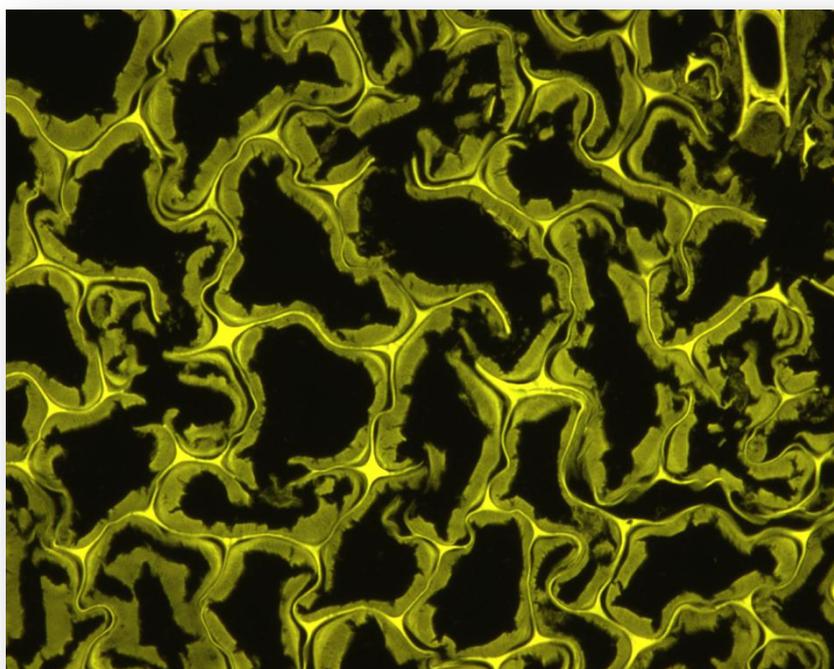




**COST Action FP0901 "Analytical tools for biorefineries"**

**Workshop on "Challenges in lignin analytics: thermal properties and quantitation"**

**BOOK OF ABSTRACTS**



August 30-31, 2012  
VTT Technical Research Centre of Finland  
in co-op. with Aalto University  
Tekniikantie 2-3, Espoo, FINLAND

Cover figure by Jenni Rahikainen (VTT). "Autofluorescence of steam pre-treated spruce after acid hydrolysis."

## COST Action FP0901 "Analytical tools for biorefineries"

### MC meeting and Workshop "Challenges in lignin analytics: thermal properties and quantitation"

#### Thursday August 30<sup>th</sup> (Hanasaari)

15.30-18.00	Opening of the workshop, practical information and reimbursements	Tarja Tamminen, Anna Sundberg
16.00-18.00	MC meeting, see separate agenda	Stefan Willför / Antje Potthast

18-19: Boat trip to Haukilahti, Espoo

19: Dinner: Haukilahti, Espoo

#### Friday, August 31<sup>st</sup> (Tekniikantie 2/3, Otaniemi)

Tekniikantie 3:

8.15-9.00	<b>Registration, coffee, poster mounting</b>	
9.00-9:15	<b>Welcome</b>	<b>Tamminen, Willför/Potthast</b>
	Chair: Bodo Saake	
9:15-9:35	Lignin analytics: need for harmonisation?	Richard J.A. Gosselink
9:35-9:55	Round Robin of lignin samples: Thermal analysis	Elisabeth Sjöholm, Anders Reimann, Fredrik Aldaeus
9:55-10:15	Comparison of the Round Robin method (ADSC) and VTT's in-house method for lignin glass transition analysis	Riku Talja, Lea Räsänen, Jarmo Ropponen, Tarja Tamminen
10:15-10:35	Thermal properties of self-binding fibreboards and sulphur-free lignins	Janis Gravitis, Ramunas Tupciauskas, Janis Abolins
<b>10:35-11:00</b>	<b>Coffee break</b>	
	Chair: Anna Jacobs	
11:00-11:20	Understanding the Variables that Define the Tg of Kraft Lignin	Dimitris S. Argyropoulos

11:20-11:40	Round Robins of lignin samples: Lignin content	Fredrik Aldaeus, Elisabeth Sjöholm
11:40-12:00	Determination of lignin content for extracts obtained by pressurised hot water	Risto Korpinen, Andrey Pranovich, Stefan Willför
<b>12:00-13:00</b>	<b>Lunch (Tekniikantie 2)</b>	
	Chair: Marco Orlandi	
13:00-13:20	Degradation reactions of lignin in pyrolysis	Thomas Elder
13:20-13:40	Round-Robin test on analytical pyrolysis-GC/MS of ligneous materials: First results	Falk Liebner
13:40-14:00	Modification of the lignin structure of eucalypt feedstocks during chemical deconstruction by kraft, soda-AQ and soda-O2 processing	Pepijn Prinsen, Ana Gutiérrez, José C. del Río, Manuel A. Berbis, Jesús-Jiménez Barbero, Angel T. Martínez, Tiina Liitiä, Taina Ohra-aho, Tarja Tamminen
14:00-14:20	The Influence of Lignin upon the Sorption Properties of Wood and Plant Fibres	Callum Hill
14:20-14:40	COST Action FP1105 "Understanding wood cell wall structure, biopolymer interaction and composition: implications for current products and new material innovation"	Philip Turner
14:40-16:00	<b>Coffee and Posters*</b>	
	Chair: Antje Potthast	
16:00-16:20	Comparative study of differently treated (cooked and bleached) industrial grass and straw fibres, respectively, by Raman and energy-dispersive X-ray spectroscopy	András Víg, István Lele, Mariann Lele, Zsolt Janowszky, János Janowszky
16:20-16:40	Extraction of phenolic and lipophilic compounds from <i>Pinus pinaster</i> knots and stemwood by pressurized solvents (STSM)	E. Conde, H. Domínguez, J. C. Parajóa, J. Hemming, S. M. Willför
16:40-17:00	Determination of carbohydrate composition in pulps and papers (STSM)	M. Becker, C. Xu, A. Sundberg, A. Potthast

### \*Poster presentations

1. Determination of lignin content in kraft black liquors using capillary zone electrophoresis,  
*Getachew Gizaw Gebremeskel, Fredrik Aldaeus*
2. New pretreatment chemical for bioethanol production of corn stalks  
*Yalçın Çöpür , Ayhan Tozluoglu*
3. Potential applications of wastes from energy and forestry industry in plant tissue culture  
*Corneliu Tanase, Smaranda Vintu , Irina Volf, Valentin I. Popa*
4. Analytical characterization of Pinus pinaster wood autohydrolysis liquors  
*María Jesús González-Muñoz, Sandra Rivas, Valentín Santos, Juan Carlos Parajó*
5. Thermal and thermo-oxidative stability of lignins  
*Z. Hromádková , A. Ebringerová, V. Sasinková , N. Vrchotová, L. Matisová-Rychlá, K. Csomorová*
6. HPLC-analysis of anthocyanins in coloured wheat hybrid  
*Sharifah Nabihah Syed Jaafar, Johanna Baron, Stefan Böhmdorfer, Heinrich Grausgruber , Thomas Rosenau, Antje Potthast*
7. Environmental friendly production of cellulose fibres from one year plants with three variables experimental design  
*Mariann Lele ,István Lele, Attila Rab , András Víg, Istvan Rusznak*
8. Microscopy as a tool in lignin research: a case study of thermally treated wheat straw  
*Ulla Holopainen-Mantila, Kaisa Marjamaa, Anna-Stiina Jääskeläinen, Kristiina Kruus, Tarja Tamminen*
9. Fractionation of brewer's spent grain and characterization of the obtained lignin-rich fractions  
*Piritta Niemi, Tarja Tamminen, Taina Ohra-aho, Stella Rovio, Craig Faulds, Marco Orlandi, Kaisa Poutanen, Johanna Buchert*
10. TG/DSC/FT-IR/MS study on biomass I. Grape seeds  
*Tugce Uysal, Manuela Tatiana Nistor, Mihai Brebu, Elena Părpăriță, Jale Yanik, Cornelia Vasile*
11. Analysis of small molecules in bark extracts of coniferous trees  
*Petr Marsik, Jan Rezek, Tomas Vanek*
12. Novel TD-GC/MS method to simulate VOC formation in the temperature range of thermoplastic processing  
*Taina Ohra-aho, Anna Kalliola, Tarja Tamminen*
13. Utilization of waste hydrolysis lignin from the Razlog area  
*Stoyko Petrin , Ivo Valchev, Sanchi Nenkova, Temenuzhka Radoykova*
14. Effect of the ultrasound and UV radiation on lignin depolymerisation  
*Raquel Prado, Xabier Erdocia, Jalel Labidi*
15. Characteristics of lignocellulosic hydrolyzed agricultural crops and isolated alkaline activated lignin  
*Temenuzhka Radoykova , Sanchi Nenkova, Ivo Valchev, Ekaterina Serafimova*
16. Lignin isolation for cellulase inhibition studies  
*Jenni Rahikainen, Harri Heikkinen, Stella Rovio, Tarja Tamminen, Kaisa Marjamaa, Kristiina Kruus*

17. Impact of steam explosion on the lignin structures in spruce and wheat straw- 2D NMR study

*Harri Heikkinen, Jenni Rahikainen, Hannu Maaheimo, Tarja Tamminen*

18. Analysis of lignans polymerized by a versatile peroxidase from *Pleurotus eryngii*

*Davinia Salvachúa, Majja-Liisa Mattinen, Tiina Liitia, Tarja Tamminen, Stefan Willför, Angel T. Martínez, Alicia Prieto, Craig B. Faulds, María Jesús Martínez*

19. Proposal of catalytic process for lignin upgrading

*Luis Serrano, Ana Toledano, Xabier Erdocia, Jalel Labidi*

20. Investigation of lignin samples by means of multifunctional Pyrolysis-GC/MS

*Windeisen E., Heigenmoser A., Richter K.*

21. Analysis of the lignin modified under cold plasma conditions

*Oana Chirila, Marian I. Totolin, Georgeta Cazacu, Cornelia Vasile*

22. Effects of the operational conditions on the composition of aqueous extracts from *Pinus pinaster* wood

*E. Conde, H. Domínguez, J. C. Parajó, J. Hemming, S. M. Willför*



**ORAL PRESENTATIONS**

## **Lignin analytics: need for harmonisation?**

Dr. Richard J.A. Gosselink  
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In order for lignin to become a renewable aromatic resource for the chemical industry, the basic chemical and physical characteristics of technical lignins need to be characterised in a reliable and reproducible manner to elucidate its structure dependent properties. Successful introduction of lignin derived products into new markets is highly dependent on these structure related properties and functionalities, because these are decisive for further conversions and application in performance products. To accurately measure the lignin properties, reliable and well established analytical protocols for lignin characterisation need to be developed. This is an ongoing and relevant subject during the last decades. At WUR extensive efforts have been performed to develop suitable analytical methods for lignin characterisation and WUR took part in several European round robins. Results of these round robins (including COST FP0901 lignin quantification) related to lignin analytics will be presented. Additionally, preliminary results on lignin analytics from the ongoing EU Biorefinery project IP Biocore (Biocommodity refinery, FP7-241566, 2010-2014, [www.biocore-europe.org](http://www.biocore-europe.org)) will be presented. These results are among others related to analyses of lignin purity, molar mass and functional groups determination. The question: 'Is there a need for harmonisation?' will be discussed in this presentation.

## **Round Robin of lignin samples**

### **Part 2. Thermal analysis**

Sjöholm, E., Reimann, A. and Aldaeus F.  
Innventia, Box 5604, SE-114 86 Stockholm

In order to increase the competitiveness of the forestry industry, efficient use of the raw material is necessary. In this context, isolated lignins have been identified as a potential new value-added product. The common bulk characterization of e.g. kraft lignin has been developed to be used for following the delignification in pulp production. For utilization of lignin as a product, more consistent analytical methods needs to be developed to evaluate its potential applicability.

Thermal properties is one of the main characteristics of lignin, and thus of increasing importance. As for other analytical methods, the actual result of well-known techniques is depending on how the specific protocol os designed. One of the thermal properties that lacks a common procedure is the determination of the glass transition temperature ( $T_g$ ) of lignin. In order to settle a common method, a Round Robin have been conducted within Cost Action FP0901. Five lignin samples, representing lignins of different origin and processing, were selected, all being considered of interest in today's research & development. The samples were: Hardwood (birch/aspens) Kraft lignin (KLHM); Softwood (pine/spruce) Kraft lignin (KLSM); Spruce Organosolv lignin (Org.solv.); Soda (wheat straw) lignin (Soda) and Enzymatic treated Steam Explosion lignin (poplar) (ESEL). About ten laboratories have participated, with the mission to perform a common suggested protocol for  $T_g$  determination preferably with differential scanning calorimetry (DSC) and an optional in-house method. At the same time determination of the decomposition temperature ( $T_d$ ) according to a distributed procedure have been performed.

The presentation will summarize and discuss the results from this Round Robin, along with fundamental sample characteristics.

## **Comparison of the Round Robin method (ADSC) and VTT's in-house method for lignin glass transition analysis**

Riku Talja<sup>1</sup>, Lea Räsänen<sup>2</sup>, Jarmo Ropponen<sup>1</sup> and Tarja Tamminen<sup>2</sup>

1) VTT, Valta-akseli, P.O. Box 21, FI-05201 Rajamäki, Finland

2) VTT, Biologinkuja 7, Espoo, P.O. Box 1000, FI-02044 VTT, Finland

Different types of lignins are formed as side products in biomaterial processing. Unfortunately, these technical lignins are typically crosslinked during processing and unreactive structures are enriched, which renders them challenging as raw materials for material applications. The heterogeneity of lignin causes variations in thermograms based on the heating programs.

In this paper, VTT's in-house and COST FP0901 ADSC methods for the analysis of the glass transition temperature ( $T_g$ ) were compared. The methods differ e.g. by heating rate and amount of heating cycles which both are known to affect thermal transitions. The analyzed lignins were spruce (*Picea abies*) organosolv lignin, Soda lignin from wheat, enzymatically treated steam explosion poplar lignin, and softwood and hardwood LignoBoost kraft lignins. The obtained results may help to verify the appropriate heating program for  $T_g$  determination of lignin.

## **Thermal properties of self-binding fibreboards and sulphur-free lignins**

Janis Gravitis, Ramunas Tupciauskas, and Janis Abolins

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Importance of sulphur-free lignin (steam exploded, soda lignin, organosolv, etc.) as a replacement for adhesives from crude fossil oil and of producing self-binding wood composites (e. g., fibreboards) is rising with the dramatic rise of oil prices increasing company production costs. The sulphur-free lignin as an added-value chemical is a crucial phenol binder substitute in self-binding steam-exploded materials for biomass fibreboards or as a component of plywood adhesives.

Recent studies with respect to the biorefinery concept and sustainable energy consumption at biomass pre-treatment by steam explosion technology are reported.

Physical parameters of sulphur-free lignin extracted from steam-exploded biomass have been tested by means of a Toledo TGA/SDTA 851 thermal gravimeter and a Toledo DSC 822 differential scanning calorimeter (DSC). The DSC allows detecting the glass transition temperatures of lignin, which is important for acting adhesive components. Results show that energy at hot-pressing the boards and plywood is saved by steam explosion pre-treatment reducing the glass transition temperature.

The sulphur-free lignin from different species including classical lignin samples has been examined. The sulphur free lignins have been examined from different species including classical lignin samples.

All lignins were studied using elemental analysis, chemical group analysis and pyrolysis/gas chromatography/ mass-spectrometry (PY-GC/MS, FRONTIER LAB PY-2020 ID, Shimadzu GCMS-QP 2010) instrument providing structural data of guaiacyl and syringyl derivatives.

The steam-exploded fibres have been analysed by scanning electron microscopy.

The obtained data will be analysed and discussed.

### **References**

1. J. Gravitis, R. Tupciauskas, J. Abolins, A. Veveris. In *Self-Binding Boards and Lignin Adhesives from Steam-Exploded Wood Biomass*, 11th European Workshop on Lignocellulose and Pulp EWLP'10. Proceedings. August 16-19, 2010, Hamburg, Germany, 2010, pp 49-52.
2. J. Gravitis, J. Abolins. *Environmental Footprint of Sustainable Use of Biomass*. In: *Recent Developments in Energy and Environmental Research*. Ed. Eva Maleviti, ATINER, 2011, ISBN: 978-960-85411-2-2, 206 pages.

## Understanding the Variables that Define the Tg of Kraft Lignin

By

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### **Abstract**

This effort will describe our work toward understanding the variables that define the Tg of Kraft Lignin. Our experimental work has defined the effect of heating on the development of the molecular weight averages in lignin as a function of heating time and heating rate. This information when supplemented with different derivatization protocols underlines the significance and the mechanism of thermal gelation processes that set in when technical lignins are subjected to heating during either a Tg measurement or potential processing regimes.

# Round Robins of lignin samples

## Part 1: Lignin content

Fredrik Aldaeus, Elisabeth Sjöholm

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A Round Robin test on the determination of lignin content in lignin samples were performed during 2010–2011 by 12 laboratories within COST Action FP0901. Five different samples were analysed, each sample using two different protocols. The samples were hardwood kraft lignin, softwood kraft lignin, organosolv lignin, soda lignin and enzymatic treated steam explosion lignin. The two protocols employed were based on a conventional acid hydrolysis method, and a simplified acid dissolution protocol, respectively.

It was shown that both protocols were easily executed, and the obtained results were comparable for all participants. The results achieved using the procedure based on standard acid hydrolysis was very reproducible, with a relative standard deviation of only 3–6%.

Furthermore, it was shown that hydrolysis may be omitted when determining the total lignin content in lignin samples. However, some caution should be taken, and the method should be verified for each sample type. Due to the higher throughput when using the procedure without hydrolysis, this method should be especially good for screening purposes.

## **DETERMINATION OF LIGNIN CONTENT FOR EXTRACTS OBTAINED BY PRESSURISED HOT WATER**

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In this study, commonly used lignin determination methods were applied for different hemicellulose-rich extracts obtained by pressurised hot water extraction (PHWE). The chosen methods are mainly used for analysing wood and pulp samples. However, this study focused on the suitability of these methods for PHW extracts. Additionally, lignin mass balances obtained by different methods in respect to dry solids content were taken into account when comparing different methods.

Industrial spruce and birch sawdust were PHW extracted using a flow-through extraction technique. The obtained hemicellulose-rich fractions were further ultrafiltered using a regenerated cellulose membrane having 10 kDa cut-off. The ultrafiltration generated two fractions; a concentrate containing high Mw hemicelluloses with increased dry solids content, and a permeate containing low Mw hemicelluloses with low dry solids content. The obtained hemicellulose-rich fractions—feed, concentrate and permeate—were freeze-dried and determination of chlorine consumption or chlorine number, the acetyl bromide method and modifications of the Klason lignin method were employed to the studied materials. Furfural and hydroxymethylfurfural (HMF) content were also determined for the filtrates containing acid-soluble lignin. Further, hemicellulose content and acetyl content of the hemicellulose-rich extracts were determined.

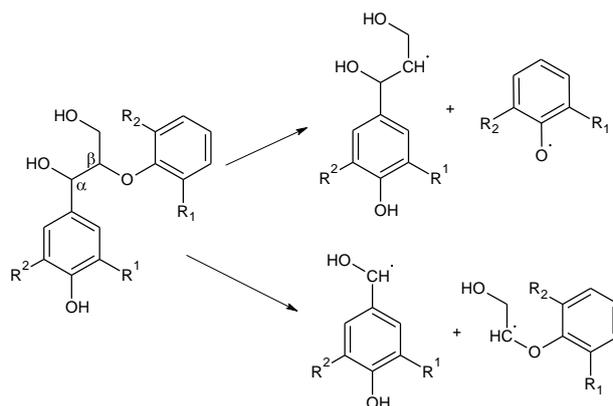
Acid-insoluble lignin contents obtained by all the studied methods were relatively constant for the feed, concentrate and permeate fractions. However, a certain Klason lignin method generated substantial amounts of acid-soluble lignin. The furfural and HMF analysis showed that the particular method generated large amounts of furfural and HMF. These compounds absorb UV-radiation and thus caused the analysis method to show significantly higher lignin content in the filtrate. The chlorine number and acetyl bromide methods gave somewhat higher lignin contents than the other Klason lignin methods. Milled wood lignin was used for calibration in the acetyl bromide method, which may explain the higher lignin content. Chlorine gas that was liberated during the chlorine number method may have been reacting also with carbohydrates and therefore giving higher lignin content.

Lignin mass balances obtained with all the different methods showed similar results, i.e. the lignin content in input was roughly the same as in output. Therefore the studied methods cannot only be evaluated based on the lignin mass balances. However, when hemicellulose content and acetyl content were taken into account, the chemical composition of the materials exceeded 100% when applying the lignin content obtained by certain lignin determination methods. We suggest that the lignin content of PHW extracts can be determined using common lignin determination methods but the complete chemical composition must be taken into consideration.

## Degradation Reactions of Lignin in Pyrolysis (Computational Applications)

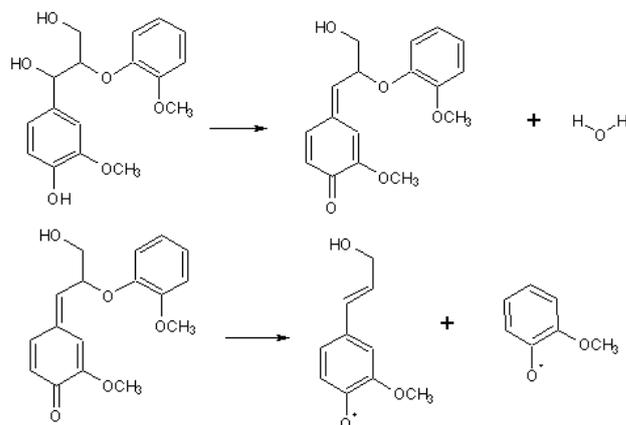
Thomas Elder  
USDA-Forest Service  
Southern Research Station  
USA

The reported reactions of lignin pyrolysis have been examined using compound computational methods to evaluate the steps associated with thermal degradation as shown below.



Results from G3MP2 and CBS-4m calculations were found to be consistent with experiment and density functional results from the literature. CBS-4m calculations on  $\beta$ -O-4 dilignols, with varying methoxyl contents reveal that bond dissociation enthalpies of competing unimolecular decompositions are quite similar, such that the reactions are relatively non-selective.

It has also been proposed in the experimental literature that free phenolic lignin models may undergo  $\beta$ -O cleavage subsequent to quinone methide formation. The bond dissociation energy of the  $\beta$ -O bond from the quinone methide is indeed lower than for homolytic cleavage, and the total energy, including quinone methide formation is slightly lower.



## Round-Robin Test on Analytical Pyrolysis-GC/MS of Ligneous Materials: First Results

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*University of Natural Resources and Life Sciences Vienna, Department of Chemistry,  
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Analytical pyrolysis-GC/MS (Py-GC/MS) is a powerful tool that has been extensively used to investigate the chemical composition of organic polymers and composites, to reveal the type of an unknown synthetic polymer, or to study chemical alterations of biopolymer-based composites, such as wood, caused by chemical pulping, enzymatic treatment, natural degradation, or thermal modification. The results of Py-GC/MS measurements largely depend on various parameters, such as sample amount and properties (particle size, thermal conductivity) or pyrolysis time.

Furthermore, the results are largely affected by the pyrolysis technique employed (e.g., filament pyrolysis, Curiepoint pyrolysis). Even for one particular technique considerable variations with regard to the chromatographic fingerprint can occur between instruments of different manufacturers because of different sample holder geometries, different ways of pyrolysis gas transfer into the inlet of the gas chromatograph, or auxiliary heating to avoid condensation of pyrolysis gases inside the transfer line being implemented in a different way.

The current paper reports the first results of a round-robin test (RR) on analytical pyrolysis-GC/MS of ligneous materials aiming at a better understanding and assessment of instrument- and setup-specific differences in the results, obtained from different groups. This activity, running within the COST FP0901 action was joined by Innventia, KTH (Sweden), Abo Academy (Finland), BOKU University Vienna (Austria), Munich University of Technology (Germany), University of Glasgow (UK), Scion Research (New Zealand), University of Ljubljana (Slovenia), and University of Grenoble (France).

The following samples were distributed amongst the RR participants for analysis:

1) Sarkanda grass soda lignin

- Measurement of the ratio of P-, G-, and S units; with and without derivatisation with tetramethylammonium hydroxide (TMAH)

2) Lignocellulosic residue from bagasse after steam explosion and partial lignin extraction

- Determination of the pyrolytic lignin and polysaccharide content
- Determination of the degree of lignin demethylation / hydroxylation by online-derivatisation with tetraethylammonium hydroxide (TEAH)

3) Pine kraft lignin

- Semi-quantitative determination of the relative amounts of pyrolysis products deriving from beta-O-4 bond cleavage; derivatisation with tetramethylammonium hydroxide (TMAH).

Standard pyrolysis conditions were 600°C, 5s of pyrolysis time, 3 repetitions per sample.

# MODIFICATION OF THE LIGNIN STRUCTURE OF EUCALYPT FEEDSTOCKS DURING CHEMICAL DECONSTRUCTION BY KRAFT, SODA-AQ AND SODA-O<sub>2</sub> PROCESSING

Pepijn Prinsen<sup>1\*</sup>, Ana Gutiérrez<sup>1</sup>, José C. del Río<sup>1</sup>, Manuel A. Berbis<sup>2</sup>, Jesús-Jiménez Barbero<sup>2</sup>, Angel T. Martínez<sup>2</sup>, Tiina Liitiä<sup>3</sup>, Taina Ohra-aho<sup>3</sup> and Tarja Tamminen<sup>3</sup>.

<sup>1</sup> Instituto de Recursos Naturales y Agrobiología de Sevilla, CSIC, PO Box 1052, E-41080 Sevilla, Spain; <sup>2</sup> Centro de Investigaciones Biológicas, CSIC, Ramiro de Maeztu 9, E-28040 Madrid, Spain;

<sup>3</sup> VTT Technical Research Centre of Finland, Biologinkuja 7, Espoo, P.O. Box 1000, FI-02044 VTT, Finland

The modification of the lignin structure in an eucalypt feedstock after chemical deconstruction by the alkaline processes kraft, soda-AQ and soda-O<sub>2</sub> was assessed. For this purpose, the residual lignins were isolated by acidolysis from the kraft, soda-AQ and Soda-O<sub>2</sub> pulps at kappa levels 50, 35, 20 and 15. The isolated residual lignins were freeze-dried and extracted with n-pentane. In addition, dissolved lignins precipitated from the black liquors of the kraft and soda-AQ processes at kappa 20 and 15 were also analysed. All isolated lignins were analyzed by an array of analytical techniques, including 2D-NMR, <sup>31</sup>P-NMR and Py-GC/MS.

2D-NMR can provide information about the fate of non-condensed and condensed structures originally present in the MWL, as well the structures formed during condensation reactions.

Py-GC/MS can provide a more exact S/G calculation. Comparison of the Py-GC/MS results of the residual lignin and the corresponding pulp can provide additional information about the representativeness of the extracted lignin (residual lignin extraction yields were between 26 and 82 %, based on the theoretical lignin content of the pulps in function of their kappa number).

During the deconstruction of the lignin polymer in pulping processes, degradation of preferentially non-condensed moieties is the main reaction, although the accumulation of condensed moieties may also result from condensation reactions. This accumulation lowers the delignification rate drastically and makes the residual lignin more difficult to eliminate during posterior bleaching processes.

<sup>31</sup>P-NMR after phosphitylating derivatisation provides quantitative information about the aliphatic, phenolic and carboxylic hydroxyl groups in lignin. In addition, the proportion of non-condensed and condensed phenolic moieties can also be deduced. Condensed phenolic guaiacyl moieties have similar substitution patterns as phenolic syringyl moieties, so in hardwoods the signal of syringyl structures needs to be separated from the signals of condensed guaiacyl structures. Deconvolution techniques may be helpful to quantify these two groups separately from the overlapping total peak area.

The residual – and black liquor lignins were also analysed by GPC to investigate the overall depolymerisation characteristics of the different pulping processes.

Keywords: Alkaline deconstruction, Kraft, Soda-AQ, Soda-O<sub>2</sub>, Eucalyptus pulp.

The research leading to these results has received funding from the European Community's Seventh Framework Programme FP7/2007-2013 under grant agreement no KBBE-2009-3-244362 LignoDeco (EU/Brazil co-operation).

## **The Influence of Lignin upon the Sorption Properties of Wood and Plant Fibres**

Callum Hill, Forest Products Research Institute, Edinburgh Napier University

The sorption behaviour of wood and natural fibres is dependent upon the morphology and chemical composition of the substrate. Extensive studies of a wide range of natural materials have shown that hysteresis in the water vapour sorption isotherms is related to the lignin content. Plant fibres with a high lignin content exhibit greater levels of hysteresis between the adsorption and desorption branches of the sorption isotherm. There are well known models in the polymer science literature which explain the existence of hysteresis by consideration of sorption processes occurring with a polymeric material that is below its glass transition temperature ( $T_g$ ). Recent work has also shown that the sorption kinetics is also related to the composition and morphology of the sample and that Fickian models are inappropriate to explain the kinetics processes. Instead, a parallel exponential kinetics (PEK) model gives exceptionally good fits to the data. The PEK model has been interpreted in terms of relaxation-limited kinetics. Polymer relaxation processes within the cell wall control the rate at which sorbate can enter or exit the cell wall. Lignin is a glassy polymer that is plasticised by the presence of sorbed water and it is therefore logical to suppose that the presence or absence of lignin should control both the sorption kinetics and the extent of hysteresis. This hypothesis will be explored in the presentation.

## **COST Action FP1105 “Understanding wood cell wall structure, biopolymer interaction and composition: implications for current products and new material innovation”**

Philip Turner, Director of the Forest Products Research Institute, Edinburgh Napier University

The presentation offers an update on the New COST Action FP0901 “Understanding wood cell wall structure, biopolymer interaction and composition: implications for current products and new material innovation”

The goal of the Action is to integrate a number of multidisciplinary research activities.

Working group 1 of the Action starts with the latest findings on the structure of the wood cell wall, which reveal a common link with a broad range of biological (organic) and inorganic materials. The Action starts with the hypothesis, that “biological structures” are driven by fundamental physical processes, rather than “complex biological systems”, which can be modeled from first principles using quantum mechanics. A second hypothesis to be tested is that the role of genes is to “switch” between different “physical processes” rather than driving the underlying structures we observe in biological systems (A principle first proposed by Erwin Schrodinger in 1943). Working group 1 focuses on collaboration between physics, biochemistry, genetics, analytical chemistry and microscopy to understand better the interrelationships between these fields of study. The aim is to determine what drives not only structure of the cell wall but also biopolymer composition and interactions between these biopolymers within the cell wall.

Working group 2 focuses on two areas of research. The first involves using new knowledge of cell wall structure and composition combined with Finite Element modeling (FEM) to try and better understand the physical properties of the cell. The second involves using this combined knowledge to support ongoing improvement of existing processes such as pulping, recycling and papermaking. The working group will also investigate new chemical, enzymatic and mechanical processes that can be used to completely break down the wood cell wall into individual biopolymer components that can be used as biorefinery feedstock.

Working group 3 looks at developing self-assembly processes that could lead to the development of a platform of new biopolymer based materials and products. This will take three approaches:

- Reviewing and sharing state of the art self-assembly processes. This knowledge will be used as a starting point for the development of new self-assembly processes.
- Functionalisation of biopolymers which can be used as building blocks in the development of new materials
- Using new knowledge of the underlying physics driving self assembly processes found in biological and inorganic materials as a theoretical platform (which could simplify our understanding of complexed biological systems) for the development of new self assembly processes (possibly based on biological structures) that could lead to the development of new materials.

COST Action FP1105 was officially started in May 2012 with the first workshop to be held in Stockholm on December 2<sup>nd</sup> and 3<sup>rd</sup> 2012.

The success of the Action is predicated on participation of a wide range of scientific disciplines. The objective of this presentation is to inform a broader audience and to encourage wider participation in the Action.

**COMPARATIVE STUDY OF DIFFERENTLY TREATED (COOKED AND BLEACHED) INDUSTRIAL GRASS AND STRAW FIBRES, RESPECTIVELY, BY RAMAN AND ENERGY-DISPERSIVE X-RAY SPECTROSCOPY**

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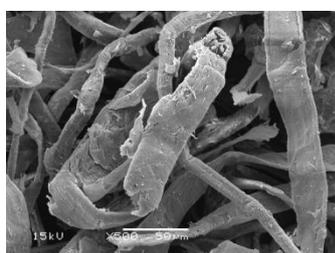
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**ABSTRACT**

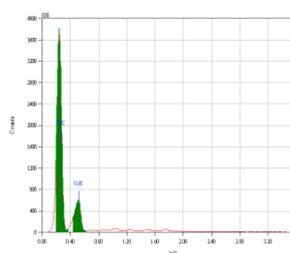
For the elemental analyses of the samples at a given surface places has been used an Energy-Dispersive X-ray Spectroscopy connected to the Scanning electron microscope. Raman spectra has been determined on a Raman micro-spectroscopy type of Jobin Yvon Labram with Olympus BX41 microscope.

**Figure 1: EDS results of industrial grass cellulose**

Element	Place of the signal keV	Amount	
		%, mass	%, atom
<b>C</b>	0.277	90.62 ± 3.2	93.25 ± 1.6
<b>O</b>	0.525	7.73 ± 1.3	5.97 ± 0.9



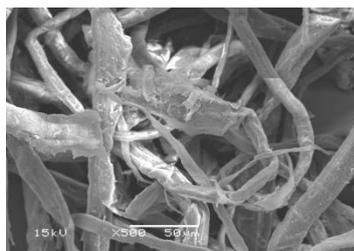
*500× enlargement*



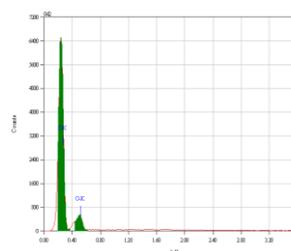
*EDS-spectrum*

**Figure 2: EDS results of bleached industrial grass cellulose**

Element	Place of the signal keV	Amount	
		%, mass	%, atom
<b>C</b>	0.277	90.38 ± 3.6	92.58 ± 2.8
<b>O</b>	0.525	9.62 ± 3.6	7.42 ± 2.8



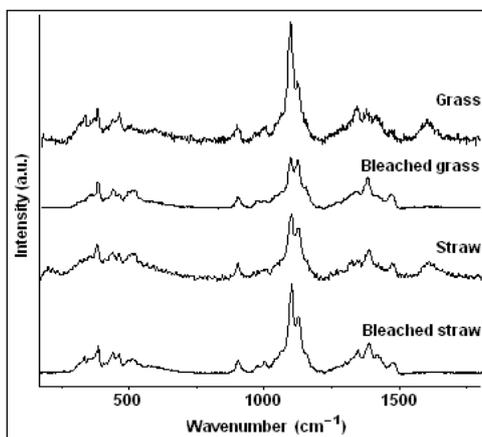
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EDS-spectrum

It could be established from the EDS elemental analyses data that the ratio of the carbon and oxygen on the surface is different from that of in the cellulose. It could be assumed that different contaminating compounds and modified cellulose molecules should be present on the surface. Spectra [Eichhorn H. (2001), Mishra S. P. (1999), Stuart B. H. (1995)] elucidated supermolecular structural information for selection of most suitable processing for pulp and paper making.

**Figure 3: Raman spectra of Industrial Grass Szarvas-1 and straw**



No essential differences could be observed between the Raman spectra of grass and straw cellulose fibres.

The amount of contaminating compounds (lignin, hemicellulose) of cellulose easily can be followed by Raman spectra.

## REFERENCES

Eichhorn H. et al.: Textile Research Journal, 2001, 71, 121-129.  
 Mishra S. P. et al.: Asian Textile Journal, 1999, 8, 64-67.  
 Stuart B. H.: Polymer Bulletin, 1995, 35, 727-733.

# EXTRACTION OF PHENOLIC AND LIPOPHILIC COMPOUNDS FROM *PINUS PINASTER* KNOTS AND STEMWOOD BY PRESSURIZED SOLVENTS

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## ABSTRACT

Knots and stemwood of several softwoods have been found to contain exceptionally large amounts of extractable polyphenols, specially lignans, stilbenes and flavonoids. Knot wood of *Pinus* species is an abundant source of stilbenes, such as pinosylvin and its derivatives. Lignans are of great interest in the search for novel agents with antioxidant and antitumor effects, and have potential for cancer chemoprevention. Stilbenes and flavonoids may also have bioactive and pharmacological effects.

An excellent method for extraction of phenolic compounds (mainly lignans and stilbenes) is the accelerated solvent extraction (ASE). Consecutive extractions with a non-polar solvent and with acetone or ethanol are recommended to separate the target compounds from the plant matrix.

This work reports on the potential of different environmentally friendly technologies for extracting bioactive compounds (such as pinosylvins and lignans) from the major pine species growing in the North West of Spain (*Pinus pinaster*). Different extraction procedures, including hot water extraction and ASE, were employed. The autohydrolysis treatment was carried out under optimized conditions. ASE was performed using hexane in the first stage (90 °C, 1500 psi, 3 x 5 min static cycles) and acetone:water (95:5, v:v, 100 °C, 1500 psi, 3 x 5 min static cycles) in the second one. The various extracts were assayed for hydrophilic and lipophilic extractives. Stilbenes, flavonoids, jubaviones, lignans, resin acids and free fatty acids were measured by GC after evaporation and silylation of non-volatile solutes, using a 25 m x 0.20 mm i.d. column coated with crosslinked methyl polysiloxane (HP-1). Heneicosanoic acid and betulinol were used as internal standards.

The different treatments gave different yields for different extractives. *Pinus pinaster* knots contain large amounts of lignans, with nortrachelogenin been the most abundant one. The lignin nortrachelogenin was recently identified in knot and branch heartwood of Scots. The large amount of lignans in *Pinus pinaster* knots offers a potential source of bioactive substances with applications in the fields of pharmacy and nutrition. Pinosylvin monomethyl ether was the most abundant stilbene in all knots and stemwood samples. Flavonoids, jubaviones, resin acids and fatty acids were also identified.

The whole set of experimental data provides quantitative information on the amounts of lipophilic and hydrophilic compounds present in extractives of *Pinus pinaster* wood, as well as on the comparative ability of the technologies considered to obtain the target compounds from wood.

## Determination of carbohydrate composition in pulps and papers - STSM

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The analysis of carbohydrate composition of pulps and papers is essential for their characterization and further processing. Hydrolysis is a necessary tool to cleave the glycosidic bond of polysaccharides. Enzymatic and acid hydrolysis are known to generate monomers units of a polysaccharide. Acid hydrolysis is a common method and involves sulfuric acid, trifluoroacetic acid and hydrochloric acid under strong or mild conditions (Bertaud et al. 2002).

Strong hydrolysis with sulfuric acid leads to a release of reducing monosaccharides /neutral sugars. Methanolysis represents a hydrolysis method under mild conditions, which converts the free monosaccharides into their corresponding methyl glycosides and the carboxyl groups of uronic acids are esterified with methyl groups (Huang et al. 1992).

The advantages of methanolysis compared to sulfuric acid hydrolysis are considerably less degradation of fragile hemicelluloses and the possibility to quantify uronic acids (Chambers and Clamp 1971). However, mild acid hydrolysis does only slightly affect highly ordered cellulose structures, therefore hydrolysis methods with stronger acids are recommended (Sundberg et al. 1996). A simple single step analysis to obtain total amounts of glucose, pentose and uronic acids is so far not available. The method applied thus mainly depends on the target of interest, either cellulose or hemicellulose.

The current study involved the analysis of 22 polysaccharides and 14 pulps and papers by acid methanolysis, followed by silylation and gas chromatography (GC) according to (Sundberg et al. 1996). The determination of cellulose content is carried out using sulfuric acid hydrolysis according to Bose et al. (2009). The amount of glucose units determined by acid methanolysis were subtracted from glucose units determined by acid hydrolysis (Alves et al. 2010; Kilic et al. 2010) in order to obtain glucose units in hemicelluloses.

The polysaccharides analyzed by methanolysis showed typical carbohydrate composition with characteristic differences between the individual carbohydrate ratios. For example, high molar ratios of arabinose were observed at all three arabinans (>60%) and in gum arabic, as expected. The glucomannan, glucogalactomannan and galactomannans showed the highest molar ratio of mannose within the polysaccharides analyzed. While the highest amounts of galacturonic acids (>50%) were observed in pectins, polygalacturonic acid and rhamnogalacturan. The polysaccharides showed a recovery of 54.96% – 102.19% of released sugar units after acid methanolysis, except for Inulin (4.29%) and Avicel (4.67%).

The primary monosaccharides identified in pulps and papers by acid hydrolysis were glucose, xylose and mannose, followed by arabinose and galactose. Recovery rates below 22.71% were observed in pulps and papers after depolymerization, (except wheat bran with 37.1%), due to the absence of the non-analyzed lignin part and the non-hydrolyzed crystalline cellulose fraction, which is not affected by the mild condition of acid methanolysis in these samples.

Datasets of sub unit patterns from depolymerized polysaccharides were compared with results from pulp and paper samples. Therefore, principal component analysis (PCA), an unsupervised clustering method, was used to compare different polysaccharides and pulps and papers samples.





**POSTER PRESENTATIONS**

# Determination of lignin content in kraft black liquors using capillary zone electrophoresis

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The simplest method to determine the lignin content in black liquor is to dilute the sample with an alkaline solution and measure the absorbance at 280 nm. However, several disadvantages are associated with this method since the absorptivity depends on the origins of the lignin, the concentration of lignin and solvent as well as on the lignin molecular mass and polydispersity. Furthermore, there may be interferences from UV-absorbing low-molecular mass compounds from the wood. A method considered more reliable than the alkali dissolution method is to precipitate the lignin using sulfuric acid, measure the precipitate gravimetrically, and measure the still dissolved lignin spectrophotometrically. This approach reduces the error arising from uncertain absorptivity, but is labor intensive and suffers from interferences due to co-precipitating compounds.

Sidestreams of the pulping mill have previously also been analyzed using capillary electrophoresis. That technique has mainly been used for on-line process monitoring of water-soluble ions, and low-molecular mass lignin degradation products have also been quantified. It is also possible to study macromolecular lignin using capillary electrophoresis, but previous studies have been limited to determinations of charge density and mobility distribution.

In this presentation, we demonstrate two methods for the determination of lignin content in kraft black liquors using capillary electrophoresis with an alkaline electrolyte. Both methods are based on simple dilution of the sample in an alkaline solvent (50 mM glycine, 25 mM NaOH), followed by injection (50 mbar, 20 s) and a run (30 minutes, 15 kV) with glycine electrolyte (100 mM glycine, 50 mM NaOH, pH 10) in a fused silica capillary (inner diameter 50  $\mu\text{m}$ , effective length 40 cm, total length 46 cm) with direct detection at 280 nm.

In the first method, external standards are employed. The black liquors used in this method were from kraft pulping of mixed hardwood (Sweden) and mixed softwood (Sweden). One lignin sample was precipitated from each of the black liquors by means of the LignoBoost process, and used as external standards (calibration curves  $R^2 > 99,6\%$ ). The results were repeatable (relative standard deviation around 2%) and comparable with results obtained by the sulfuric acid method. Furthermore, the limits of detection was approximately two orders of magnitude lower, and depending on how the integration limits are set, it is possible to exclude lignin degradation products and other interferences from the determination.

Multivariate calibration is utilized in the second method. The lignin content in 17 black liquors was determined using the sulfuric acid method. Using two principal components without any data pretreatment, it was possible to accurately predict the lignin content in most black liquors with a deviation of less than 15%.

# A NEW PRETREATMENT CHEMICAL FOR BIOETHANOL PRODUCTION OF CORN STALKS

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## ABSTRACT

Corn stalk having high carbohydrates content in structure is an abundant agricultural waste in Turkey and this waste could be utilized to produce bioethanol. Due to high cellulose crystallinity and complex lignin and hemicelluloses distribution, waste stalks were first steam exploded and then chemically pretreated to achieve efficient hydrolysis. The effects of conventional chemicals of sodium hydroxide (NaOH), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and an alternative chemical of sodium borohydrate (NaBH<sub>4</sub>) were examined. Results showed that NaOH (83.9%) and NaBH<sub>4</sub> (82.9%) pretreated samples resulted in higher glucan conversion in enzymatic hydrolysis compared to H<sub>2</sub>O<sub>2</sub> (74.5%) and H<sub>2</sub>SO<sub>4</sub> (56.6%). A significant correlation between lignin removal and enzymatic digestibility was observed. The highest ethanol yield of 97.4g/kg (based on untreated stalk) was found when the stalks were pretreated with 4% NaBH<sub>4</sub> (90 min) and the theoretical ethanol yield was found to be 72.5%.

**Keywords:** Ethanol production; enzyme; corn stalk; hydrolysis; pretreatment

## INTRODUCTION

There are studies on bioethanol production using corn stover (Kadam and McMillan, 2003) and corn kernel (Tang et al., 2011) but limited studies have been done on bioethanol production from corn stalks (Fei and Hongzhang, 2009). Corn stalks may be a promising fermentation waste for Turkey and increasing the ethanol yield after fermentation by effective pretreatment methods may make the corn stalks an economically valid biomass for bioethanol production. NaBH<sub>4</sub> was utilized for the first time ever in the chemical pretreatment step and results were compared with the conventional pretreatment chemicals NaOH, H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> (Treatments were made at 0.5, 2 and 4% (w/v) concentrations. Solid loading applied was 10% (w/v). Duplicate samples were treated at 121°C (15 psi) for residence times of 30, 60 and 90 min). After chemical pretreatments, enzymatic hydrolysis was also examined for factors of temperature, concentration and time. Enzymatically hydrolyzed stalks were fermented to produce ethanol.

## RESULTS AND DISCUSSION

In this study, corn stalk was steam exploded and then pretreated with some chemicals to improve the enzymatic digestibility and the ethanol fermentability. Results showed that steam explosion dissolved and removed some material from the stalk structure and the total solid recovered after steam explosion was of 85.3% (w/w). Glucan (44.0±0.34%), xylan (24.2±1.77%), arabinan (2.40±0.66%), acid insoluble (33.3±0.09%) and soluble lignin (1.33±0.07%), ash (4.73±0.16%), alcohol-benzene solubility (20.1±2.10%) and holocellulose (51.0±0.12%) was determined in steam exploded stalk. The solubilization of glucan in NaOH pretreated stalks was between 18.5% (0.5% NaOH, 30 min) to 42.5% (2% NaOH, 60 min) and the xylan solubility ranged from 33.8% (0.5% NaOH, 30 min) to 80.2% (4% NaOH, 60 min). An increase in alkali concentrations and times reduced lignin content (25.7%, 0.5% NaOH, 30 min; 83.0%, 4% NaOH, 90 min). Compared with steam exploded feedstock, 74.4% and 92.3% of the glucan were preserved after 0.5% (90 min) and 4% (60 min) acid pretreatment in corn stalks, respectively and acid concentration had a significant

effect on xylan solubility and 71.7% (4% H<sub>2</sub>SO<sub>4</sub>, 90 min) of xylan was removed from the structure. Also lignin reduction of acid pretreated stalks was ranged from 8.28% to 21.7%. The glucan dissolved to liquid in H<sub>2</sub>O<sub>2</sub> pretreatment varied from 8.30% (0.5% H<sub>2</sub>O<sub>2</sub>, 30 min) to 16.9% (0.5% H<sub>2</sub>O<sub>2</sub>, 90 min) and the solubilization of xylan was between 17.5% (2% H<sub>2</sub>O<sub>2</sub>, 60 min) and 34.4% (0.5% H<sub>2</sub>O<sub>2</sub>, 30 min). Increase in concentration and residence time with H<sub>2</sub>O<sub>2</sub> pretreatment resulted in more lignin reductions of 18.6% (0.5% H<sub>2</sub>O<sub>2</sub>, 30 min) and 39.1% (4% H<sub>2</sub>O<sub>2</sub>, 90 min) in samples. The glucan solubilization for NaBH<sub>4</sub> pretreated samples varied from 2.03% (0.5% NaBH<sub>4</sub>, 30 min) to 18.9% (2% NaBH<sub>4</sub>, 30 min) and the xylan solubilization was 32.5% (0.5% NaBH<sub>4</sub>, 30 min) and 49.4% (4% NaBH<sub>4</sub>, 90 min). A significant decrease in lignin content was observed when the samples were pretreated with NaBH<sub>4</sub>, and the maximum lignin reduction of 62.2% was observed for the 4% NaBH<sub>4</sub> (90 min) treated sample.

The selected samples for enzymatic hydrolysis regarding glucan to lignin ratio were of 4% NaOH (90 min), 4% H<sub>2</sub>SO<sub>4</sub> (60 min), 4% H<sub>2</sub>O<sub>2</sub> (90 min) and 4% NaBH<sub>4</sub> (90 min) and were enzymatically processed up to 72 h. Results showed that NaOH (83.9%) and NaBH<sub>4</sub> (82.4%) pretreated samples had almost similar glucan conversions. The differences of glucan conversions in samples might be due to the lignin content in the stalk's structure after chemical pretreatments. On the other hand, NaOH (69.9%) and NaBH<sub>4</sub> (69.2%) pretreated samples had higher xylan to xylose conversions compared to H<sub>2</sub>O<sub>2</sub> and H<sub>2</sub>SO<sub>4</sub>.

The fermentation performance of *S. cerevisiae* in treated hydrolysates was also studied. The maximum theoretical ethanol yield of 72.5% (97.4g ethanol/kg) was found for corn stalks (4% NaBH<sub>4</sub>, 90min.) in this study.

## CONCLUSIONS

Results showed that, NaBH<sub>4</sub> is an effective pretreatment chemical compared to NaOH, H<sub>2</sub>SO<sub>4</sub>, and H<sub>2</sub>O<sub>2</sub>. A significant decrease in lignin content was observed with NaBH<sub>4</sub> pretreatments, which significantly improved the enzymatic hydrolysis.

## REFERENCES

- Fei, C., Hongzhang, C. (2009). Absorption of ethanol by steam exploded corn stalk. *Biores. Technol.* 100, 1315-1318.
- Kadam, K.L. and McMillan, J.D. (2003). Availability of corn stover as a sustainable feedstock for bioethanol production. *Biores. Technol.* 88, 17-25.
- Tang, Y., Zhao, D., Cristhian, C., Jiang, J. (2011). Simultaneous saccharification and cofermentation of lignocellulosic residues from commercial furfural production and corn kernels using different nutrient media. *Biotechnol. Biofuels.* 4, 1-10.

# POTENTIAL APPLICATIONS OF WASTES FROM ENERGY AND FORESTRY INDUSTRY IN PLANT TISSUE CULTURE

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## ABSTRACT

Plant tissue culture refers to growing and multiplication of cells, tissues and organs on defined solid or liquid media under aseptic and controlled environment. Plant tissue culture technology is being widely used for large-scale plant multiplication (Ying et al. 2012). The aim of this study was to evaluate the effect of deuterium depleted water and spruce bark polyphenolic extract as callus culture bioregulator of maize plants. Two sorts of by-products were used in experimental: spruce bark and deuterium depleted water. Spruce bark is a major waste product from forestry and paper pulp industries. Spruce bark extracts contains considerable quantities of bioactive aromatic compounds, especially catechine and phenolic acids such as gallic acid and vanillic acid. The concentration of these compounds were determined based on HPLC. Chromatographic profile of spruce bark aqueous extract is: 1- gallic acid ( $3.19\pm 0.81$ ); 2- catechine ( $31\pm 1.9$ ); 3- vanillic acid ( $39.4\pm 0.2$ ) (Ignat 2009). On the other hand, deuterium depleted water or light water is a distilled water microbiologically pure, with an isotopic concentration of 25 ppm, obtained by isotopic distillation, in vacuum, of natural water with an isotopic concentration of 145 ppm D / (D + H) (Somlyai 2001). In Romania deuterium depleted water (DDW) is obtained in the heavy water plant at Halanga Caras Severin, in the Vest part of Romania, where daily discharge as waste tons. Recent research has shown that spruce bark extract and DDW have a great influence on growth and development plants (Tanase et al. 2011), namely in developing cells and tissues and changes that occur in normal water features lead to significant changes in fundamental processes of the cells (Olariu et al. 2007, Somlyai 2001).

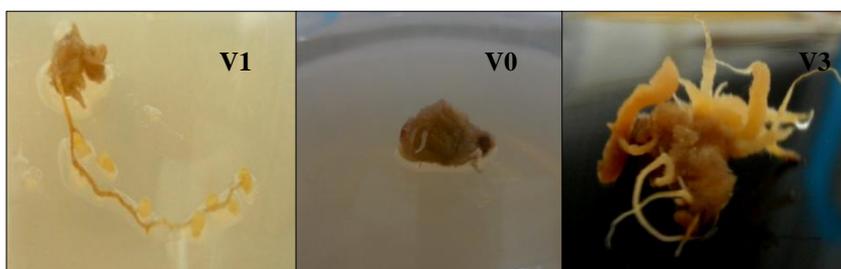
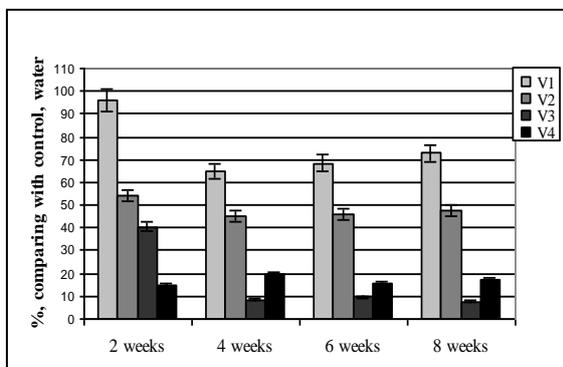


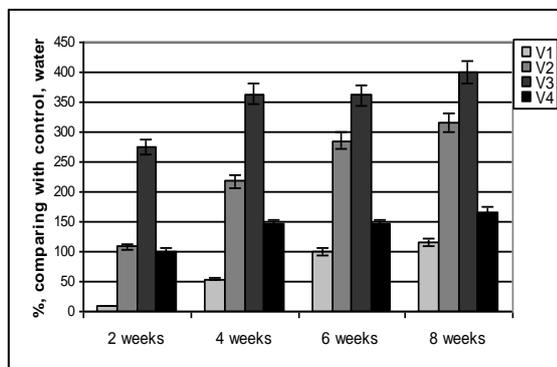
Figure 1: Aspect of callus after four weeks

In this context, the effects of DDW or in combination with spruce bark extract on callus of maize plant growth and development *in vitro* were analyzed. (Fig.1). The following specific parameters were closely monitored: fresh biomass accumulation, adventitious roots and aerial adventitious roots organogenesis and the formation of new callus. Thus, in MS culture medium (Murashige – Skoog 1962) water (V0) was replaced with deuterium depleted water (V1), deuterium depleted water in combination with spruce bark extract (V2), spruce bark extract – 25 mg GAE/100g (V3) and spruce bark extract 25 mg GAE/100g without 2, 4 D (V4). The results shown that the highest stimulating effect on biomass accumulation was registered in the presence of deuterium depleted water and in combination with spruce bark aqueous extract (Fig. 2). For adventitious roots organogenesis it was observed that the spruce bark extract (V2, V3) favors their formation, comparing with control (Fig. 3). New callus formation of adventitious roots was recorded in V1 and V2 variants. From presented data we can say that there are sufficient arguments to state that

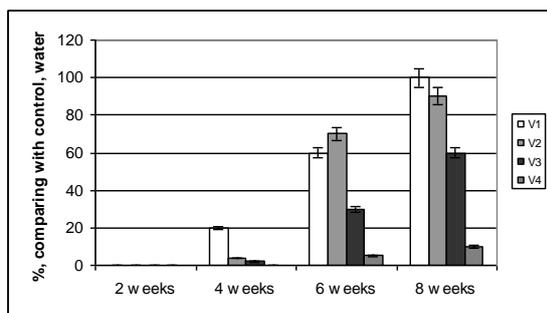
polyphenolic products and deuterium depleted water can act as allelochemicals. The results shown, that the tested solutions stimulated fresh biomass accumulation, adventitious roots organogenesis (Fig. 4) and formation of new callus (Fig. 5), comparing with Control. The most significant stimulatory effect on callus biomass accumulation was identified in the case when the deuterium depleted water and spruce bark polyphenolic extract in combination with DDW, was used for the treatments.



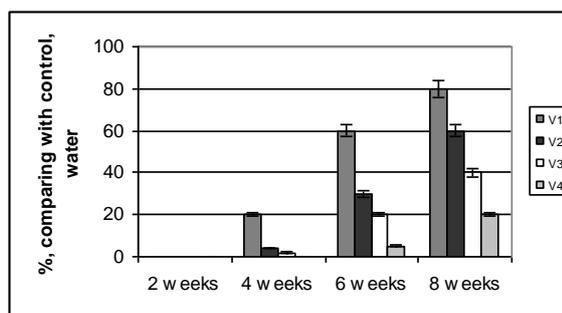
**Figure 2: Fresh biomass accumulation**



**Figure 3: Adventitious roots number**



**Figure 4: Aerial adventitious roots number**



**Figure 5: New callus formation of adventitious roots**

## REFERENCES

1. Ignat I., Stîngu, A., Volf, I., Popa, V. I., 2009. Natural bioactive compounds as plant growth regulator, *Lucrări științifice - vol. 52, seria Agronomie*, 187- 192.
2. Murashige T, Skoog FA (1962) Revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant Physiol* 154:73–479
3. Olariu, L., Petcu, M., Tulcan, C., Chis-Buiga, I., Pup, M., Florin, M., Brudiu, I. (2007) Deuterium depleted water – antioxidant or prooxidant, *Lucrari Stiintifice Medicina Veterinara*, Vol. XL, 265-269.
4. Somlyai, G., (2001) *The biological effects of Deuterium Depletion*, HYD Ltd., ISBN, 0-7596-9261-0
5. Tanase, C., Stîngu, A., Volf, I., Popa, V. I., 2011. The effect of spruce bark polyphenols extract in combination with deuterium depleted water (DDW) on *Glycine max* L. and *Helianthus annuus* L. development”, *Analele științifice ale Universității „Al. I. Cuza” din Iași, Genetică și Biologie Moleculară*, Tom XII, fasc 3, 115-120.
6. Ying, B., Guofeng, L., Xueping, S., Wen, X., Guogui, N., Juan, L., Manzhu, B., (2012) Primary and repetitive secondary somatic embryogenesis in *Rosa hybrida* ‘Samantha’ *Plant Cell Tiss Organ Cult*, 109:411–418 DOI 10.1007/s11240-011-0105-6

# ANALYTICAL CHARACTERIZATION OF *Pinus pinaster* WOOD AUTOHYDROLYSIS LIQUORS

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## ABSTRACT

In this work, autohydrolysis liquors from *Pinus pinaster* wood were treated sequentially with membranes of different molecular weight cut-off (MWCO). Combined concentration and diafiltration steps were carried out using 10, 5, 3, 1 and 0.3 kDa MWCO membranes to obtain fractions of liquors with different molecular structure and molecular weight distribution. The compositions of the different streams were determined by chromatographic (HPLC, HPSEC) and spectrometric (MALDI-TOF) methods.

In a first aqueous stage, *Pinus pinaster* wood was treated at 130 °C to remove water-soluble extractives. The resulting solids were processed in a pressurized reactor under the following conditions: liquid to solid ratio, 8 kg/kg; temperature, 175 °C; reaction time at the target temperature, 24.5 min.

The liquors were analyzed by HPLC and MALDI-TOF. HPLC-RI analyses of liquors (without further treatment and after acidic posthydrolysis) were performed with a CARBOsep CHO-682 column (Transgenomic) using distilled water as mobile phase at 0.4 mL/min flow rate and 80 °C. MALDI-TOF MS analyses were performed using an Ultraflex workstation (Bruker Daltonics, Bremen, Germany) equipped with a 337 nm nitrogen laser. Measurements were performed in the positive mode. Sample solutions (1 µL) were mixed with 1 µL of matrix, applied on the instrument target plate and dried under a stream of warm air. The matrix solution was prepared by dissolving 10 mg of 2,5-dihydroxybenzoic acid (Bruker Daltonics, Bremen, Germany) in a mixture of 700 µL of water and 300 µL of acetonitrile.

HPSEC-RI analyses were carried using two in series TSKGel G3000PWXL columns (Tosoh Bioscience). Ultrapure water was used as mobile phase at 0.6 mL/min and 40 °C.

The MALDI-TOF analysis shows a broad range of compounds. Oligomeric and low molecular weight polymeric saccharides with backbones made up of hexoses or pentoses (denoted POHS) were present in the medium. According to literature and to the composition obtained by HPLC, POHS with backbones formed by hexoses correspond to galactoglucomannan hydrolysis products, whereas POHS with backbones formed by pentoses (in some cases, with substitution by uronic acid groups) were formed by arabinoxylan. The simplest POHS detected by MALDI-TOF was made up of 3 hexoses (denoted H3), followed by an oligomer containing 4 pentoses (denoted P4). The most complex compounds detected for each type of backbone were low molecular weight polymers (19 hexoses bearing 7 acetyl groups (H19+Ac7) and 14 pentoses (P14)).

## THERMAL AND THERMO-OXIDATIVE STABILITY OF LIGNINS

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Lignins are commonly produced as by-products in pulp&paper industry, but also in various hemicellulose extraction processes, and, nowadays, in bio-ethanol industry (Sahoo et al., 2011; Stewart, 2008). Due to their aromatic structure, they are useful in various applications, however, mainly as antioxidants (Lora & Glasser, 2002; Gregorova et al., 2006). Next to the composition and structural features of lignins, there has been a need for a proper investigation of their thermal and thermo-oxidative behavior which are necessary and formidable tasks to widen the knowledge of these biopolymers. The antioxidant activity of lignin ascribed to their free radical-scavenging properties was tested mainly by colorimetric and EPR assays (Lu et al., 1998; Dizhbite et al., 2004).

This study reports on the thermal and thermo-oxidative stability of three lignin preparations isolated from woody materials. TGA measurements in combination with the chemiluminescence (CL) technique performed in presence of nitrogen and oxygen were used and which were applied in studies on various materials (Zlatkevich, 1999), synthetic polymers and polysaccharides (Sterlič et al., 2000; Rychlý et al., 2004, Matisová-Rychlá et al., 2008 and references therein).. The lignin preparations were characterized by Klason lignin content and FT-IR spectral patterns. The radical-scavenging activity of the lignin preparations was tested by the DPPH assay ((Rao and Muralikrishna, 2006). The lignins showed different behavior in both the TG and CL experiments and in the DPPH test. In contrast to the last method, both the TG and CL ones, evaluating the thermo-oxidative potential of the lignins, are not chemical- and time-consuming methods. As example, the commercial lignin as additive to cellulose was tested by these methods. The results revealed a decrease of the CL intensity of cellulose after lignin addition proceeding in a dose-dependent manner, thus onfirming the antioxidant potential of the lignin.

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Dizhbite T., Telysheva G., Jurkjane V., Viesturs U. *Bioresource Technology* 95 (2004) 309–317.

Lora J. H., Glasser W. G. *Journal of Polymers and the Environment* 10 (2002) 39–47.

Lu F.-J., Chu L.H., Gau R. J. *Nutrition and Cancer* 30 (1) (1998) 31–38.

Matisová-Rychlá L., Rychý J., Ebringerová A., Csomorová K., Malovíková A. *Polymer Degradation and Stability* 93 (2008) 1674–1680.

Sahoo S., Seydibeyoglu M.O., Mohanty A.K., Misra M. *Biomass and bioenergy* 35 (2011) 4230–4237.

Strlic M., Kolár J., Pihlar B., Rychlý J., Matisová-Rychlá L. *European Polymer Journal* 36 (2000) 2351–2358.

Rao, R. S. P., Muralikrishna, G. *Phytochemistry* 67 (2006) 91–99.

Rychlý J., Matisová-Rychlá L., Lazar M., Slovák K., Strlič M., Kočar D., Kolár J. *Carbohydrate Polymers* 58 (2004) 301–309.

Stewart, D. *Industrial Cops and Products* 27 (2008) 202–207.

Zlatkevich L. *Journal of Polymer Science Part B: Polymer Physics* 28 (1990) 425–429.

## HPLC-ANALYSIS OF ANTHOCYANINS IN COLOURED WHEAT HYBRIDS

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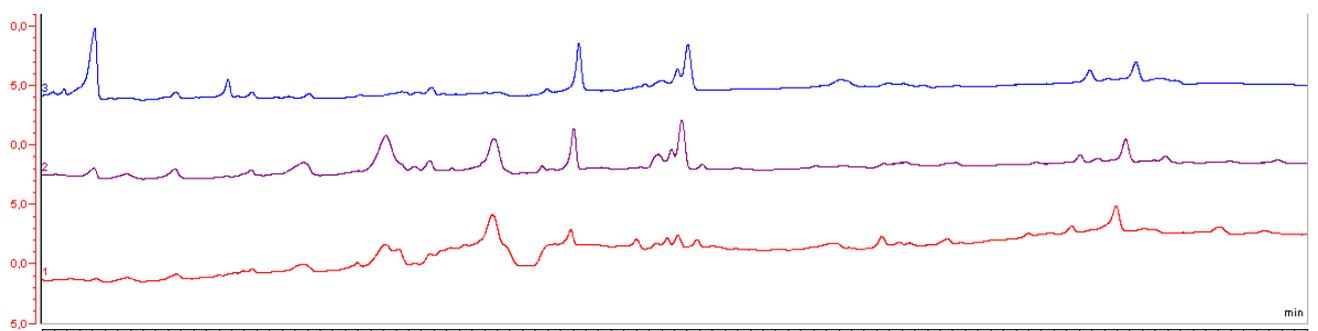
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### ABSTRACT

One strategy to increase the benefit of biorefineries is the isolation and of rare and valuable compounds. Anthocyanins are natural blue and red dyes that commonly occur in plant material, e.g. in petals or fruits. They show potential for several health improving effects (Antal et al. 2003), and more importantly, they are approved as a food colourant of natural origin (E163). Wheat is one of the most important sources for starch and offers several hybrids that have a blue or purple colour. This colour arises from anthocyanins that are located either in the aleuron (blue wheat) or the pericarp (purple wheat). We are currently pursuing a project to increase the total concentration of anthocyanins in the grain by crossbreeding blue and purple varieties. The obtained wheat variety could serve as a source for anthocyanins for both specialty food products and purified anthocyanin extracts.

After harvesting, the hybrid grain samples were visually graded according to colour into 6 groups – white, red, blue, light purple, purple and dark purple. 64 of the new crossbreeds were selected, their anthocyanins extracted and analyzed by HPLC. The extraction was conducted on a Dionex ASE 350 pressurized liquid extraction device with 3:2 methanol : 0.1% aqueous TFA as solvent. The total anthocyanin content of the extracts was determined by photometry according to (Giusti et al. 2001). The concentrations were in a range from 4 – 280 µg/g wheat flour, considerably exceeding those of selected check varieties used as references (Baron et al. 2011).

The anthocyanin extracts were analyzed on a Dionex Ultimate 3000 HPLC system connected to an Agilent 6320 IonTrap MS with an ESI-source. The following separation conditions are a modification of parameters given by the manufacturer (Dionex, 2011).



**Figure 1: Exemplary chromatograms of two reference samples and a hybrid (middle trace). The hybrid features peaks of both references.**

The obtained chromatograms revealed distinctly different patterns in pigment composition. A set of seven reference samples was selected to reclassify the hybrids following the established pattern of

blue, light purple, purple, dark purple with the addition of dark blue and two new classes. The analysis demonstrated that the visual grading of the grains did sometimes not correspond to the chemical composition of the extract. The hybrids that showed an increased total anthocyanin content in fact contained anthocyanins of both purple and blue varieties (see Fig. 1), which should correspond to an anthocyanin deposition in both the aleuron and the pericarp. Furthermore, the composition of anthocyanins in the samples clearly reflects the pattern used in the hybridization. This allows using the anthocyanins as molecular markers for changes in the genotype of coloured wheat.

## REFERENCES

- Antal, D.S, Gârban, G., Gârban, Z. (2003). The anthocyanins: biologically-active substances of food and pharmaceutical interest. *The Annals of the University Dunrea de Jos of Galati, Fascicle VI – Food Technology*, 26, 106-115.
- Baron, J., Siebenhandl-Ehn, S., Syed Jaafar, S. N. B., Böhmendorfer, S., Rosenau, T., Grausgruber, H. (2011). Increase of the total amount of anthocyanins in progenies of blue aleurone×purple pericarp wheat crosses. In: *Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs, Tagungsband 62. Irdning, Austria*. pp. 87-90.
- Dionex Corporation (2011). *Rapid and Sensitive Determination of Anthocyanins in Bilberries using UHPLC. Application Note 281, 2011, Sunnyvale, CA.*
- Giusti, M. M., Wrolstad, R. E. (2001) Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. *Current Protocols in Food Analytical Chemistry*, Unit F1.2.1-F1.2.13.

## **Environmental friendly production of cellulose fibres from one year plants with three variables experimental design**

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As forest are not in high percentage in Hungary consequently our local Paper Research Institute has been highly interested in projects of gaining cellulose from one year plants with special respect to environmentally friendly procedures.

The following one year plants have been applied in our experiments:  
wheat, barley, panic grass and hemp.

Our experiments have been performed partly for selecting the appropriate raw materials otherwise for elaborating the most suitable procedures for gaining the wanted cellulose fibres. Naturally the environmentally friendly requirements have also been rigorously followed. Sulphur free procedures have been elaborated in the experiments. An experimental design has been applied for determining the respective optimal parameters.

Experimental design with three variables has been elaborated. Based upon it minimum number of experiments could be achieved. Their evaluation was rather easy.

Although the lignin content of the so far discussed raw materials was rather small, its separation is inevitable. Strong chemical interaction is necessary for its decomposition. The wanted reaction is catalysed by anthraquinon establishing less serious conditions in the procedure.

Three variables (Temperature °C, NaOH concentration and Anthraquinone concentration) have been varied in the following ranges: 86 – 154 °C, 6,3-19,7 % and 0-0.14 % respectively.

The higher efficiency of the procedure with the anthraquinon could be concluded: the yield was higher by 5-10 % and simultaneously the Kappa numbers decreased by 8-9%.

Comparative differences of selected physical characteristics between procedures assisted by sulphate and anthraquinon respectively performed with four raw materials (wheat, barley, panic grass and hemp).

The procedure with anthraquinon was more effective than that with sulphate, generating better physical characteristics in all aspects: tensile index (7-10 %), burst index (6-10%) tear index (2-3 %).

Summarising: comparing the products of sulphur free and sulphurous procedures the following advantages on the site of sulphur free technology can be formulated:

- Kappa number of the fibres: less by 2-3 percentages
- Yield: higher by 4-5 percentages
- Strength index of fibres: higher by 8-12 percentages
- Cooking temperature: less by 15-20 °C

## **Microscopy as a tool in lignin research: a case study of thermally treated wheat straw**

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The structure of thick-walled sclerenchyma cells of natural and hydrothermally pre-treated wheat straw sample were examined using different light microscopy techniques in order to understand the differences observed in the chemical analyses and improved enzymatic digestibility. For light microscopy, the samples were fixed, embedded into methylacrylate resin and cut into 2  $\mu\text{m}$  sections. Lignin and cellulose were localised in the sections by staining with phloroglucinol and Calcofluor, respectively. Xylans were immunolabelled with commercially available, monoclonal antibody. In addition, the autofluorescence properties of the samples were examined in pH 7 and 10. With these microscopy techniques, clear differences were observed in the composition and structure of thick-walled sclerenchyma cells of wheat straw. Less lignin was stained in thermally treated samples compared to the control, and the staining was also reduced by the severity of the treatment. More cellulose, in turn, was detected in the cell walls of more treated samples indicating the loss of other cell wall components. The autofluorescence imaging and immunolabelling revealed the reorganisation of cell wall structures: brightly autofluorescing substance that was assumed as lignin, was detected inside the cell cavities especially in the optimally treated sample and xylans were reorientated in the cell wall. The structural and compositional differences observed by microscopy were in line with the chemical data. In addition, the usability of conventional lignin stains has to be carefully examined, because the stainability of lignin seems to be affected by the processing conditions.

The work carried out in research projects funded by Academy of Finland and European Commission FP7 (Targeted DISCOvery of novel cellulases and hemicellulases and their reaction mechanisms for hydrolysis of lignocellulosic biomass". Liisa Änäkäinen and Ritva Heinonen are thanked for technical assistance.

## **Fractionation of brewer's spent grain and characterization of the obtained lignin-rich fractions**

Piritta Niemi, Tarja Tamminen, Taina Ohra-aho, Stella Rovio, Craig Faulds, Marco Orlandi, Kaisa Poutanen, Johanna Buchert

Brewer's spent grain (BSG) is an abundant, lignocellulosic by-product generated in the brewing process. It contains the hulls and outer layers of the barley grains, and is rich in carbohydrates, lignin and protein. BSG was fractionated with enzymatic and chemical means to produce lignin-rich fractions. The obtained fractions were characterized with pyrolysis-GC/MS and <sup>31</sup>P NMR. BSG was a challenging material to fractionate due to the presence of protein and lipids, and also the carbohydrates were partially resistant to enzymes and seemed to be cross-linked with lignin. BSG lignin was found to be composed mainly of G units but also small amounts of S and H were detected. The low amount of phenolic hydroxyl groups suggests that BSG lignin is bound to carbohydrates.

# TG/DSC/FT-IR/MS STUDY ON BIOMASS

## I.GRAPE SEEDS

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### Introduction

The utilization of grape residue as a renewable energy resource is of great importance in responding to concerns over the protection of the environment and the security of energy supply. For example, in 2005, over 67 million tons of wine grapes were harvested all over the world, of which more than 20% typically becomes waste during wine production.

Grape seeds contain several antioxidants, including oligomeric proanthocyanidin complexes, which show some health benefits. However, cold-pressed grape seed oil itself does not contain significant amounts of these antioxidants since proanthocyanidins are insoluble in lipids. They are present in much higher concentrations in other parts of the grape, such as grape seed extract, grape skins, and in particular red wine.

Grape seed extracts are industrial derivatives from whole grape seeds that have a great concentration of vitamin E, flavonoids, linoleic acid. Typically, the commercial opportunity of extracting grape seed constituents has been for chemicals known as polyphenols, including oligomeric proanthocyanidins that are recognized as antioxidants.

Grape seed oil (also called grapeseed oil or grape oil) is a vegetable oil pressed from the seeds of various varieties of *Vitis vinifera grapes*, an abundant by-product of winemaking.

Grape residues consist mainly of the redundant skins, stalks and seeds that remain once the juice has been extracted. It must be treated effectively to avoid a number of environmental hazards, ranging from surface and groundwater pollution to foul odours. The grape residue contains a large amount of organic constituents (i.e., cellulose, hemicellulose and lignin) and has a high-energy content. Therefore, its conversion to renewable energy would be attractive since it would solve pollution problems, reduce greenhouse gases emissions and provide a clean, low sulphur fuel. (Xu R.,2009)

### Experimental

Grape seeds were supplied by Bozacada, Turkey as the dried residue from the juice extraction process of a wine industry. They contain 8.1 wt% moisture and leave 2.9 wt% ash. Samples before and after extraction with various solvents have been studied.

### Investigation methods

Thermal degradation was followed by simultaneous thermal analysis TG/DSC – FTIR – MS. The thermal study was performed on a STA 449 F1 Jupiter apparatus (Netzsch-Germany). The heating program started from 30 °C to 600 °C with 10 °C/min heating rate and under nitrogen as purge and protective gas for a flow rate of 70 ml/min. The temperature reproducibility of TGA recordings was  $\pm 2$  °C and  $\pm 3\%$  for mass loss. The thermobalance was coupled with a Vertex 70 IR spectrophotometer and Aeolos QMS 403C mass spectrometer (Netzsch-Germany) for *in situ* recording of the spectral characteristics of the degradation gaseous products. For each recording, 7-8 mg of sample was placed in Al<sub>2</sub>O<sub>3</sub> crucibles. Volatile degradation products in a controlled temperature environment were directly transferred both into a electron impact ion source of mass spectrometer type QMS 403C Aëolos (Netzsch) and in infrared (FT- IR) spectrometer via a heated capillary tubing and analyzed by *in-situ* vapor phase FTIR. The transfer of the degradation gaseous products was realized through two isothermal lines.

The transfer to QMS spectrometer was made of a quartz capillary heated at 290 °C and with a 75 µm diameter. The QMS spectrometer works under 10<sup>-6</sup> mbar vacuum and electron impact

ionization energy of 70 eV. The data was acquired using Aeolos 7.0 software, in the range of  $m/z$  1÷300, at a measuring time of ca. 0.5 s for one channel, resulting in a time/cycle of approximately 100 s.

The kinetic parameters were processed on a temperature range selection from 200 to 450 °C with Kinetic3 software from Netzsch-Germany. For the kinetic analysis Friedman equation was used.

## Results and Discussion

Comparative examination of TG/DTG curves, 3D and 2D FT-IR spectra and MS spectra reveal the decomposition pathway of organic mass of grape seeds and differences between thermal characteristics and the composition of the decomposition products at various stages of heating and also between unextracted and extracted biomass – Figures 1 -3.

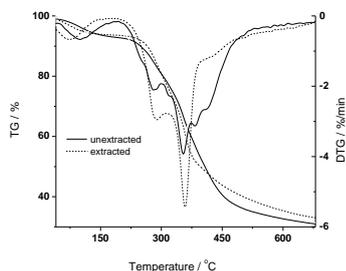


Fig. 1. TG/DTG curves of unextracted and extracted grape seeds

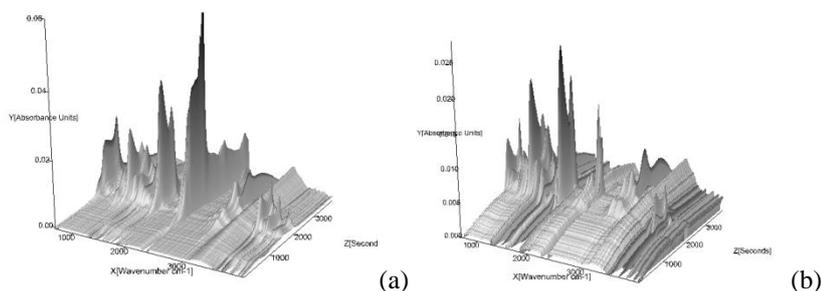


Fig. 2. 3D Spectra of unextracted (a) and extracted grape seeds (b)

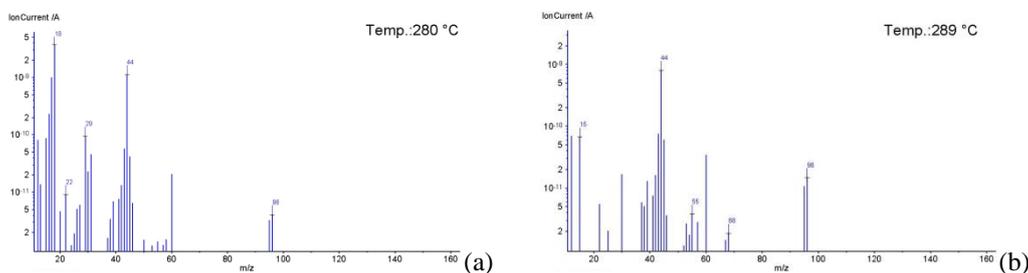


Fig. 3 MS of unextracted (a) and extracted grape seeds (b)

Both compounds from cellulose and lignin have been identified and their evolution during heating was followed.

## References

Xu R., Ferrante L., Briens C., Berruti F., 2009, Flash pyrolysis of grape residues into biofuel in a bubbling fluid bed, *J. Anal. Appl. Pyrolysis* 86, 58–65

## Analysis of small molecules in bark extracts of coniferous trees

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Pine (*Pinus sylvestris*) and European larch (*Larix decidua*) belong to coniferous species with the highest importance for agroforestry and wood processing industry in Czech Republic. Both of these species are investigated as a source of important metabolites potentially exploitable in pharmaceutical, cosmetic and food processing industry [1]. There are many studies concerning chemical composition of small molecule fraction of both species, which are previously focused on quantification and profiling of terpenic compounds [2].

Bark together with foliage is the biggest portion of waste of wood processing. In spite of the fact, there are plenty of compounds with high potential for industry [3]. In our study we compared content of simply extractable small molecules in bark extracts of larch and pine trees growing in Czech Republic. Preliminary profiling of the extractable compounds was measured on GCxGC/TOF (Pegasus 4D, Leco) apparatus after derivatization with MSTFA. The identification of trimethylsilyl derivatives was carried out by comparison of commercial spectra library (NIST) and retention indexes. Quantification of polar constituents (phenolic acids, maltol) contained in purified water and methanol extracts was performed using HPLC with PDA detection and LC/MS/MS (Q-Trap 4000) system. Number of measured compounds as well as their concentration was higher in extracts of larch bark. The most abundant compound in the larch extracts was maltol (between 1.2mg/g and 1.7mg/g FW), which is used as flavour enhancer (E number E636), followed by catechin (about 1 mg/g FW). Both of them were identified in larch bark only. Concentration of free phenolic acids in larch was considerably lower and varied between 9µg/g (caffeic acid) and 90µg/g (ferulic acid) FW, respectively. In the pine bark, the highest concentration was found for vanillic acid (26µg/g FW). The amount of all measured compounds did not differ significantly in water and methanol extracts.

Acknowledgment: This grant was supported by Ministry of Education, Youth and Sports CR, project No. OC10026.

<sup>1</sup> Killic A et al. (2011) Eur J Wood Prod, 69:37-40

<sup>2</sup> Orav A et al. (1996) Chromatographia, 43:215-219

<sup>3</sup> Ushanova VM et al. (1998) Chem Nat Compd, 34:104-105

## **Novel TD-GC/MS method to simulate VOC formation in the temperature range of thermoplastic processing**

*Taina Ohra-aho, Anna Kalliola and Tarja Tamminen*

The potential of lignin to replace oil-based raw materials is being actively investigated for various material applications, such as composites. However, there are several difficulties in applying lignin. One of them is the volatile organic compounds (VOCs), either present in the isolated lignin, or formed as they are processed at high temperature in thermoplastic processes. VOCs in kraft lignin are typically lignin originated phenolic structures, *e.g.* guaiacol or reduced sulfur compounds formed in cooking. The problem related to VOC's in kraft lignin is their relatively low odor threshold value. In order to overcome the odor problem softwood kraft lignin was treated by different methods to reduce VOCs.

Thermal desorption (TD) method can be used to determine volatile compounds released from the high molecular weight materials. In the method material is heated at constant temperature for selected time and the released volatiles are determined by gas chromatography/mass spectrometry. In our study TD-GC/MS method was used to simulate and quantify the VOC formation in the temperature range of thermoplastic processing. TD experiments were carried out in a pyrolysis unit. Temperatures 150 and 190 °C were used for the VOC measurement. Lower temperature was selected because it is close to the softening temperature of lignin, whereas the higher temperature was more close to the thermoplastic processing temperature. Heating time of 5 minutes was selected for the treatment time, because the longer heating time did not significantly increase the VOC formation [1].

The result showed that higher amount of volatiles were released at 190 °C than at 150 °C. Guaiacol was the main phenolic volatile released from the untreated and treated lignins at both temperatures. Other phenolic volatiles detected mainly at 190°C were vanillin, homovanillin and dihydroconiferylalcohol. In addition to phenolic products also substantial amount of sulphur compounds was detected. Methanethiol was detected at both temperatures whereas dimethyldisulfide and dimethyltrisulfide were detected only at 190 °C. Quantification of guaiacol was done in order to make comparison between odor threshold value and released guaiacol. Even if the amount of released guaiacol was decreased in different treatments of lignin, it was still rather high in comparison to the odor threshold value. In addition to TD-GC/MS measurement, odor intensity of selected materials was evaluated using an odor test. There was no big difference between the odor values evaluated for the untreated and treated lignin samples. However, the obtained odor values were decreased simultaneously with decreasing VOCs released from the samples.

[1]. Anna Kalliola, Anne Savolainen, Taina Ohra-aho, Greta Faccio, Tarja Tamminen, Reducing the content of VOCs of softwood kraft lignins for material applications, *Bioresources*, in press.

## UTILIZATION OF WASTE HYDROLISYS LIGNIN FROM THE RAZLOG AREA

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In the area of Razlog close to the famous Bulgarian ski resort Bansko is located 140 acres landfill for hydrolysis lignin waste. The generated lignin from the hydrolytic plant which is not operated at present is evaluated to about 350 000 - 400 000 tones. The technology process was on the basis of high temperature diluted sulphuric acid hydrolysis of sawdust to sugars which were further subjected to yeast fodder production. The energy potential of this waste hydrolysis lignin is a substantial one, but it has not yet been used.

The aim of resent study is chemical and thermal analysis of waste hydrolysis lignin from area of Razlog in respect to its utilization for energy purposes.

It is found different moisture and solid content in central and surface zones of the stockpile, while the sulphur content is about 0.7 %. It is established some decreasing of cellulose content in the landfill. The hydrolysis lignin is subjected to FTIR spectroscopy and the calorific value is determinate. It is obtained briquettes and pellets from the lignin waste.

The results from this investigation will be used for proposal of technologies for waste hydrolysis lignin utilization, mainly for district heating in ski resort Bansko.

***Keywords:*** *waste hydrolysis lignin , utilization, calorific value; district heating.*

### ***Acknowledgements***

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## **Effect of the ultrasound and UV radiation on lignin depolymerization**

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Lignin is the second most abundant natural polymer, it is obtained by renewable sources and its structure is composed by the combination of three different phenylpropane derived units (guaiacyl alcohol, para-coumaryl alcohol and syringyl alcohol). These phenylpropane units form an amorphous three-dimensional structure and are linked mainly by an aryl-aryl ether linkage. This unique and complex structure makes lignin a suitable candidate to be catalytically transformed into low molecular weight phenolic compounds.

Lignin can be extracted from lignocellulosic biomass by several processes. Organosolv process which employs a mixture of ethanol/water as solvent is already used in the pulp and paper industry as the Alcell® process, that allows obtaining black liquor very rich in lignin. Production of high-added value phenolic compounds from black liquor involves the transformation of lignin into much smaller molecules. Organosolv lignins have been found to be an appropriate raw material for producing LMW compounds like vanillin, simple and hydroxylated aromatics, quinines, aldehydes, aliphatic acids and many others. A number of thermochemical conversion methods have been proposed to depolymerize lignin to low-molecular weight compounds. Among them, base-catalyzed depolymerization, pyrolysis (lignin to liquid, LtL), and Lewis acid-catalyzed solvolysis have received a considerable amount of attention. Lignin is a good candidate for photocatalytic cracking due to the presence of hydroxyl groups. The photocatalytic cracking reaction involves  $\text{TiO}_2$  as heterogeneous catalyst.

In the present work lignin is treated under UV radiation and ultrasound to be depolymerized. Different exposure time to UV radiation and to ultrasound were carried out. The results of GS-MS revealed the degradation products of the hemicelluloses contained in the lignin samples. One hour of exposure to UV radiation shows the best yield in the obtaining of lignin degradation derived compounds; the main products obtained are syringaldehyde, pirocatechol and raspberryketone, the concentration of syringaldehyde increases 20% and pirocatechol 30%. The obtained products were characterized by uv-vis spectroscopy, GPC, ATR-IR and GS-MS.

# CHARACTERISTICS OF LIGNOCELLULOSIC HYDROLYZED AGRICULTURAL CROPS AND ISOLATED ALKALINE ACTIVATED LIGNIN

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The main goal of the present industry is the most effective utilization of the resources and materials. Chemical and biotechnological processing of wood waste, agricultural waste of plant origin and secondary fibrous materials is one of the main aspects of their comprehensive utilisation as valuable natural raw materials.

The investigations on bioethanol production from agriculture lignocellulosic raw materials (wheat straw and maize stalks) were carried out in our previous study. The dilute acid hydrolysis are used as pre-treatment methods, followed by enzyme hydrolysis with cellulase enzyme complex combined with  $\beta$ -glucosidase. The solid lignocellulosic hydrolyzed mass is in fact a waste product.

A comparative study of lignocellulosic hydrolyzed agricultural crops and isolated alkaline activated lignin after alkaline treatment of these materials was carried out. The process conditions of the treatment were- 4 hours duration of treatment, 5% NaOH, lignin: aqueous solution NaOH ratio 1:8. The temperature of the process was 180°C.

The samples were subjected to DSC analysis and FTIR spectroscopy. The determination of the specific surface area of the samples was carried out by nitrogen adsorption at 77.4 K. Nitrogen adsorption isotherms were used to calculate the specific surface area (ABET) using the BET equation. On the base of BET analysis (determination of specific surface area) are assessed the opportunities for using these waste materials and isolated alkaline activated lignin as sorbent.

**Keywords:** *lignocellulosic hydrolyzed agricultural crops, alkaline treatment, specific surface area;*

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## LIGNIN ISOLATION FOR CELLULASE INHIBITION STUDIES

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### ABSTRACT

Enzymatic hydrolysis of lignocellulosic feedstocks is restricted due to the presence of lignin in the raw material. Lignin shields the cell wall polysaccharides from enzymatic attack but enzyme adsorption on lignin-rich surfaces is also known to inhibit the hydrolysis. The extent of lignin-derived inhibition is known to depend on the origin of lignin: softwood-type lignin is considered more detrimental for hydrolytic enzymes compared to lignin from annual plants. Furthermore, little is known how biomass pretreatment affects lignin structure and its inhibitory properties. In this work, lignin was isolated from spruce and wheat straw before and after steam explosion pretreatment using the EMAL protocol involving ball-milling, enzymatic hydrolysis of structural carbohydrates and subsequent mild acidolysis/extraction of lignin. The paper will discuss isolation, characterisation and use of the different lignins in enzyme inhibition and adsorption studies.

# IMPACT OF STEAM EXPLOSION ON LIGNIN STRUCTURE- 2D NMR STUDY OF WHEAT STRAW

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Steam explosion (SE) of lignocellulosic materials is considered to be one of the promising pretreatment methods to enhance the reactivity of lignocellulosic fibers in enzymatic hydrolysis for producing fermentable sugars. [1] During SE, high-pressure steam followed by a rapid decompression in the absence of any additional chemicals results in changes mainly of the ether bonds found in lignocellulosic materials (polysaccharides, lignin). [2] The impact of steam explosion on the structure of lignin, especially in changes between different inter-unit linkages have been only addressed in the literature. [3,4]

In this study, highly sophisticated 2D NMR methods combined with state of the art instrumentation were used to elucidate further chemical structures of the lignin in wheat straw after steam explosion treatment. Enzymatic mild acidolysis lignin (EMAL) was isolated from the ball milled materials for the analysis [5]. According to our results, the content of  $\beta$ -O-4-structures decreased after SE. Our results are in good agreement with the literature data, where the phenomenon has been explained by depolymerization and recondensation reactions. [6] We also concluded that the spectral resolution of NMR plays an important role especially when part of the lignin structure is used as an internal standard as such. [7]

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<sup>1</sup> Ramos, L.P., Breuil, C., Kuschner, D.J., Saddler, J.N., *Holzforschung* 46 (1992) 149-154.

<sup>2</sup> Martin-Sampedro, R., Capanema, E.A., Hoeger, I., Villar, J.C., Rojas, O.J., *J.Agric. Food Chem.* 59 (2011) 8761-8769.

<sup>3</sup> Robert, D., Bardet, M., Lapierre, D., Gellerstedt, G., *Cell. Chem. Technol.* 22 (1988) 221-230.

<sup>4</sup> Jakobsons, J., Hortling, B., Erins, P., Sundquist, J., *Holzforschung* 49 (1995) 51-59.

<sup>5</sup> Rahikainen, J., Tamminen, T., Marjamaa, K., Kruus, K. EWLP 2012, Espoo, Finland

<sup>6</sup> Li, J., Henriksson, G., Gellerstedt, G., *Bioresour. Technol.* 98 (2007) 3061-3068.

<sup>7</sup> Sette, M., Wechselberger, R., Crestini, C., *Chem. Eur. J.* 17 (2011) 9529-9535.

## ANALYSIS OF LIGNANS POLIMERIZED BY A VERSATILE PEROXIDASE FROM *Pleurotus eryngii*

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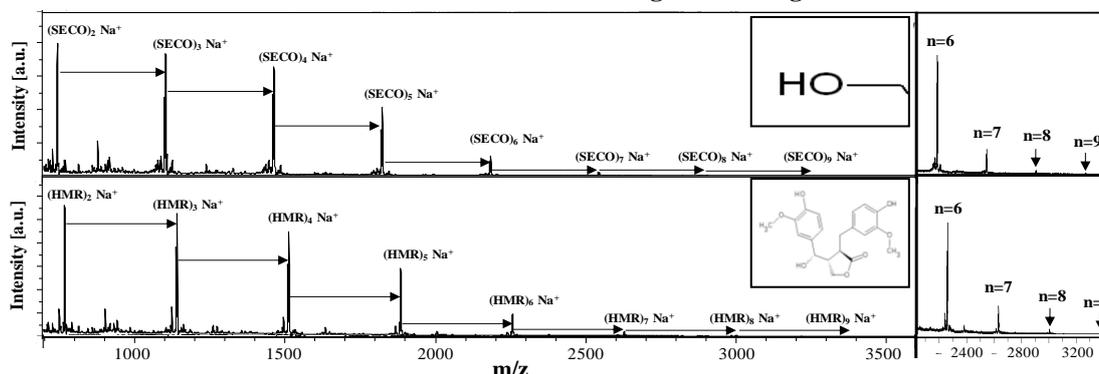
Lignans are polyphenolic compounds commonly found in wholegrain cereals, seeds, nuts, fruits, and beans (Milder et al. 2005), and many of these lignans are present in agro-industrial sidestreams which are potential raw ingredients for biorefineries. Several hundred lignans have been identified so far, and many of these [e.g. secoisolariciresinol (SECO), hydroxymatairesinol (HMR), matairesinol (MR), cyclolariciresinol (CYCLO), and 7-Hydroxysecoisolariciresinol (7-HSECO)] have been shown to demonstrate health-promoting benefits, such as a reduction of the risk of different type of cancers, virus, inflammations and osteoporosis in humans due mainly to their antioxidant properties and influence on estrogen metabolism (Mattinen et al. 2009; Saleem et al. 2005). The modification or generation of new polymeric structures by homo- and hetero-cross-linking through chemical or enzyme treatments could improve further these functional properties. Horseradish peroxidase has been widely studied for this purpose, however other enzymes, as the versatile peroxidase (VP) from the ligninolytic whit-rot fungus *Pleurotus eryngii*, are capable to oxidize compounds of even higher redox-potentials than HRP (Heinfling et al. 1998; Martínez et al. 1996; Pérez-Boada et al. 2005). Moreover, VP is an interesting example of fungal enzyme multifunctionality, as it shares the catalytic properties of LiP and MnP, becoming an interesting tool for biotechnological processes.

The polymerization/modification of the different lignans was performed in 1.5 mL Eppendorf tubes containing 200  $\mu$ L final mixture reaction (3 mM lignan, VP dosage of 1.5 U mL<sup>-1</sup>, 0.1 mM H<sub>2</sub>O<sub>2</sub>). After initiating the reaction with H<sub>2</sub>O<sub>2</sub>, an extra dosage was added after 1 hour of incubation. Aliquots of each reaction were removed after 0.5, 2 and 24 hours incubation and were analysed by matrix-assisted laser desorption ionisation-time of flight-mass spectroscopy (MALDI-TOF) and size exclusion chromatography (SEC). Antioxidant activity was also analysed through an adaptation of DPPH method since enzymatic modification can also enhance the bioactive properties of phenolic compounds

Results showed how up to 9 units of SECO and HMR were polymerized reaching 3265 and 3373 Da at 24 and 2 hours of VP-incubation respectively (Fig.1). Heterogeneous cross-linking between lignans and other small-size molecules as peptides was also detected. The weight average molecular mass ( $M_r$ ), studied by SEC, also showed a significantly increase in the  $M_r$  of SECO, HMR, and MR upon VP-induced polymerization, reaching mass increases of approximately 500, 110, and 190 Da, respectively after 24 hours. Due to the versatile activity of VP, polymerization treatments could be elongated after the addition of Mn<sup>+2</sup> in presence of H<sub>2</sub>O<sub>2</sub>, generating successive oxidative reactions that improved the process yield. Moreover, when H<sub>2</sub>O<sub>2</sub> was 5-fold higher, HMR polymers gave one degree of polymerization more at each timepoint compared to the equivalent products obtained with 0.1mM of H<sub>2</sub>O<sub>2</sub>. Regarding to the antioxidant capacity of VP-untreated lignans, the polymerization produced different effects on this property, dependant on the lignan and time of VP-incubation, but was not abolished.

In this work, VP has been shown for the first time to be competent modifying small-size lignans. These results can have scientific impact since higher molecules as lignin, which have undesirable properties due to its low reactivity and its dark colour (Moya et al. 2011), could also be modified, producing value-added polymers and materials with improved quality.

**Figure 1: MALDI-TOF mass spectra from VP-treated SECO and HMR. The smallest peaks are highlighted by an arrow. The number of monomer (n) in the (lignan)<sub>n</sub> homopolymers is shown above the corresponding peak. The structure of the lignans is framed and represented in each case. An enlargement of the smallest peaks is also shown at the right of the Figure.**



## REFERENCES

- Heinfling, A., Martínez, M.J., Martínez, A.T., Bergbauer, M., Szewzyk, U. (1998). Transformation of industrial dyes by manganese peroxidase from *Bjerkandera adusta* and *Pleurotus eryngii* in a manganese-independent reaction. *Applied and Environmental Microbiology*, 64, 2788-2793.
- Martínez, M.J., Ruiz-Dueñas, F.J., Guillén, F., Martínez, A.T. (1996). Purification and catalytic properties of two manganese-peroxidase isoenzymes from *Pleurotus eryngii*. *European Journal of Biochemistry*, 237, 424-432.
- Mattinen, M.L., Struijs, K., Suortti, T., Mattila, I., Kruus, K., Willfor, S., Tamminen, T., Vincken, J.P. (2009). Modification of Lignans by *Trametes Hirsuta* Laccase. *Bioresources*, 4, 482-496.
- Milder, I.E.J., Feskens, E.J.M., Arts, I.C.W., de Mesquita, H.B.B., Hollman, P.C.H., Kromhout, D. (2005). Intake of the plant lignans secoisolariciresinol, matairesinol, lariciresinol, and pinoresinol in Dutch men and women. *Journal of Nutrition*, 135, 1202-1207.
- Moya, R., Saastamoinen, P., Hernandez, M., Suurnakki, A., Arias, E., Mattinen, M.L. (2011). Reactivity of bacterial and fungal laccases with lignin under alkaline conditions. *BioresourceTechnology*, 102, 10006-10012.
- Pérez-Boada, M., Ruiz-Dueñas, F.J., Pogni, R., Basosi, R., Choinowski, T., Martínez, M.J., Piontek, K., Martínez, A.T. (2005). Versatile peroxidase oxidation of high redox potential aromatic compounds: Site-directed mutagenesis, spectroscopic and crystallographic investigations of three long-range electron transfer pathways. *Journal of Molecular Biology*, 354, 385-402.
- Saleem, M., Kim, H.J., Ali, M.S., Lee, Y.S. (2005). An update on bioactive plant lignans. *Natural Product Reports*, 22, 696-716.

## Proposal of catalytic process for lignin upgrading

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Revalorization of lignin was studied by proposing an entire process scheme. Base catalyzed repolymerization (BCD) has been studied for phenolic monomeric compounds production. Repolymerization phenomenon is known to occur during BCD avoiding better results.

The black liquor solution was filtered successively decreasing the different membrane cut-offs (300, 150, 50, 15 and 5 kDa) and lignin was isolated. Lignin ultrafiltrated fractions were subjected to base catalyzed depolymerization. The reaction temperature was set at 310 °C during 30 minutes reaching pressures around 105 MPa. Lignin:solvent (water) ratio was 1:20 (w/w). The catalyst (sodium hydroxide) concentration was set at 4 % (wt.). The initial pH of all experiments was 14. Products yields were determined gravimetrically and their composition and properties were analyzed by GC-MS, MALDI-TOF and HPSEC.

Ultrafiltration process has been confirmed to be an effective fractionation and purification process. Ultrafiltrated lignin fractions presented lower hemicellulosic contamination as the used membrane cut off was smaller and their molecular weight also decreased along with the membrane cut off.

Concerning oil total phenolic monomeric yields, the total monomers yield increased spectacularly as the molecular weight of lignin ultrafiltrated fractions decreased. This study has proved that lignin properties such as molecular weight or composition (purity) strongly influenced base-catalyzed lignin hydrothermal depolymerization process. Guaiacyl intermediates were less stable and that is why it could be found guaiacol in oil but not syringol. However, the presence of cresols, phenol, catechol and 4-methylcatechol suggested that syringol was rapidly released from lignin structure but then syringylic intermediates underwent demethoxylation reaction to produce catechol type products instead of forming pyrogallol.

All experiments yielded high residual lignin content but unfortunately, no trend was observed along with the molecular weight of the treated lignin fractions. The high residual lignin content was related to repolymerization phenomenon that consisted in the reaction between instable lignin fragments and original lignin producing unconverted lignin with higher molecular weight than the initial lignin.

# INVESTIGATION OF LIGNIN SAMPLES BY MEANS OF MULTIFUNCTIONAL PYROLYSIS-GC/MS

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The presented multifunctional Py-GC/MS offers extended methods for the investigation and characterization of polymers. Especially the so called evolved gas analysis (EGA) as a screening method for unknown samples allows a thermal characterization and specification of the best conditions for the subsequent analytical pyrolysis techniques (Fuchs et al. 2010, Heigenmoser et al. 2012): conventional pyrolysis (single-shot analysis; SSA), double-shot analysis (DSA) and heart-cut analysis (HCA). The gases formed by defined heating intervals during EGA provide a thermal profile of the respective sample. Over the running time of an EGA the separating column of the GC is substituted by a short and deactivated column. EGA affords a fixed or a four-step temperature programme including the adjustment of different heating rates. Furthermore EGA can be improved in combination with “Single Ion Monitoring” (SIM), whereby it is possible to observe the development of specific compounds of a sample during thermal load. DSA enables the removal of non-polymeric constituents of a sample prior to pyrolysis. HCA can adopt the heating conditions of the four-step temperature programme of EGA and allows the identification of compounds generated at defined temperature ranges without considering other compounds occurring at lower temperatures, because they can be flushed off through the vent of an affiliated selective sample.

The current investigation is aimed at the analysis of three different lignin samples (GL: sarkanda grass soda lignin, LR: lignocellulosic residue from bagasse after steam explosion, and KL: pine Kraft lignin). In a first step, the thermal behaviour of the samples was determined by EGA to identify the temperature levels for the subsequent HCA. Additionally SSA was performed at 600°C, to obtain comparable conditions for the chemical characterization of the samples within the framework of a round robin test.

The possibility of using different pyrolytic methods including different heating rates have allowed a more detailed investigation of the lignin samples with respect to their specific thermal behaviour. EGA revealed different thermal profiles with specific maxima. The corresponding HCA of the identified temperature levels disclosed specific components with differing proportions. Altogether GL and LR yielded H-, G- as well as S-lignin derivatives, whereas KL predominately obtained only typical softwood lignin degradation products. LR has shown additionally high percentages of carbohydrate degradation products. SSA showed analogous compounds, but by reason of different heating conditions in varying proportions, which can be caused also due to a higher decomposition rate e.g. to carbon dioxide.

In the presented context the multifunctional pyrolysis-GC/MS has demonstrated to be a powerful analytical tool. Some deviations between the methods are related to different heating conditions, which need to be studied in more detail in upcoming research.

## REFERENCES

- Fuchs R., Windeisen E., Heigenmoser A., Wegener G. (2010) Evolved gas analysis in combination with thermodesorption and pyrolysis. In: 11<sup>th</sup> European Workshop on Lignocellulosics and Pulp – August 16-19. Hamburg, pp. 159-183
- Heigenmoser, A., Fuchs, R., Windeisen, E. (2012) Characterization of different wood samples using a new combined method of evolved gas analysis and pyrolysis-gas chromatography/mass spectrometry. Wood Science and Technology. Wood Sci. Technol. 46, 637-642

## ANALYSIS OF THE LIGNIN MODIFIED UNDER COLD PLASMA CONDITIONS

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### ABSTRACT

Plasma technology is an efficient and suitable way to modify the properties of the polymeric substrates. Plasma modification approaches have a number of benefits as it requires small quantities of starting materials and the plasma processes are environmentally friendly because the organic solvents are avoided. In this paper, the modification of an organosolv lignin powder (ALCELL) with different carboxylic acids such as oleic, butyric, lactic acids and also with butyrolactone under cold plasma discharge has been performed. The modified lignins were characterized by XPS, <sup>1</sup>H-NMR, ATR FT-IR spectroscopy and SEM. It has been established that by modification the structure, morphology and solution properties are specific for each kind of product obtained.

Guo et al. analysed lignins by XPS and assigned the binding energy of C<sub>1s</sub> was assigned to 283.52 (C—H or C—C), 284.58; 285.72 (C—OR or C—OH), 286.10, 286.44 (C—O or HO—C—OR), 287.65; 287.72 (O—C—O) eV and O<sub>1s</sub> binding energy was to 530.31 (O—H), 531.4; 531.72 (RC—O), 532.73; 533.74 (O—C—O) eV respectively. (Guo et al 2005)

A comparison between the survey spectra reveals that C and O are the predominant species (usually found at virgin lignin surface). Plasma exposure led to weight loss and changes in the chemical composition on the polymer surface reflected in the decrease of the C<sub>1s</sub> percentage for all modified lignins from 80.2 atomic % for pristine polymer to 75.0 atomic % for lignin modified with oleic acid which showed the highest decrease in carbon percentage in respect with all samples. Corresponding this decrease in carbon percentage is accompanied by a decrease in oxygen percentage from 19.8 atomic percentage for unmodified lignin to 25 % for sample modified with OA.

Table 1. XPS data of modified samples

Sample	C <sub>1s</sub> (atomic %)	O <sub>1s</sub> (atomic %)	O/C
Alcell lignin (L)	80.2	19.8	0.246
Modified lignin LA	80.2	19.8	0.246
Modified lignin OA	75.0	25.0	0.333
Modified lignin BA	76.4	23.6	0.309
Modified lignin BL	78.5	21.5	0.274

The O/C ration of 0.25 is characteristic to lignin and it presented an increase for samples modified with OA and BA which show probably a high degree of modification. Oxygen-carbon ratios of 0.25, 0.38, and 0.39 were reported by Ahmed et al. (1987) for lotech lignin, thiolignin, and milled wood lignin, respectively. Hon (1984) observed an oxygen-carbon ratio of 0.43 for milled wood lignin. Oxygen-carbon ratios in the range of 0.31±0.36 were found by Dorris and Gray (1978) for dioxane lignin.

The C<sub>1s</sub> spectrum can be curve-fitted with four peak components, from the chemically non-equivalent carbon atoms: two major peaks (noted C1 and C4) at 286.38 eV and 290.97 eV are related to CH<sub>2</sub> and, respectively, , and two smaller peaks (C2 and C3) at 285.07 eV (attributed to C-C/C-H) and 288.9 eV (characteristic for the C-O bond). The O<sub>1s</sub> spectrum Figure 1b is fitted in three peaks.

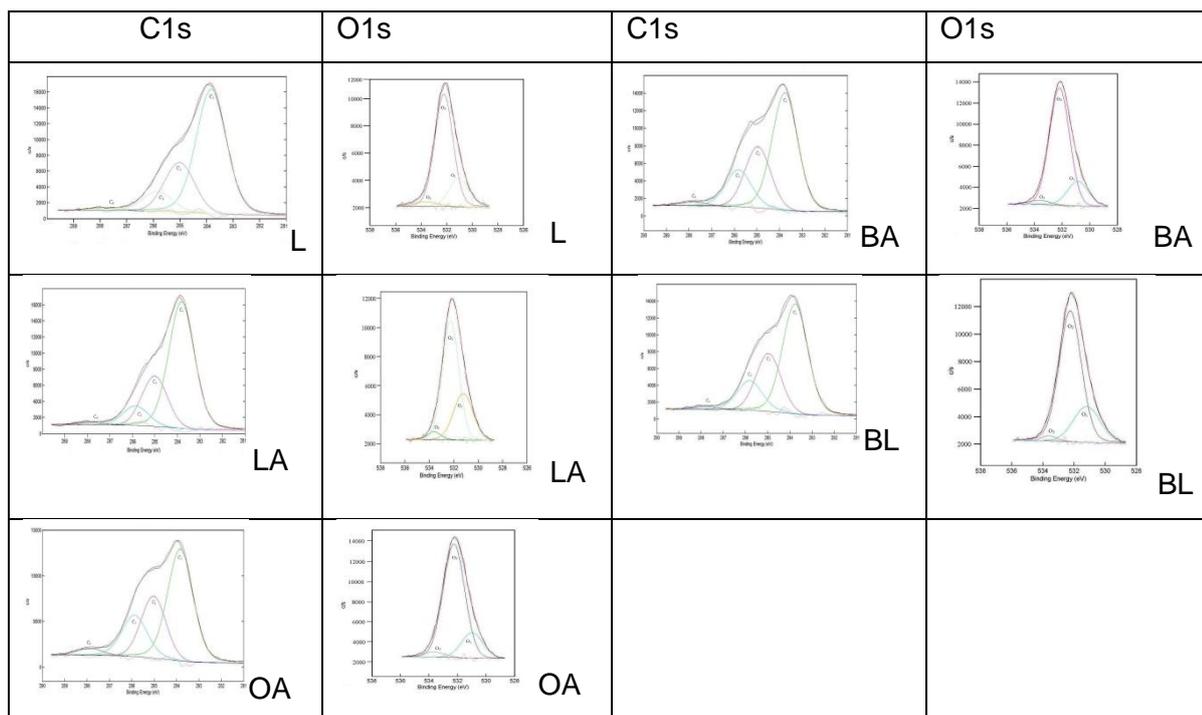


Figure 1: Fitted C 1s, and O 1s envelopes for (a) pristine lignin and modified lignins

By cold plasma functionalization surface composition is changed and also properties of modified lignin.

The cold plasma modification is an eco-friendly technique able to produce new valuable products from lignin widening its compatibility with various polymers and applications.

## REFERENCES

Guo J.-B., Tao Z.-Y, Luo X.-G. (2005), Analysis of Bamboo Lignin with FTIR and XPS, J Acta Chim. Sinica, 63(16): 1536-1540.

Ahmed A., Adnot A., Kaliaguine S. (1987), J. Appl. Polymer Sci. 34: 359-375

Hon D.N.S. (1984) J. Appl. Polym. Sci. 29: 2777-2784.

Dorris G.M., Gray D.G. (1978), The surface analysis of paper and wood fibres by ESCA. I. Application to Cellulose and Lignin. Cell. Chem. Technol. 12: 9-23.

# EFFECTS OF THE OPERATIONAL CONDITIONS ON THE COMPOSITION OF AQUEOUS EXTRACTS FROM *Pinus pinaster* WOOD

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## ABSTRACT

Knots and stemwood of pine wood have been found to contain valuable extractable compounds, including lignans, flavonoids and stilbenes, which can be used in several applications in the fields of pharmacy and nutrition. Knots and stemwood of *Pinus pinaster* (maritime pine, native to the western and southwestern Mediterranean region) contain phenolic compounds with bioactive potential.

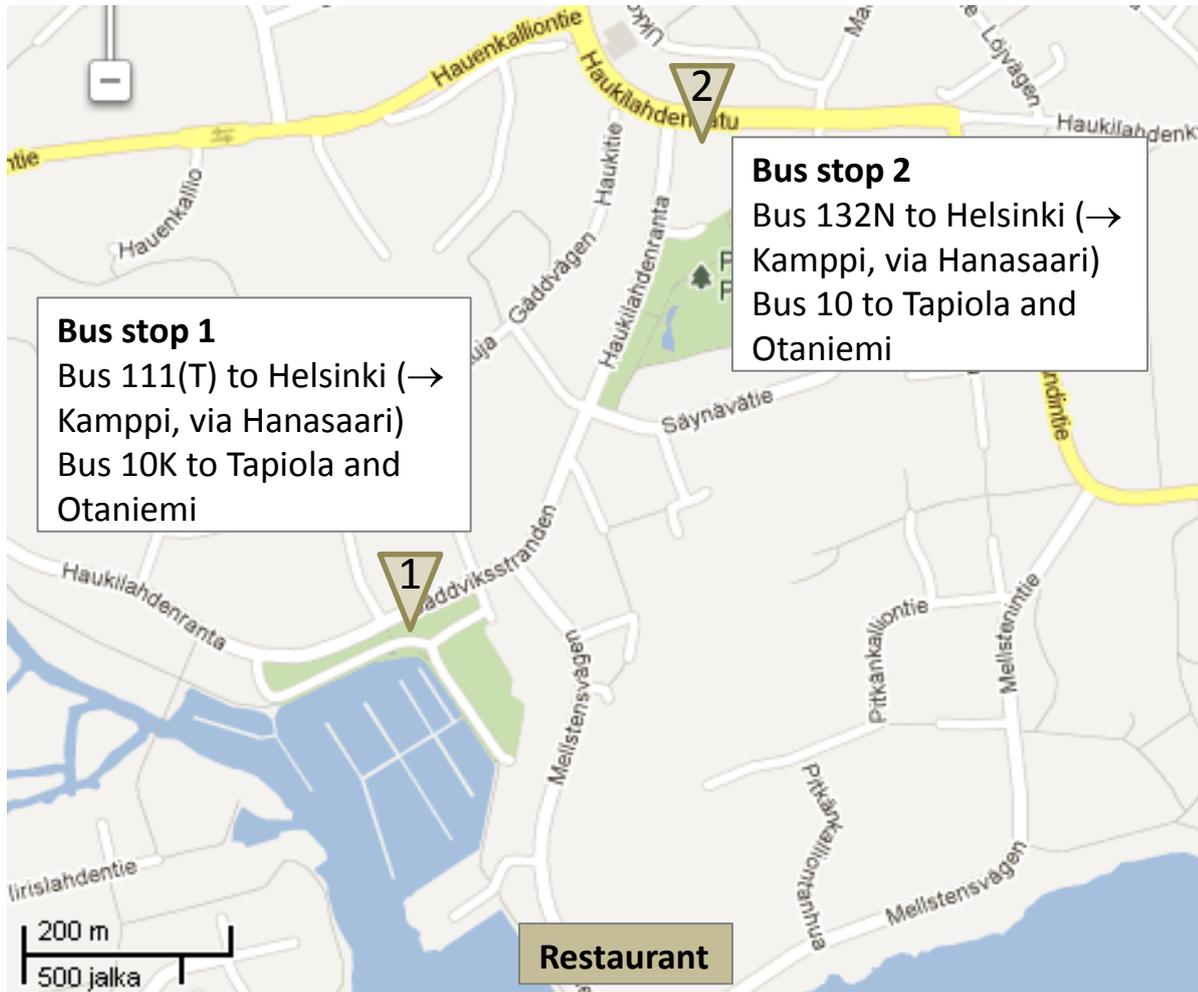
In this work, aqueous pretreatments of *Pinus pinaster* stemwood and knots were performed under a variety of operational conditions for extracting phenolic compounds (including lignans and pinosylvins). The severity of treatments was varied by operating under non-isothermal conditions up to achieve different maximum temperatures. The resulting liquors were assayed for composition and yields in the target compounds. The antioxidant activity of extracts and their contents of hemicellulose-derived saccharides was also measured.

Three trees were sampled in different positions (heartwood and sapwood of stemwood, knots from dead branches and knots from living branches). Samples were milled to pass a 10-mesh screen, and treated with hot, compressed water in a stirred, stainless steel reactor with temperature control (Parr Instr. Co.) under non-isothermal conditions up to reach maximal temperatures in the range 130-140 °C. Experiments were performed using a liquid:solid ratio of 10:1 g:g (oven-dry solid basis). The above treatments covered the range of operational conditions corresponding to negligible to moderate hemicellulose hydrolysis. Once the desired temperature was reached, the reactor was cooled by circulating water through a coil. After cooling, the reaction liquor was recovered by filtration and analyzed for total extraction yield, total phenolic content, hemicellulose-derived products (including monosaccharides and oligomeric saccharides) and Trolox equivalent antioxidant activity (TEAC).

The highest extraction yield (5-11 g extract/100 g dried sample) corresponded to heartwood from knots, whereas the highest phenolic content of the extract was determined for sapwood of dead branch knots (13-19 g gallic acid equivalents/ 100 g extract). The highest antioxidant activity (0.2-0.3 g Trolox/g extract) was determined for extracts from stem sapwood. Glucose, xylose, arabinose, galactose, and mannose were identified and quantified in the extracts. The highest monosaccharide contents (6-12 g monosaccharides/ 10 g extracts) were found in extracts from stem heartwood. Oligosaccharides contents accounted for up to 90 % of the dissolved fraction. Liquors were freeze-dried, and the resulting extracts were extracted with acetone and assayed for composition by GC-MS for non-saccharide compounds, including pinosylvins, flavonoids and lignans.

## NOTES

## Map of Haukilahti (dinner on Thursday)



We will arrive in the restaurant by boat. If you miss the boat, take the bus 111 or 111T from Hanasaari or Kamppi Bus Station (Helsinki) to the direction of Haukilahti.

### Timetables for buses on the way back from Haukilahti

#### Bus stop 1

**111(T):** 20:20, 20:50, 21:20

**10K:** 20:02, 20:51, 21:50, 22:49

Distance to bus stop 1 from the restaurant ca. 500 m.

#### Bus stop 2

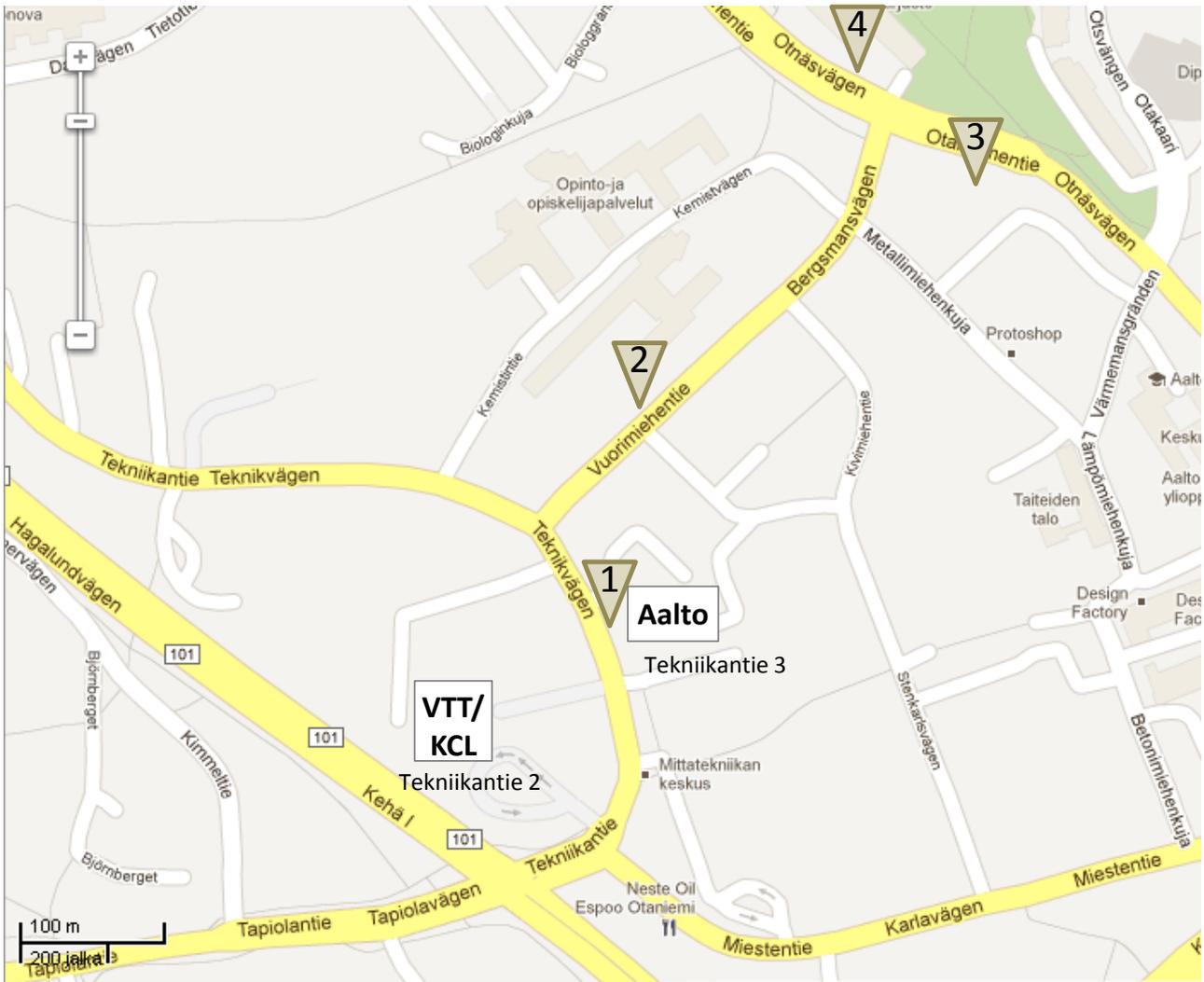
**132N:** 21:56, 22:26, 22:46, 23:26

**10:** 20:23, 21:22, 22:21

Distance to bus stop 2 from the restaurant ca. 900 m.

In addition, taxi is another (more expensive) alternative.

## Map of Otaniemi



1. Bus stop to Helsinki (194, 195 → Helsinki Railway Station)
2. Bus stop from Helsinki (194, 195)
3. Bus stop to Helsinki (102, 103 → Kamppi Bus Station via Hanasaari)
4. Bus stop from Helsinki (102, 103)

Traveling time for buses 194 and 195 from Helsinki Railway Station to Otaniemi is approx. 30 min. Traveling time for 102 and 103 from Kamppi is approx. 20 min, or if the bus is marked with letter 'T' the bus will take an extra 5 min detour through Lauttasaari.

Buses 102 and 103 run approximately every 5 minutes during rush hours.

Buses from the Railway Station  
(west side of the station/Elielinaukio):  
194: 8:01, 8:23  
195: 7:48, 8:12

From Otaniemi (stop no 1)  
194: 16:50, 17:14  
195: 17:03, 17:25, 17:45, 18:07