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Research Article

Analysis of Wood Carbohydrates: A New Scheme

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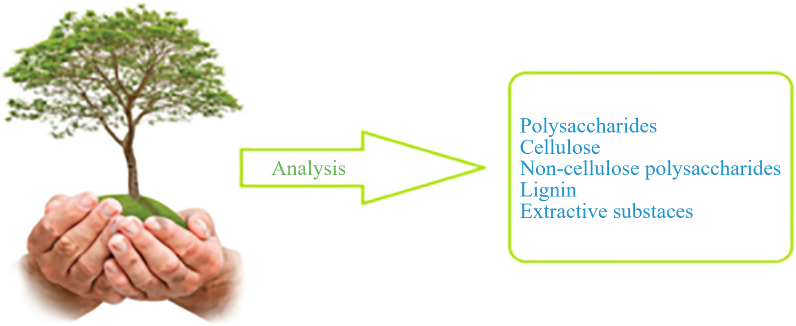
Graphical Abstract:



Abstract: A new scheme for the analysis of wood carbohydrates is proposed, which includes measurements of cellulose and non-cellulose polysaccharides. The analysis is based on the color reaction of monosaccharides in hydrolysates of polysaccharides with phenol in the presence of concentrated sulfuric acid. The method is highly sensitive: the concentration of monosaccharides in the mixture prepared for analysis is determined at the level of 1×10^{-4} M. For the analysis, 0.25 cm^3 of the hydrolysate, previously diluted 10 times, is sufficient. The total time for the analysis of the hydrolysate, including sample preparation, does not exceed 30 minutes. The method allows for a relatively simple and highly accurate determination of polysaccharides both in plant raw materials (agricultural plants, softwood, and hardwood) and in lignin samples (softly isolated and technical).

***Keywords*:** wood, polysaccharides, cellulose, non-cellulose polysaccharides, analysis

1. Introduction

Due to the depletion of non-renewable fossil resources (oil, gas, and coal), an intensive search for alternative sources of energy and chemicals for organic synthesis has been continuing in recent years. Renewable plant raw materials, including wood, are considered as such alternatives. The concept of biorefinery encompasses the utilization of

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components of tree biomass.[1-3]

Accurate feedstock compositional analysis is essential for the evaluation of conversion yields and process economics as dependent on changes in feedstock or process design. Reliable measurement of biomass carbohydrate content is of prime importance because it is directly proportional to ethanol yield in the biochemical conversion processes. One ton of coniferous wood yields 160-175 kg (45-49 gallons) of ethanol.[4]

The main component of wood, cellulose, is both a raw material for biofuel production and a precursor in the production of carbon fibers. Various lignin preparations are of great importance for the production of carbon fibers and many other materials in demand on the market.[5] The driving force behind the development of carbon fiber production is the ever-increasing demand to reduce CO_2 emissions, a significant contribution to which is made by the emissions of cars. In 2012, the average carbon dioxide emission of Volkswagen cars was 130 g CO_2/km, in 2020, the limit in Germany was 95 g CO_2/km, and in the future (2050), it could be reduced to 20 g CO_2/km. In the European Union, the USA and Japan in 2020, the limits were, respectively, 95, 114 and 113 g CO_2/km.[6]

The main means to achieve these goals is to reduce the mass of individual parts of the car by ~ 50% while maintaining its operational and safety characteristics. Therefore, it is no coincidence that carbon fibers are increasingly used in the automotive industry, allowing for a reduction in the weight of products by up to 60% with the same functionality. However, to obtain carbon fibers, an expensive raw material is used – polyacrylonitrile (PAN), which accounts for more than 50% of the production cost, and therefore lignin and cellulose are considered as an alternative source of raw material.[6,7] Relatively speaking, with the use of modern technologies, the application of wood, cellulose and lignin, could a twofold decrease in the mass of cars and provide them with biofuel. These areas are discussed in more detail in the reviews.[8,9]

Against this background, there is a growing need for the development of accurate and time-saving methods for the quantitative determination of cellulose, non-cellulosic polysaccharides and lignin in plant materials. The currently known methods of analysis of plant raw materials, including the history of their development, are described in detail in publications.[10-12] Popular are the methods of analysis used in the International Program Round-Robin on Whole Feedstock Analysis,[13] where the author of this article participated.

The Whole Feedstock Analysis is based on the Theander[14] method, which includes the following stages: preliminary extraction of the sample, acid hydrolysis, neutralization of the hydrolysate, reduction and acetylation of monosaccharides, extraction of the obtained alditolacetates with subsequent gas chromatographic analysis. The method is rather complex, multi-stage, requiring calibration and using standard samples. The total content of polysaccharides is determined by summing up the results of the analysis of monosaccharides per individual polysaccharides. The analysis takes several days.

We have developed a method based on the color reaction of monosaccharides with phenol in the presence of concentrated sulfuric acid.[15] The developed modification of the phenol-sulfuric acid method is universal in the sense that it allows determination of polysaccharides in samples both with a high content of polysaccharides (plant raw materials) and with a low content (lignin preparations). The method is highly sensitive: the concentration of monosaccharides in the mixture prepared for analysis is determined from 1×10^{-4} M. For the analysis, 0.25 cm^3 of the hydrolysate, previously diluted 10 times, is sufficient. The duration of the analysis of the hydrolysate, including the preparation of the mixture, the recording of the spectrum and the calculation by the formula, does not exceed 30 minutes. When developing the method, we used samples with a known content of polysaccharides and other components of plant raw materials provided as part of the Round-Robin program. The relative error in determining the total polysaccharide content was 0.3%, for *Populus deltoides* and *Pinus radiata*, 1.6% for wheat straw and 3.2% for bagasse.

In this work, we propose a scheme for the analysis of the component composition of wood, including the determination of the total content of polysaccharides, cellulose, non-cellulose polysaccharides, lignin, and other compounds. Using this scheme, samples of wood of industrial importance were studied: spruce (*Picea abies*), pine (*Pinus silvestris*), larch (*Larix sibirica*), aspen (*Populus tremula*) and birch (*Betula verrucosa*).

A simple and reliable method for determining the chemical composition of wood and other plant raw materials will allow us to improve existing and develop new methods for the valorization of individual components of renewable raw materials and, in general, solve the problems of biorefining.

2. Experimental

Samples. Wood samples of spruce (*Picea abies*), pine (*Pinus silvestris*), larch (*Larix sibirica*), aspen (*Populus tremula*) and birch (*Betula verrucosa*) in the form of chips were ground in a mechanical mill, then a fraction with a particle size of 0.25-0.5 mm was taken and extracted with ethanol in a Soxhlet apparatus for 6 hours (30 overflows).

Hydrolysis. To the deresinated sample (1 g), 15 cm^3 of 72% H_2SO_4 was added and kept at a temperature of 25 °C for 2.5 h with periodic stirring. After the specified time, the mixture was transferred into a 500 cm^3 flask, 200 cm^3 of distilled water was added, and the mixture was refluxed for 1 h.

Acid-insoluble lignin. The liquid sample with lignin precipitated was filtered through two 150 mm paper filters folded together and weighed on an analytical balance. The lignin precipitate and filters were washed with water until the acid was completely removed. Filters with lignin were dried at a temperature of 103 ± 2 °C to constant weight and weighed by placing the upper filter (with lignin) on the left pan of an analytical balance, and the lower one on the right. It is important to note here that the filtrate for subsequent analysis for the content of acid-soluble lignin and polysaccharides was taken before the washing stage.[16]

Acid-soluble lignin. Acid-Soluble Lignin (ASL) was determined by the method[17] at a wavelength of 204-205 nm for hardwood and 192-193 nm for softwood. After the filtrate was diluted by a factor of 10, ultraviolet (UV) spectra were recorded in 1 cm cuvettes on a Shimadzu UV-2400PC spectrophotometer. As an example, Figure 1 shows the UV spectrum of the aspen wood filtrate. The ASL content, % to an absolutely dry initial sample, was calculated using the formula

$$\mathrm{ASL} = \frac{D \times K_\mathrm{d} \times V}{\varepsilon \times 1{,}000 \times g} \times K_\mathrm{e} \times 100,$$

where D is the optical density; K_d is the dilution factor; V-volume of hydrolysate, cm^3; ε-absorptivity, $\mathrm{L \cdot g^{-1} \cdot cm^{-1}}$; g is the mass of an absolutely dry sample, g; K_e is the coefficient of extraction.

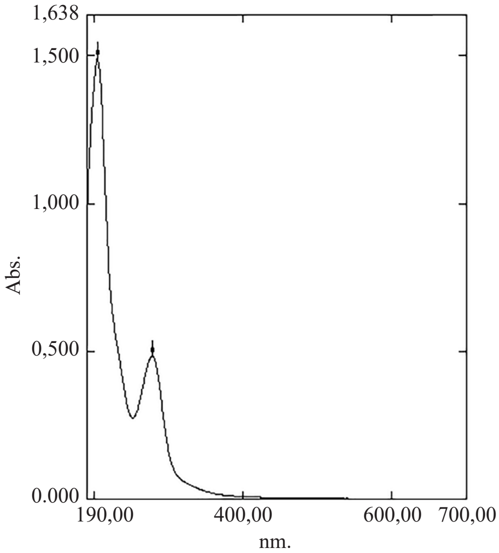

Figure 1. UV spectrum of acid-soluble aspen lignin (*Populus tremula*)

Polysaccharides. To determine polysaccharides, 0.25 cm^3 of the filtrate, previously diluted 10 times, was placed in a test tube, 1 cm^3 of a solution of freshly distilled phenol with a concentration of 0.06 mol L^{-1} and 5 cm^3 of concentrated H_2SO_4 (reagent grade) were added, the hot solution was kept for 10 min, and then cooled for 10 min at 25 °C. Then, the UV spectrum was recorded in 1 cm cuvettes, using the same reagents and at the same concentrations as the reference solution as in the working solution, except that 0.25 cm^3 of distilled water was added instead of the hydrolysate. The UV spectra were recorded on a Shimadzu UV-2400PC spectrophotometer. As an example, Figure 2 shows the UV spectrum of pine wood hydrolysate. The content of polysaccharides, % to an absolutely dry initial sample of plant raw materials, was calculated using the formula

$$C = \frac{D \times K_{\mathrm{d}} \times M_{\mathrm{m}} \times V \times K_{\mathrm{p}}}{\varepsilon_{\mathrm{c}} \times 1{,}000 \times g} \times K_{\mathrm{e}} \times 100,$$

where D is the optical density; K_d is the dilution factor; M_m is the molar mass of the monosaccharide, $g \cdot mol^{-1}$ (hexoses 180.16, pentoses 150.13); V is the volume of the hydrolysate, cm^3; K_p-coefficient of conversion of monosaccharides into polysaccharides (for hexosans 0.9, for pentosans 0.88); ε_c-average molar absorption coefficient, $L \cdot mol^{-1} \cdot cm^{-1}$; g is the mass of an absolutely dry sample, g; K_e is the extraction coefficient.

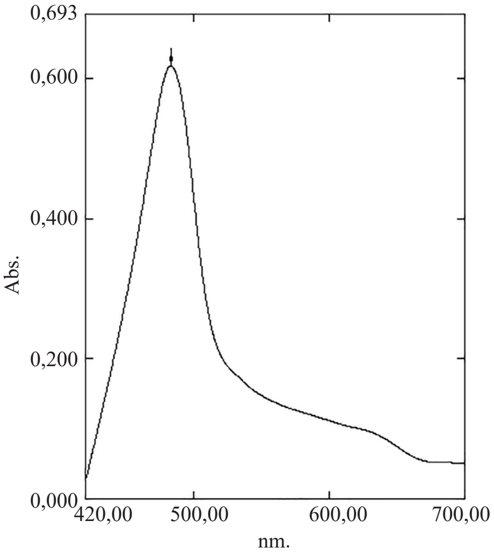

Figure 2. UV spectrum of pine wood hydrolysate (*Pinus silvestris*) after interaction with phenol in the presence of sulfuric acid

Cellulose. The determination of cellulose was carried out by the nitrogen-alcohol method (the Kürschner method).[16,18] The number of successive treatments with a nitrogen-alcohol mixture was 4 for coniferous wood, and 3 for all other samples. Kürschner cellulose isolated from wood was filtered through a PS-2 glass porous filter (pore size 40-100 μm), cellulose from wheat straw and bagasse-through the PS-3 filter (pore sizes 16-40 μm).

Pentosans. The content of residual pentosans in Kürschner cellulose was determined by the bromide-bromate semi-micro method.[16] Furfural yield, % to absolutely dry sample, was calculated using the formula

$$F = \frac{(a-b) \times 100 \times 0{,}0012}{25g} \times 100,$$

where a is the consumption for control titration of a sodium thiosulfate solution with a concentration of 0.05 mol/dm^3, cm^3; b - consumption for titration of the distillate of a sodium thiosulfate solution with a concentration of 0.05 mol/dm^3, cm^3; 0.0012 - the mass of furfural corresponding to 1 cm^3 of sodium thiosulfate solution with a concentration of 0.05 mol/dm^3, g; g is the mass of an absolutely dry weighed portion of the sample, g. Coefficients of conversion of furfural to pentosans for coniferous wood are 1.56, for other samples - 1.52.

Arabinogalactan. Traditionally, to determine arabinogalactan from larch wood, water extraction is used, followed by the precipitation of arabinogalactan with ethanol. The main disadvantage of the method is the precipitation of various impurities together with arabinogalactan, which reduces the determination accuracy. When developing a new method for the quantitative determination of arabinogalactan, we proceeded from the fact that it is an easily hydrolyzable polysaccharide, and upon complete hydrolysis, it yields galactose and arabinose in a 6 : 1 ratio.[19]

The theoretical value of the content of Reducing Substances (RS) in arabinogalactan hydrolysate can be calculated from the equation

$$\left[\left(C_6H_{10}O_5\right)_6\left(C_5H_8O_4\right)\right]_n + 7H_2O \overset{H^+}{\rightleftarrows} 6nC_6H_{12}O_6 + nC_5H_{10}O_5.$$

In accordance with this equation, upon complete hydrolysis of 1 g of arabinogalactan, 0.9782 g of galactose and 0.1359 g of arabinose are formed. Then, upon hydrolysis of a 1% solution of arabinogalactan, the theoretical value of RS = (0.9782 + 0.1359)% = 1.1141%.

Experimental verification of this value was carried out using model solutions of individual monosaccharides (galactopyranose and arabinofuranose) and their mixture by the Schoorl[15,20] method in reaction with Fehling's reagent (reduction reaction of copper Cu^{2+} with reducing sugar in a boiling alkaline solution). It was found that theoretical and experimental values of the RS were very close: 1.1141 and 1.1096%, respectively. In other words, 1% of arabinogalactan corresponds to 1.1% RS in its hydrolysate. At a preliminary stage, the optimal conditions for the hydrolysis of arabinogalactan were determined: 2% HCl, temperature 100 ℃, duration 1 h.

Based on the results obtained, a method for the quantitative determination of arabinogalactan in larch wood was developed. Larch wood (48.1 g based on absolutely dry wood) in the form of flour (fraction with a particle size of < 0.125 mm) was continuously extracted with hot water in a Soxhlet apparatus for 5 hours (30 overflows). 100 cm^3 of 4% HCl was added to 100 cm^3 of the extract so that the hydrolysis proceeded in 2% HCl. The hydrolysis was carried out at the boiling point of the solution for 1 h in a flask equipped with a reflux condenser. After the completion of hydrolysis, the flask was cooled to room temperature with running water, and the RS in the hydrolysate was determined. The content of reducing substances was 1.195%. Considering that during the extraction of larch wood with hot water, reducing substances that are not arabinogalactan can be extracted, the RS content in the extract was also determined before hydrolysis which was 0.17%. The content of arabinogalactan, % to absolutely dry larch wood, was calculated using the formula for determining easy hydrolyzable polysaccharides.[15]

$$X_{AG} = \frac{c_{AG} \times V \times K_d \times K_p}{g \times 100} \times 100,$$

where c_{AG} is the mass fraction of RS in the hydrolysate of arabinogalactan, %; V is the volume of the extract, cm^3; K_d is the dilution factor; K_p coefficient of conversion of monosaccharides into polysaccharides; g is the mass of an absolutely dry sample of wood, g.

The content of arabinogalactan calculated by this formula was 14.5%. The content of easily hydrolysable substances in the extract before hydrolysis was 1.0%. Thus, the content of arabinogalactan in larch wood was 13.5%. For comparison, we determined the content of arabinogalactan by the traditional method, i.e. by precipitation with ethanol. It was 17.1%, which is much higher, since other wood components are precipitated together with arabinogalactan. This is evidenced by the fact that after hydrolysis of this "crude" arabinogalactan and its quantitative determination in the hydrolysate, the number was 13.7%.

Since the galactose to arabinose ratio in it is 6 : 1,[19] we used the e_c value for galactose-6,730[15] as the average molar absorption coefficient for determining polysaccharides in larch wood. Using this coefficient, the polysaccharide content in the wood was 65.6%. According to the literature data,[21] the total yield of reducing substances during quantitative hydrolysis of larch wood is 73.55%. When converting monosaccharides into polysaccharides (K_p = 0.89) the content of the latter is 65.5%.

3. Results and discussion

The scheme of the analysis of wood samples is shown in Figure 3.

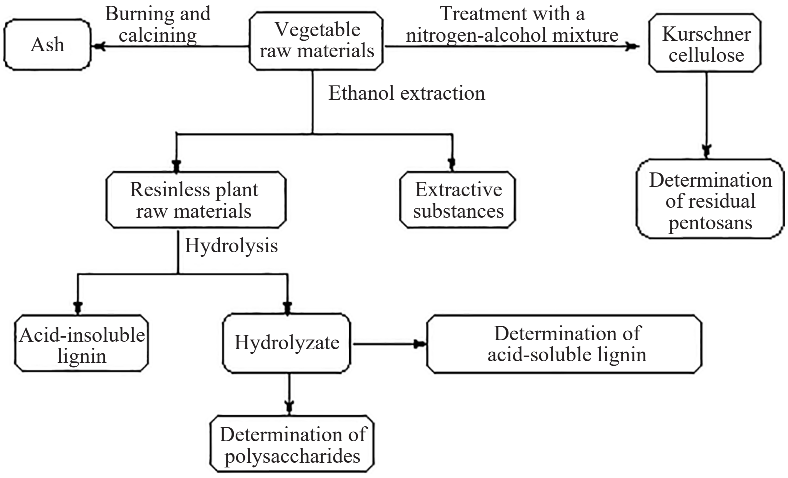

Figure 3. Scheme for quantitative assessing the main components of plant materials

The total content of polysaccharides was determined by the phenol-sulfuric acid method (see Experimental). Non-cellulosic polysaccharides can be determined by the difference between the total polysaccharide content and the cellulose content. As a method for determining cellulose, we used the nitrogen-alcohol method,[16] which is simple and does not require preliminary extraction of the sample, since ethanol is the best solvent for extractive substances among individual solvents. Cellulose (Kürschner cellulose) isolated by this method contains a small amount of non-cellulosic compounds, mainly residual pentosans, therefore they are measured and the content of Kürschner cellulose is corrected for "pure" cellulose. The methods for the determination of pentosans are based on the reaction of hydrolysis of pentosans with a 12-13% solution of hydrochloric acid to pentoses, which are converted into furfural (distilled off with water vapor).

It should be noted that, in addition to furfural, the distillate contains hydroxymethylfurfural, which is formed from residual hexosans, and methylfurfural, which is formed from methylpentose units, for example, rhamnose. Therefore, in cases where it is necessary to determine only furfural, use is made of its ability to give a color reaction with orcin, as well as spectrophotometric and chromatographic methods. When using the bromide-bromate method for the determination of furfural, corrections must be made for these reaction by-products.[15]

In the context of this work, the bromide-bromate method is preferred, since it provides the potential to determine not only residual pentosans, but also residual hexosans. The answer to the question about the accuracy with which the bromide-bromate method makes it possible to determine the amount of residual non-cellulosic polysaccharides can be obtained by analyzing samples of raw materials with a known composition of polysaccharides (Table 1).

As the results show, the content of non-cellulose polysaccharides obtained using the determination of furfural by the bromide-bromate method for all studied samples of plant raw materials slightly differs from the known one (the relative error of determination does not exceed 4.3%). In other words, the proposed method makes it possible to relatively simply and with a sufficiently high accuracy determine non-cellulosic polysaccharides both in wood and in agricultural plants.

Table 1. Results of determination of non-cellulose polysaccharides in plant raw materials

Component	Component content, %			
	Wood		Wheat straw	Bagasse
	Populus deltoides	*Pinus radiata*		
Polysaccharides*	58.6	62.4	55.7	59.6
"Raw" cellulose	50.6	48.7	46.9	51.9
Residual pentosans	7.9	6.5	13.2	14.3
Cellulose	42.7	42.2	33.7	37.6
Non-cellulose polysaccharides	15.9	20.2	22.0	22.0
Non-cellulose polysaccharides**	16.6	20.5	21.9	23.0
Relative error in determining, %	4.2	1.5	0.5	4.3

*Data from article.[15]
** The sum of non-cellulose polysaccharides (Arabinan, Xylan, Mannan and Galactan) in reference samples[13]

Returning now to the analysis of wood spruce, pine, larch, aspen and birch, we note that the average values of the molar absorption coefficient of the hydrolysate (ε_c) play a key role in determining the total polysaccharide content. The method for calculating these coefficients is described in detail in our publication.[15] The optical characteristics of the hydrolysates of the studied wood samples are given in Table 2.

Table 2. Optical characteristics of wood hydrolysates

Characteristic	Hydrolysate				
	Softwood			Hardwood	
	Picea abies	*Pinus silvestris*	*Larix sibirica*	*Populus tremula*	*Betula verrucosa*
λ_{max}, nm	483.2	483.0	483.6	479.0	479.2
ε_c, L·mol^{-1}·cm^{-1}	8,050	8,050	6,730	8,400	9,400

Table 3. Content and composition of Kürschner cellulose

Component	Component content, %				
	Softwood			Hardwood	
	Picea abies	*Pinus silvestris*	*Larix sibirica*	*Populus tremula*	*Betula verrucosa*
"Raw" cellulose	48.5	51.7	42.1	51.1	53.3
Residual pentosans	4.5	5.2	3.0	11.7	13.1
Cellulose	44.0	46.5	39.1	39.4	40.2

Larch wood has a high content of arabinogalactan. In this regard, it became necessary to develop a quantitative method for its determination in order to correctly calculate ε_c for the hydrolysate (see Experimental). The yield and composition of the isolated cellulose are given in Table 3. The wood analysis results obtained using the new scheme are presented in Table 4.

Table 4. Results of the analysis of wood components according to the new scheme

Component	Component content, %				
	Softwood			Hardwood	
	Picea abies	*Pinus silvestris*	*Larix sibirica*	*Populus tremula*	*Betula verrucosa*
Cellulose	44.0	46.5	39.1	39.4	40.2
Non-cellulose polysaccharides	18.6	15.9	26.5	25.8	24.7
Total polysaccharides	62.6	62.4	65.6	65.2	64.9
Klason lignin	28.0	24.5	25.0	18.7	18.7
ASL*	0.6	0.4	0.4	2.8	3.7
Total lignin	28.6	24.9	25.4	21.5	22.4
Extractive substances**	2.6	6.9	7.4	4.2	2.9
Ash	0.5	0.2	0.2	0.4	0.2
Sum of components	94.3	94.4	98.6	91.3	90.4

*ASL-acid-soluble lignin; **ethanol

The relative error in determining polysaccharides was 4.1% for spruce wood, 4.8% for pine wood, 3.6% for aspen wood, 0.6% for birch wood, and 0.9% for larch wood.

It should be noted that a detailed study of the composition of wood, in addition to the main components indicated in Table 4, uronic acids and acetyl groups we also determined. The content of uronic acids in softwood was on average 3-4%, in hardwood-5-8%; acetyl groups, respectively 1-2% and 3-6%.[21] In combination with these data, the sum of the components in Table 4 for each wood species approaches 100%, which once again indicates the accuracy of the developed method for the determination of non-cellulose polysaccharides and the analysis scheme.

Analyzing the experimental data obtained using the new scheme, some regularities can be noted. The cellulose content is higher in softwood (spruce, pine) compared to hardwood (aspen, birch). The content of non-cellulosic polysaccharides, on the contrary, is higher in hardwood. For lignin, the opposite is observed. Among the studied wood species, larch occupies a special place. Being a coniferous tree, in terms of the content of cellulose and non-cellulosic polysaccharides, it is more of a deciduous tree. This is another feature of larch, along with the fact that, like deciduous trees, it drops needles every year.

A distinctive feature of the proposed scheme from the known schemes[19,22] is the stage of determining the total content of polysaccharides, and with its help the total content of non-cellulosic polysaccharides – an important criterion in assessing the suitability of plant materials for chemical processing, for example, to obtain biofuel. It is also important that all non-cellulose polysaccharides (hemicelluloses and water-soluble polysaccharides) are determined.

Currently, the total content of polysaccharides in wood is estimated by the amount of isolated holocellulose. There are three known methods for isolation of holocellulose: chlorine,[23] chlorite[24] and the method using peracetic acid.[25] All of them have drawbacks, the most important being the loss of polysaccharides during the release of holocellulose (pentosans and hexosans), as well as the loss of uronic acids, acetyl groups and methoxyl groups. In addition, the isolated holocellulose contains residual lignin.[15]

Hemicelluloses are determined either in hydrolysates of plant raw materials with the determination of monosaccharides using chromatography, or by dissolving hemicelluloses in base solutions with their subsequent precipitation from previously neutralized solutions with ethanol and other solvents.[22] Chromatographically, individual monosaccharides can be quantified, but this approach does not allow determining the content of individual hemicelluloses, which are mainly heteropolysaccharides (for example, arabinoglucuronoxylan, galactoglucomannan, etc.), since the hydrolysate contains monosaccharides formed from various polysaccharides, including cellulose.

According to the second method for determining hemicelluloses, in fact, their fractionation occurs, since in this case bases with different concentrations or solutions of different bases are used. This method cannot determine with high accuracy the total content of hemicelluloses, since in the process of isolation and subsequent purification, part of the sample is inevitably lost. Therefore, the alkaline extraction method is usually used for the isolation of hemicellulose preparations, and not for their quantitative measurement.

4. Conclusion

The current level of biorefining of wood and other renewable plant raw materials has made it possible to determine new directions for the valorization of carbohydrates, such as the production of biofuels (bioethanol),[26] carbon fibers,[6] hydrogels,[27] 3D printing components,[28] and gamma-valerolactone.[29,30] The developed scheme for the analysis of polysaccharides can be useful in choosing the most suitable raw materials when developing technology for new types of alternative energy sources and precursors.

Conflict of interest

The author declares no conflict of interest.

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