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Rapid semi-quantitative determination of aspen lignin in lignocellulosic products

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Abstract. In the present study different methods for the determination of aspen lignin were investigated. In experiments, dried samples of extractive-free aspen wood and grinded bleached-chemo-thermo-mechanical-pulp (BCTMP) of aspen were used. For isolating lignin from aspen wood and BCTMP, Klason method was used. For the quantification of lignin content, a series of aspen wood powder/microcrystalline cellulose binder were mixed and analysed with FTIR-ATR and UV-VIS. To compare with colour reactions, an average content 21% of lignin was assigned to experimental content in 100% aspen wood powder. FTIR-ATR absorbance maximum between 1234–1237 cm^{-1} and UV-VIS pseudo-absorbance of measured samples maximum at 280 nm were taken as measurement points for the calibration of lignin content. Nearly linear dependence was established with both methods. Weisner and Mäule colour tests were used for staining to detect lignin in samples. Suspensions, containing samples with staining solution, were prepared and photographed. Best positive Weisner reaction with violet colour, both with aspen wood and BCTMP samples, were established with the 1 : 1 phloroglucinol/HCl staining solution. Carefully mixed suspension of 0.1 g samples and 5 min reaction time were applied. In Mäule reaction, intensive red colour appeared to samples after 10 min treatment with 1% $KMnO_4$. Samples were washed with distilled water and treated with 3% aqueous HCl until the colour changed from black to beige/yellow. Then samples were treated with concentrated $NH_3 \cdot H_2O$ for 2 min to achieve the most intensive colour. A simple semiquantitative method for detection of lignin in BCTMP was worked out.

Key words: quantitative lignin determination, colour reactions, Weisner reaction, Mäule reaction, staining reaction, BCTMP, lignocellulosic product.

1. INTRODUCTION*

Lignin is an organic substance, binding the plant cells with a complex structure with distinctive variations among wood species. Depending on the pulp manufacturing method, there is certain share of lignin left in differently treated pulps. Quality of the paper depends on the content of residual lignin, therefore it is important to detect it in different stages of pulp making. The main objective of this work was to find an effective, simple but quantitative method for estimating the presence of lignin in aspen bleached-chemi-thermo-mechanical pulp (BCTMP).

Lignin is the second main component in wood, after polysaccharides. Normal hardwood contains between 20 and 30 wt%, whereas normal softwood contains from 26 to 32 wt% of lignin. Due to different types of linkages, the structure of lignin is rather complex and the composition of functional groups of lignin shows variations among the wood species. Therefore there is no generally applicable method for the determination of lignin in lignocellulosics [1,2].

The determination of lignin in lignocellulosic material is important for characterizing and evaluating the effects of different treatments (chemical, mechanical, and biological) on wood and pulp. In addition, determination of lignin is needed for observing effluents in wood processing industries and for estimating chemical requirements of bleaching [3].

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A number of spectral methods for determining lignin content are based on totally dissolving the sample in a suitable solvent and measuring the spectral absorbance of the solution. These methods are dependent upon the specificity with which lignin is hydrolysed, as well as the yield of the solubilized lignin. The experimental difficulty in studying the macromolecular properties of lignin is due to the fact that lignin has a very low solubility in most solvents. Furthermore, lignin behaves quite differently in solutions in comparison with cellulose [4].

There are several standard methods, but not a generally applicable method for the determination of the total amount of lignin in wood and pulp samples. Probably the most used is the Klason method, in which acid-insoluble lignin is determined gravimetrically. Indirect methods include mainly instrumental, such as spectroscopic, methods, but also chemical methods using staining reactions for lignin detection. Staining is typically used for wood and pulp samples. Spectroscopic methods are generally considered the best basis for identification of lignin [5].

Klason method is probably the simplest and most reliable, therefore it is the most widely used one for lignin determination. The standard Klason lignin protocol has been well covered in [3]. Method is based on digestion of samples with 72% sulphuric acid, then with dilute sulphuric acid following to hydrolyse and solubilize the polysaccharides. The insoluble residue is dried and weighed as lignin [3,6].

The major disadvantages of the procedure are as follows: other components, including proteins and suberins, may condense and be analysed as Klason lignin. On the other hand, some lignins, notably those highly enriched for syringylpropane units, is partially solubilized. The lignin remaining in the solution, after the sulphuric acid has been diluted, may represent as much as 3–5% of the total lignin (in angiosperms). Given the variability in syringyl lignin content across various species of angiosperms, a single version of the Klason procedure cannot be generalized for all angiosperms [7].

Certain stains and colour reactions, like Weisner and Mäule reaction, have emerged as the most widely used diagnostic tests for lignin. The coniferyl and sinapyl aldehyde groups of lignin appear to react with phloroglucinol-HCl (the Weisner reaction) to give a red-violet colour. These reactive groups are present only in small quantities in lignin, but the test is quite sensitive and because of the ease of staining, this procedure is still often used as one of the tests for identification the presence of lignin in plant cell wall. The test should be used carefully, because coloration may be weak or even absent in lignin containing high amounts of syringyl units [3,8,9].

In Mäule test, lignin reacts differently in hardwoods and softwoods, therefore lignin can be differentiated using this reaction. In this test, sequential wood treatments with potassium permanganate and hydrochloric acid convert guaiacyl and syringyl residues to catechol and methoxycatechol moieties, respectively. Subsequent treatment with concentrated ammonium hydroxide gives a red colour if derived from a hardwood species, but only a dirty brown colour if derived from a softwood species. The red colour is due to the higher content of units derived from sinapyl alcohol in hardwoods [9,10].

The preceding methods are convenient for studying lignin at the macroscopic or optical microscopy levels. One method is to take a photo of the lignin-containing suspension. This method is invaluable if the aim is to determine the nature of living plant cells, but can be also used for the detection of lignin in dead cells of wood and pulps. Recently, Christiernin and Ohlsson used this method to show presence of lignin in the hybrid aspen cell cultures cultivated up to 21 days [11]. Discretion is needed with these reactions, because almost all the components of wood will produce coloured products upon application of selected reagents, therefore sometimes it is necessary to remove one or more of wood constituents (polysaccharides and extractives) in order to accurately detect lignin or to use multiple tests. Another important thing to be taken into consideration is that the chemical nature of a particular constituent within the cell wall may be quite different from its nature after removal from the wall. Also different treatments can affect lignin colour reaction and negative reaction does not necessarily mean that lignin is absent. Therefore it is advisable to use several methods on the same material in order to be reasonably sure of detecting lignin [6,10].

Since lignin is the predominant UV-absorbing material in wood, UV spectrometry is an appealing and simple method for lignin quantification and has been widely used. However, the type of lignin, the solvent for lignin, and the pH of the solution and lignin structure, may have a considerable influence on the spectra [12,13].

In the qualitative and quantitative UV spectroscopic determination of lignin the typical maximum at a wavelength of 280 nm indicates the presence of benzene ring in the lignin structure. Because lignin molecule contains no large portion of unsaturated aliphatic units in addition to its aromatic structure, it is considered that there are two characteristic bands in the lignin spectrum at 200–230 and 260–280 nm, useful for analysis [12].

Since lignin absorbs also light relatively strongly in the IR region (compared with cellulose or hemicelluloses), infrared spectroscopy has become a preferred technique for rapid in situ analysis, widely used for both characterization and qualification of lignin. FTIR spectroscopy is valuable for analysing the chemical structure of lignin: lignin type (the phydroxyphenyl, guaiacy and syringyl units), methoxyl groups,

carbonyl groups, and the ratio of phenolic hydroxyl to aliphatic hydroxyl groups [14,15]. FTIR spectra can be obtained directly (with no sample preparation) on solid samples such as wood, pulp, and paper by attenuated total reflectance (ATR), diffuse reflectance (DRIFT), and photoacoustic (PAS) techniques.

The presence and content of lignin in wood is determined by chemical or instrumental methods or by a mixture of these methods, which can be divided into direct and indirect methods. A chemical method such as direct Klason method is the most common for lignin determination. In contrast to the direct determination of lignin content, indirect methods do not involve the isolation of the lignin residue. For the rapid and routine detection of the presence of lignin there are techniques that may be applied to lignocellulosic materials in situ. For example, staining reactions like Weisner and Mäule tests are widely used for the identification of lignin in woody tissues.

The aim of the present study was to find an effective and simple method for estimating the presence of aspen lignin and for quantifying it. Main focus was on the staining reaction and finding the best methods for the identification of lignin in aspen BCTMP samples. Klason method was used for isolating lignin from samples. Spectroscopic methods were used for quantification and for calibration of lignin for colouring reaction.

2. MATERIALS AND METHODS

Dried samples of extractive-free aspen wood and grinded and oven-dried BCTMP pulp of aspen were used in experiments. Aspen BCTMP was kindly provided by Estonian Cell Company. Cellulose binder of 100% cellulose powder with a particle size of less than 20 μm, chemical formula $(C_6H_{10}O_5)_n$, was used for making quantification standards of lignin. Benzene, purity higher than 99% and 96.0% sulphuric acid, were used for lignin isolation from wood and pulp and were supplied from Sigma Aldrich.

A solution of phloroglucinol in strong hydrochloric acid is known as Weisner reagent. Phloroglucinol (126.11 g/mol), purity 99.0% was supplied from Sigma Aldrich and used for staining reactions.

The 37% hydrochloric acid, ACS reagent, and ammonium hydroxide solution ACS reagent, 28.0–30.0% NH_3 basis, were supplied from Sigma Aldrich and used for staining reactions. Potassium permanganate and ethanol were of analytical grade. Distilled water was readily used from the laboratory's own distilled water system.

For the quantification of lignin, a series of aspen wood powder/microcrystalline cellulose binder were mixed and analysed with FTIR-ATR. For UV-VIS experiment, samples were pressed into pellets. To compare with colour reactions, 21% [7] of lignin was assigned to experimental content in 100% aspen wood powder.

Weisner and Mäule tests were used for staining reaction to detect lignin in samples. Suspension containing samples with staining solution were prepared, photographed, and analysed. Reaction time and ratio between the mass of the sample and the volume of the solvent used for staining, which give the best colour intensity, were determined. For Weisner reaction, the difference between the ratio of phloroglucinol/HCl and mixed and separately added phloroglucinol and reagent HCl was investigated.

Spectroscopic measurements were performed with JASKO UV/VIV/NIR spectrophotometer V-670 and FTIR Alpha spectrometer using Platinum ATR, a single reflection diamond ATR accessory from Bruker.

Before the determination of lignin in samples, acid-insoluble lignin was isolated from the extractive-free aspen wood and from the BCTMP pulp. The isolation procedure was performed according to TAPPI Standard T222 [16]. Acid-insoluble part was weighed by analytical scale and analysed with FTIR spectrometer.

The preparation of extractive-free wood for the lignin isolation procedure was performed according to TAPPI Standard T264. Method involves three extraction steps; at first with ethanol-benzene solution, then with 95% ethanol, and finally with distilled water. Before the extraction, fresh sawdust was made of aspen wood with a handsaw. Fresh sawdust of about 30 g (80 mesh) was extracted with 400 mL of ethanol-benzene solution (1 : 2 by volume) in a flask reactor under a reflux condenser for 6 h, keeping the liquid stably boiling. After extraction with ethanol-benzene, the excess solvent was removed with suction on a Büchner funnel and the samples were washed with ethanol to remove benzene. After that, sawdust was returned to the extraction flask and extracted with 95% ethanol for 4 h. Then the sample was filtered and washed with distilled water to remove ethanol. Finally, the sample was transferred to a 1000 mL Erlenmeyer flask and 500 mL of boiling distilled water was added. The flask was heated for 1 h in a boiling water bath. After extraction, the sawdust sample was washed with 500 mL of boiling distilled water and filtered on a Büchner funnel. Then the sample was allowed to air-dry thoroughly at room temperature [17].

For lignin determination, FTIR-ATR spectra were recorded over the wavelength range from 400 to 4000 cm^{-1}. All samples were dried to stable weight before the analysis to avoid absorbance caused by water molecules. The sample was measured directly with the ATR technique, and no further sample preparation was needed. The spectra of aspen wood and BCTMP were recorded, analysed, and compared. For calibration of lignin for staining reactions, microcrystalline cellulose binder and aspen extractive-free powder were used.

Cellulose with different amount of aspen (0, 25, 50, 75, 100 wt% of aspen) were mixed together and analysed with FTIR. A calibration was made, as described in results, assigning a lignin content of 21% to the pure aspen. The UV-VIS spectroscopic experiments for solid samples were performed by using the diffuse reflectance spectra from samples pressed into pellets. The results were recorded at room temperature on a JASKO UV/VIV/NIR spectrophotometer V-670 equipped with an integrating sphere. The reflectance spectra were recorded against $BaSO_4$ as a white (R∞) optical standard. The study was carried out over the wavelength range from 250 to 600 nm. The reflectance spectra of woody samples were converted into pseudo-absorption k/s spectra using the Kubelka–Munk equation [18]

$$f(R) = \frac{(1-R)^2}{2R} = \frac{k}{s}, \quad (1)$$

where R is the absolute reflectance of the sampled layer, k is the molar absorption coefficient, and s is the scattering coefficient.

3. RESULTS AND DISCUSSION

First, FT-IR spectra of aspen wood and BCTMP were taken and compared (Fig. 1). For better readability, mean spectra were offset along the absorbance axis and presented on the same scale. The horizontal grey line indicates the zero value for aspen wood spectrum.

The spectrum of aspen wood and BCTMP were broadly similar and showed main peaks which are for wood spectra–strong broad OH stretching (3500–3100 cm^{-1}), C–H stretching in methyl and methylene groups (2800–3000 cm^{-1}), and a strong broad superposition with sharp and discrete absorptions in the region 1000 cm^{-1} [19,20].

Comparison of the aspen wood and BCTMP spectra revealed main differences at a few regions. The band of 1735–1740 cm^{-1} showed the difference in carbonyl group contents. There were more aliphatic aldehyde and ester groups present in aspen wood than in BCTMP. Also absorption intensity in aspen wood spectrum was stronger at 1234–1237 cm^{-1} range, which, according to literature [19], is due to asymmetric stretching vibrations of the C–O–C linkages in ethers and esters or to phenolic hydroxyls, which are associated with lignin.

Secondly, by using Klason method it was determined that aspen wood sample contained 15% lignin and BCTMP 10% lignin. According to findings by Dean [7], there is approximately 21% of lignin in aspen wood, as aspen BCTMP contains 17% lignin. Undesirably, some of the lignin, isolated from BCTMP, could not be filtered out using the above described method. Some part of the total lignin (3–5%) remained in the solution of diluted sulphuric acid [7,21].

Thirdly, calibration of lignin for staining reactions was performed by using FTIR and UV-Vis spectrometer. An average content 21% of lignin was assigned to experimental content of 100% aspen wood.

FTIR absorbance of measured samples showed maxima at 1234–1237 cm^{-1} (Fig. 2) and this was taken as measurement point for the calibration of the lignin content. For better readability, mean spectra were offset along the absorbance axis and presented at the same scale. The horizontal grey lines indicate the zero value for each spectrum (Fig. 2). Nearly linear dependence was established (Fig. 3).

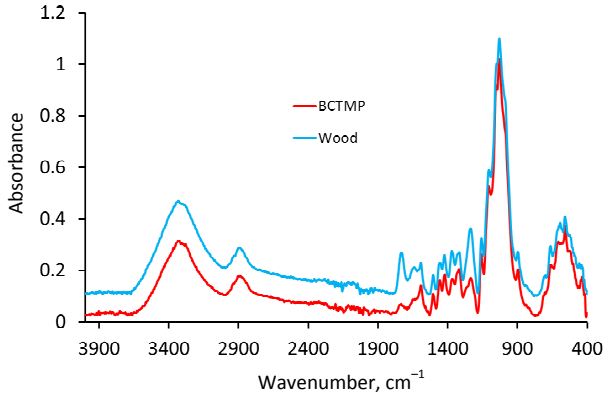

Fig. 1. The IR spectra of aspen wood (blue) and BCTMP (red).

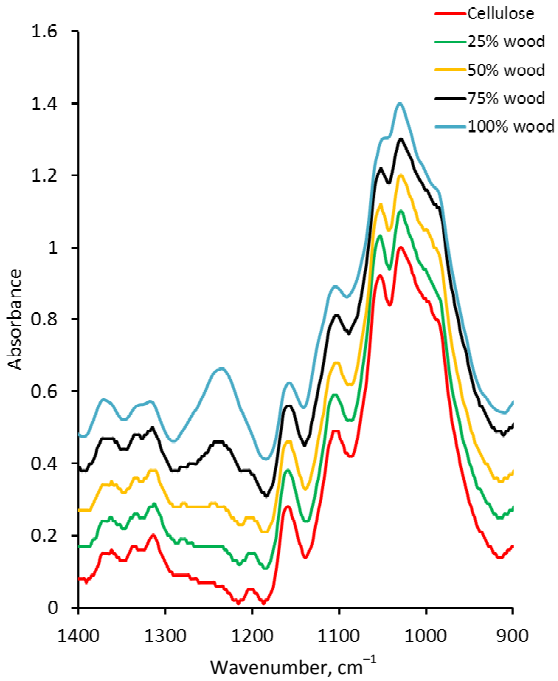

Fig. 2. FTIR spectra of: 100% cellulose binder (red), 25% wood + 75% cellulose binder (green), 50% wood + 50% cellulose binder (yellow), 75% wood + 25% cellulose binder (black), 100% wood (blue).

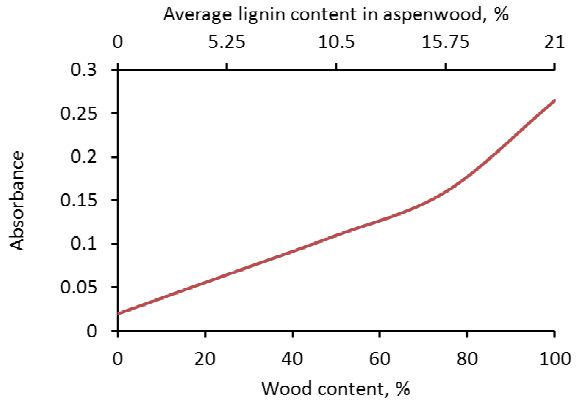

Fig. 3. Calibration curve for the detection of lignin content at maxima between 1234–1237 nm for the comparative experiments of lignin colouring.

UV-VIS pseudo-absorbance of measured samples showed distinct maximum at 280 nm (Fig. 4) and this was taken as measurement point for the calibration of lignin content. Nearly linear dependence was established (Fig. 5).

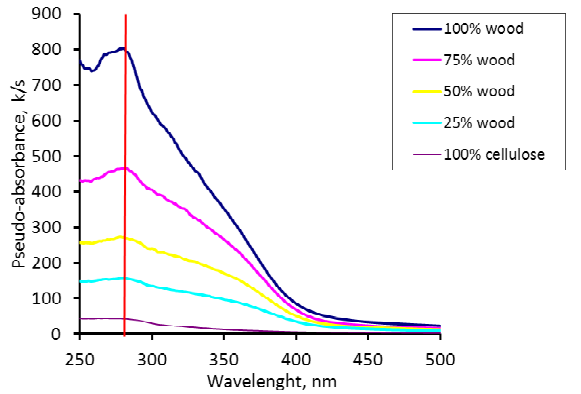

Fig. 4. Pseudo-absorbance spectra of aspen wood powder/ microcrystalline cellulose binder mixtures. Distinct absorbance maximum at 280 nm was detected.

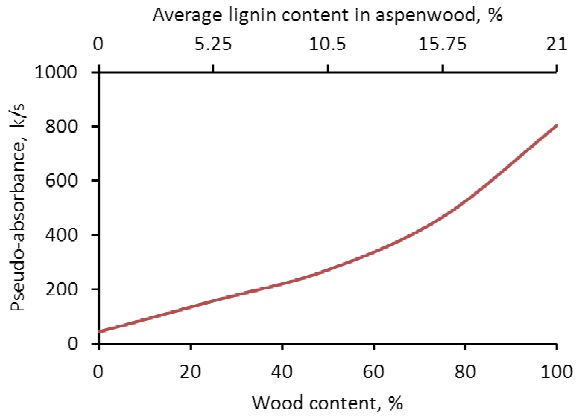

Fig. 5. Calibration curve for the detection of lignin content at 280 nm for the comparative experiments of lignin colouring.

3.1. Weisner reaction

For Weisner reaction, first, the difference between mixed and separately added phloroglucinol and reagent HCl (with 2 : 1 ratio) to 0.05 g of aspen wood (a) and BCTMP (b) samples were investigated. It was noticed, that after 3 min of reaction time, practically no visual difference occurred between the samples in which phloroglucinol and HCl were mixed together before adding it or added separately to the samples. Therefore staining solution components could be applied to samples separately which made the procedure quicker.

The next aim was to find best reaction time and ratio between phloroglucinol/HCl. Literature [7] suggests to add two parts of phloroglucinol and one part of HCl to samples. Gray [10] suggested to add more reagent if colour starts to fade. Our previous experiments showed that adding more HCl to samples will increase the intensity of the colour in the suspension, therefore three different phloroglucinol/HCl ratios: 2 : 1, 1 : 1, and 1 : 2 were investigated. Our tests showed that both aspen wood and BCTMP samples established the fastest intensive colour with phloroglucinol/HCl ratio 1 : 1. The ratio 2 : 1 that was suggested in literature, turned out to be the slowest and did not establish the same intensity as 1 : 1 even in 5 min. Colour intensity for the ratios 1 : 1 and 1 : 2 was stabilized after 2 min reaction for aspen wood and after 5 min for BCTMP. After 20 min, colour started to move from the fibres into the solution. Therefore it is advisable to add more reagent if the suspensions are investigated.

Dependence between the mass of the sample and staining solution volume was also investigated. It turned out that by increasing the mass of the sample, the colour became more intense. Best results, both for aspen wood and BCTMP, were obtained with 0.1 g of sample in 1 mL staining solution. Also it was noticed, that careful mixing of the sample during the staining reaction resulted in an even distribution of the colour in suspension.

Visual test suspension series for Weisner reaction made with mixtures of extractive-free aspen wood powder/microcrystalline cellulose binder (0, 25, 50, 75, 100 wt% of aspen) can be seen in Fig. 6. Since aspen wood powder and microcrystalline cellulose binder had different particle sizes, calibration series were not as clearly distinguishable as expected.

3.2. Mäule reaction

Compared to Weisner reaction, Mäule colouring test is more complicated and involves staining with three different solutions: $KMnO_4$, HCl, and finally $NH_3 \cdot H_2O$. Between and after treatments, washing with distilled water is required.

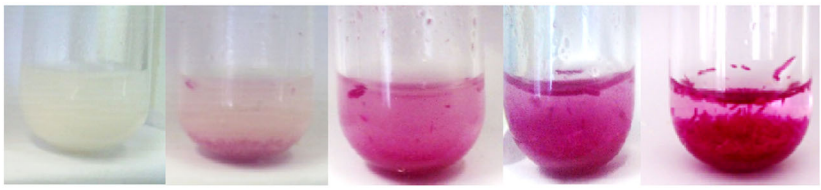

Fig. 6. Weisner reaction. Sequential photos show suspension of aspen wood powder/microcrystalline cellulose binder (0, 25, 50, 75, 100 wt% of aspen) samples stained with phloroglucinol/HCl.

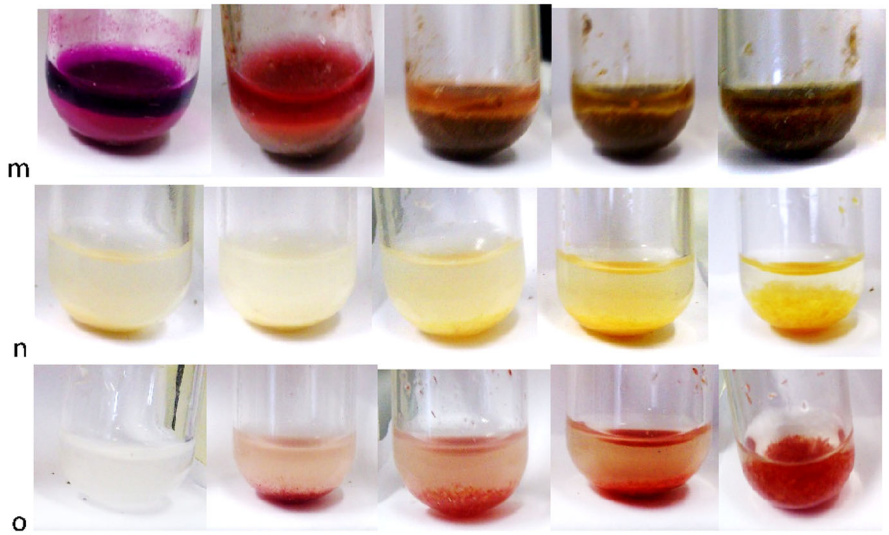

Fig. 7. Mäule reaction. Photos show suspension of aspen wood powder/microcrystalline cellulose binder (0, 25, 50, 75, 100 wt% of aspen) samples treated with 1% $KMnO_4$ solution (m) 3% HCl solution (n) $NH_3 \cdot H_2O$ solution (o).

In the first step of Mäule reaction, samples of aspen wood and BCTMP turned brown/black in 10 min when 1% $KMnO_4$ solution was added. Treatment with 3% HCl turned aspen wood and BCTMP colour to beige/yellow in 2 min. An intensive red colour came out in 1 min at the treatment of both, aspen wood and BCTMP samples, with 1 mL and 2 mL of $NH_3 \cdot H_2O$ solution. It started to diffuse out from the tissue into solution in 10 min. There was only a slight difference between colour intensities for aspen wood and BCTMP samples. But remarkable difference between staining solutions turned out with treatment volumes 1 and 2 mL – samples stained with 2 mL $NH_3 \cdot H_2O$ had more intensive colour in suspension than samples coloured with 1 mL solution.

Visual test suspension series for Mäule reaction was prepared with mixing extractive-free aspen wood powder and microcrystalline cellulose binder at different ratios (0, 25, 50, 75, 100 wt% of aspen). Mäule reaction that involved three steps of treatment with solutions of 1% $KMnO_4$ (m), 3% HCl (n), and NH_3H_2O (o) resulted in different colouration of samples (Fig. 7).

4. CONCLUSIONS

Lignin is an organic substance binding the cells with complex structure with variations among the wood species and appears differently in differently treated pulps. There is no generally applicable method for the determination of lignin in these media.

In this work an attempt was made to find effective, simple, but at least semi-quantitative method for estimating the presence of lignin in aspen BCTMP. To achieve it, different methods for the determination of aspen lignin were applied.

Particular focus was put on staining reactions. For that Weisner and Mäule tests were used to visually detect lignin in wood product samples. Until now, colouring reactions have been used for the detection of lignin mainly in wood matrix and less in other wood products. Even though these methods have been used for an extensive period of time, it has not been calibrated to this day. In this work quantification of the staining reactions was carried out using FTIR and UV-VIS spectroscopy.

For isolating lignin from aspen wood and BCTMP, Klason method was used. It was found that aspen wood sample contained 15% lignin and BCTMP 10% lignin. According to the literature, there should be approximately 21% of lignin in aspen wood and aspen BCTMP should contain about 17% lignin. The reason of this discrepancy is due to the dissolution of some lignin (3–5%) in sulphuric acid solution after diluting it with water.

To compare starting materials, spectroscopic methods were used. Main differences between spectra of the aspen wood and BCTMP samples occurred in absorption values at 1735–1740 cm^{-1} and 1234–1237 cm^{-1} (detected with FTIR-ATR).

For the calibration of the lignin content, a series of aspen wood powder/microcrystalline cellulose binder samples were mixed and analysed with FTIR-ATR and UV-VIS. Measurements were performed at FTIR-ATR absorbance maximum between 1234–1237 cm^{-1} and at UV-VIS pseudo-absorbance maximum 280 nm. Nearly linear dependence was established with both methods.

The best positive Weisner reactions with violet colour, both with aspen wood and BCTMP samples, were established with 1 : 1 phloroglucinol/HCl staining and appeared after 5 min of reaction. It was established that as small as 5% differences in aspen lignin content can be distinguished by this method.

With Mäule reaction, both aspen wood and BCTMP samples established intensive red colour. Therefore with this method only the presence of lignin was detectable, but small differences between lignin content were not distinguished.

As Mäule reaction was more sensitive to hardwood residual lignin than Weisner reaction, future development involves determination of lignin, based on it in smaller quantities.

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Kiire poolkvantitatiivne meetod ligniini määramiseks haava puitmassis

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Käesoleva töö eesmärgiks oli leida efektiivne ja lihtne, kuid samal ajal kvantitatiivne meetod ligniini tuvastamiseks haavapuitmassis. Katseteks kasutati kuivatatud haavapuitu (ekstraktiivained eraldatud) ja jahvatatud haava puitmassi (BCTMP). Ligniini eraldamiseks proovidest kasutati Klasoni meetodit. Spektroskoopilisi meetodeid (FTIR ja UV-VIS) kasutati ligniinisisalduse määramiseks, värvireaktsioonide kalibreerimiseks ning haavapuidu ja BCTMP struktuuri võrdluseks ligniini seisukohast. Töös keskenduti peamiselt värvireaktsioonidele ja nende abil haavapuitmassis ligniini määramiseks parimate parameetrite väljaselgitamisele. Klasoni meetodiga määrati ligniinikoguseks haavapuidus 15% ja puitmassis 10%. Spektromeetriga FTIR-ATR uuriti haavapuitu ja BCTMP-d. Spektrite analüüsi tehti, toetudes kirjanduses toodud ligniini neeldumismaksimumide paiknemisele. Suurim erinevus haavapuidu ja BCTMP-proovide neeldumisspektrites paiknes vahemikes 1735–1740 ning 1234–1237 cm^{-1}, kus haavapuidu neeldumismaksimumid olid BCTMP omadest intensiivsemad. Antud maksimumid on seostatavad ligniiniga, järelikult on FTIR-i abil võimalik kaudselt võrrelda ligniini koguse erinevust haavapuidus ja puitmassis.

Värvireaktsioonide jaoks ligniinikoguse kalibreerimiseks kasutati haavapuidupulbri ja mikrokristallilise tselluloosi segusid vastavalt haavapuidu mahuprotsendi sisaldusega 0, 25, 50, 75 ning 100% ja neeldumisspektrid määrati FTIR-ATR-i ning UV-VIS-i spektromeetriga. FTIR-ATR-iga oli võimalik mõõta neeldumisspekter otse proovilt, UV-VIS-i spektromeetri jaoks pressiti proovidest tabletid ja proovide peegeldumisspektrid muudeti pseudoneeldumise k/s-spektriteks. Värvireaktsioonidega võrdlemiseks valiti kirjanduse põhjal keskmiseks haavapuidu ligniinisisalduseks 21%. Ligniinikoguse kalibreerimiseks valiti mõõtepunktideks mõõdetud proovide FTIR-ATR-i neeldumismaksimum vahemikus 1234–1237 cm^{-1} ja UV-VIS-i pseudoneeldumismaksimum 280 nm. Mõlema meetodiga saavutati peaaegu lineaarne sõltuvus.

Värvireaktsioonidest kasutati Weisneri ja Mäule teste. Selleks valmistati proovi ja värvimislahust sisaldavad suspensioonid, mida fotografeeriti ning seejärel analüüsiti. Klassikaliste meetodite edasiarendamiseks ja täiendamiseks uuriti värvireaktsiooni intensiivsuse sõltuvust proovi massi ning värvilahuse mahu suhtest, reaktsiooniajast ja Weisneri reaktsiooni puhul värvaine ning reagendi suhtest ja nende eelnevast kokkusegamisest.

Parim lilla värvusega positiivne Weisneri reaktsioon saavutati floroglutsinooli/HCl-i suhtega 1 : 1 ja korralikult segatud suspensiooniga (reaktsiooniajaga 5 minutit) nii haavapuidus kui ka BCTMP-s. See, kas floroglutsinool ja HCl olid enne proovile lisatud või mitte, ei andnud värvireaktsioonis erinevust. Selgelt oli ka näha, et proovikoguse suurendamisega kasvab ka värvi intensiivsus suspensioonis. Haavapuidu suspensiooni värvi intensiivsus oli tugevam BCTMP puhul. Selle meetodiga oli võimalik näha kuni 5% ligniinisisalduse vahet.

Intensiivne punane värvus tekkis Mäule testil nii haavapuidu kui ka BCTMP 0,1 g massiga proovides. Algul töödeldi proove 10 minutit 1% $KMnO_4$ lahusega kuni pruuni värvuse tekkimiseni, seejärel pesti destilleeritud veega. Edasi töödeldi kiude 3% HCl-i lahusega, kuni pruun värvus muutus kollakasbeežiks, ja pesti uuesti destilleeritud veega. Lõpuks töödeldi proove 2 minutit $NH_3 \cdot H_2O$ vesilahusega, mis muutis nii haavapuidu- kui ka BCTMP-proovid üsna sarnase intensiivsusega punaseks. Seega on antud meetodiga võimalik ligniini olemasolu hästi tõestada, kuigi ligniinikoguse eristamine on keeruline.

Käesoleva töö uudsus on värvireaktsioonide osaline kvantiseerimine. Kalibreeriti värvireaktsioonide ligniinisisaldus UV ja FTIR-i instrumentaalsete meetodite abil. Senini on värvireaktsioone kasutatud peamiselt ligniini visuaalseks määramiseks *in situ* puidu struktuuris valgusmikroskoobis, kuid antud töös leiti, et värvireaktsioonide abil on võimalik visuaalselt kiiresti tuvastada ka jääkligniini olemasolu puitmassis. Töö tulemusena leiti lihtne meetod, mille abil on võimalik kvantitatiivselt tuvastada jääkligniini olemasolu haavapuitmassis. Weisneri ja Mäule reaktsioonid tõestasid, et nende abil on võimalik jääkligniini poolkvantitatiivselt määrata.