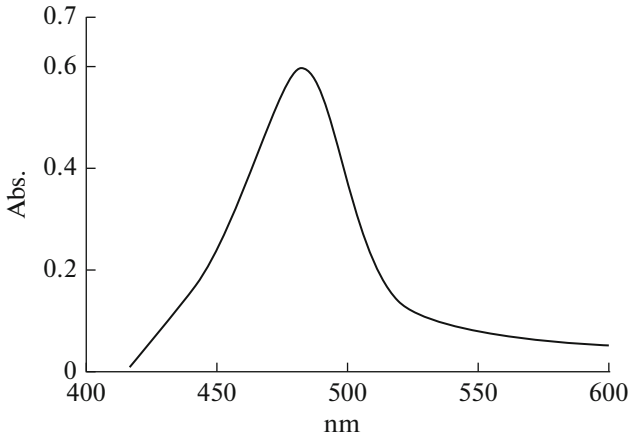

Fig. 1. UV spectrum of hydrolysate of pine wood (*Pinus radiata*).

in both vegetable raw materials (softwood, hardwood and agricultural vegetables) and in different lignin preparations (laboratory-scale and technical preparations), which differ by a relatively low content of polysaccharides (2–7%).

CONCLUSIONS

(1) The photocolorimetry method that is based on the color reaction between monosaccharides and phenol in the presence of sulfuric acid makes it possible to relatively simply and quite reliably evaluate the content of polysaccharides in wood, agricultural vegetables, and lignin preparations.

(2) The method may be used for the analysis of the chemical composition of renewable vegetable raw materials when developing technologies for obtaining alternative energy sources.

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