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Wheat straw lignin – Isolation and characterization of a plant cell wall networking polymer

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KURZZUSAMMENFASSUNG

Beim Lignin von einkeimblättrigen Gräsern wie Weizen handelt es sich um ein hochgradig heterogenes Makromolekül, das in der Pflanze in einem ausgeklügelten Zellwandkomplex vorliegt, wo es starke Quervernetzungen mit den Zellwandpolysacchariden ausbildet. Eine geschickte Kombination von verschiedenen Isolierungsmethoden, die an unterschiedlichen Ebenen der Pflanzenzellwandorganisation ansetzen, wie konventioneller Lignin-Isolierungsmethoden und kürzlich entwickelter Protokolle zur Isolierung von Lignin-Kohlehydrat-Komplexen, ermöglicht es anhand der erhaltenen Daten ein erweitertes Bild von Weizenstroh-Lignin und seiner Organisation innerhalb des Zellwandverbundes zu entwerfen.

Im Zuge dieser Thesis wurden bei der Isolierung des Weizenstroh-Lignins zwei unterschiedliche Ansätze verfolgt. Im ersten Fall wurden etablierte Lignin-Isolierungsmethoden in einer neuartigen Abfolge kombiniert. Nach einer Vorbehandlung des Stroh in einer Vibrationskugelmühle wurde die erste Lignin-Fraktion mit neutralem Dioxan-Wasser extrahiert. In weiterer Folge wurde zur Isolierung der zweiten Lignin-Fraktion das verbleibende Stroh in mild-saurem Dioxan-Wasser extrahiert. Diesem ersten Ansatz folgend wurden 82% des Klason-Lignins (KL) im Ausgangstroh in vier getrennten Fraktionen isoliert: „Free Lignin“, zugänglich durch Dioxan-Wasser Extraktion bereits ohne Vorbehandlung des Stroh durch Kugelmahlen, zeichnet sich durch eine sehr niedere mittlere Molmasse aus, ist verknüpft mit Glucan, enthält hohe Anteile von *p*-Hydroxymethylsäuren und Tricin und repräsentiert 14% des KL des Ausgangstrohs. „Dioxan Lignin“ wurde nach einer Mahldauer von 8h durch neutrale Dioxan-Wasser Extraktion gewonnen und steht für 18% des KL. Es liegt verknüpft mit linearem Arabinoxylan vor und aufgrund seiner monomeren Zusammensetzung wird als sein Ursprung die Mittellamelle angenommen. „Acidolysis Lignin“, die Fraktion mit dem größten Anteil am KL im Ausgangstroh (38%), wurde in der zweiten, mild-sauren Extraktionsstufe gewonnen. Es zeigt Verknüpfungen mit verzweigtem Arabinoxylan und wurde aufgrund hoher Vorkommen von Syringyl- und *p*-Hydroxymethylsäure-Einheiten der Sekundärzellwand zugeordnet. Die vierte Fraktion dieses Isolierungsprotokolls enthält das im restlichen Stroh verbliebene Lignin, das sich als resistent gegenüber aller angewendeter Methoden herausstellte.

Der zweite Ansatz hatte eine höhere Ebene der Zellwandorganisation zum Ziel und bestand aus einem Fraktionierungsprotokoll für Holz bzw. Holzpulpe in sogenannte Lignin-Kohlehydrat-Komplexe, das hier zum ersten Mal für Weizenstroh verwendet wurde. 95% des Ausgangstrohs konnten in zwei Fraktionen wiedergefunden werden, die je nach Polysaccharidanteil als „Glucan-Lignin“ und „Xylan-Lignin“ bezeichnet wurden. Die

Monomerenverhältnisse der Ligninanteile dienen zur Zuordnung der Fraktion zu entweder Mittellamelle („Xylan-Lignin“) oder Sekundärzellwand („Glucan-Lignin“).

In einem dritten Versuch wurde „Dioxan Lignin“ präparativ nach seiner Molmasse fraktioniert: Fraktionen hoher Masse zeigen gehäuft *p*-Hydroxyphenyl- und *p*-Hydroxymethylsäure-Einheiten und sind mit linearem Arabinoxylan verknüpft, während Fraktionen niedriger Masse höhere Anteile von Syringyl- und Guajacyl-Einheiten aufweisen. In einer der Fraktionen wurde ein verstärktes Auftreten von Dibenzodioxocin-Bindungen und Ferulasäure beobachtet, während der Gehalt an freien Carboxylgruppen sehr niedrig ist. Gleichzeitig besteht der Polysaccharidanteil dieser Fraktion beinahe ausschließlich aus Arabinoxylan, was darauf hindeutet, dass sich in dieser Fraktion vermehrt strukturelle Motive finden lassen, die über obengenannte Komponenten das Lignin mit den Zellwandpolysacchariden vernetzen. Tricin wurde ausschließlich in den Fraktionen niedriger Masse gefunden, wo außerdem auch Glucan-Anteile festgestellt wurden. Diese beiden Charakteristika der Fraktionen niedriger Masse unterstützen die These, dass „Free Lignin“ innerhalb der „Dioxan-Lignin“ Fraktion vorliegt, wie es in Chapter 1 vorgeschlagen wird.

In der vorliegenden Arbeit wurden mehrere Lignin-Fraktionen aus Weizenstroh isoliert und detailliert charakterisiert. Dabei zeigten sich signifikant unterschiedliche Strukturen und Zusammensetzungen bezüglich des Lignins selbst als auch bezüglich der jeweiligen Polysaccharidkomponenten. Die gesammelten Daten liefern essentielle Informationen über den molekularen Aufbau von Weizenstroh-Lignin und dienen auf diese Weise als Basis für das Design zukünftiger Experimente zu seiner Anwendung unter Berücksichtigung seiner strukturellen Charakteristika.

ABSTRACT

Lignin of monocot grasses like wheat is a highly heterogenic macromolecule participating in an elaborated cell wall network compound *in planta* where it is strongly cross-linked with cell wall polysaccharides. The combination of different isolation methods which target different levels of plant cell wall organization, namely conventional isolation protocols aiming specifically for lignin on one side and recently developed isolation techniques for lignin-carbohydrate-complexes on the other, provides useful data for constructing an enhanced picture of wheat straw lignin variation and its organization inside the plant cell wall.

In this thesis wheat straw lignin was isolated using two different approaches: the first as a novel combination of established lignin isolation techniques comprised of an initial ball-milling step, followed by one neutral and eventually one acidolysis-assisted dioxane-water extraction. This approach provided four distinct lignin fractions recovering in total 82% of the starting material, based on Klason lignin (KL): “Free Lignin”, accessible by neutral dioxane-water extraction without prior ball-milling, is of very low molar mass, linked to glucan, contains high amounts of *p*-hydroxycinnamic acids and tricetin, and accounted for 14% of KL. “Dioxane Lignin” was isolated after 8 hours of vibratory ball-milling with neutral dioxane-water representing 18% of KL. It is connected to linear arabinoxylan and its monomeric composition indicated its origin from the middle lamella. The biggest fraction (38% of KL), “Acidolysis Lignin”, was isolated by acidolysis-assisted dioxane-water extraction of the residual straw after “Dioxane Lignin” isolation. It is connected to branched arabinoxylan and was assigned to the secondary wall based on high ratios of syringyl and *p*-coumaric acid units. The fourth fraction contained the core glucan-associated lignin in the residual wheat straw that was resistant to all applied extraction methods (12% of KL).

In the second isolation approach targeting a higher level of plant cell wall organization, a fractionation protocol for wood and pulp into lignin-carbohydrate complexes (LCCs) was successfully applied to wheat straw recovering 95% of the straw in two LCC fractions, denominated as “Glucan-Lignin” and “Xylan-Lignin”. While in the first LCC fraction the lignin moiety was mainly connected to glucan and only small parts to branched arabinoxylan, lignin in the second fraction was linked to linear arabinoxylan and contained a high proportion of ferulic acid. “Glucan-Lignin” was related to secondary wall regions and “Xylan-Lignin” instead to middle lamella regions, based on literature reports about monolignol deposition during lignifications and the monomeric compositions of the lignin inside the LCC fractions determined by 2D NMR spectroscopy.

In a third study Dioxane lignin was subjected to preparative molar mass fractionation and fractions of higher molar mass were found to be enriched in *p*-hydroxyphenyl and *p*-hydroxycinnamic acid units and associated with linear arabinoxylan. However, in the lower molar mass fractions guaiacyl and syringyl units were more abundant. One fraction showed a high ratio of dibenzodioxocin bonds, ferulic acid and almost exclusively arabinoxylan in the carbohydrates part while carboxylic OH groups were low suggesting their involvement in structural motifs cross-linking lignin with hemicelluloses. Tricin was detected exclusively in lower mass lignin where also additional glucan contributions were found. These results confirm the presence of earlier described “Free Lignin” inside the “Dioxane Lignin” preparation which was characterized as a low mass glucan-associated lignin with high abundance of guaiacyl units and triclin.

In the presented studies several wheat straw lignin fractions were isolated and thoroughly characterized showing significant variation in their molecular structure and their specific association to certain polysaccharides providing essential information and orientation for designing respective lignin utilization strategies. The described protocols provide straightforward routes for accessing lignin fractions that exhibit less heterogeneous structures compared to bulk wheat straw lignin in order to utilize their specific structural characteristics for future wheat straw lignin application and upgrading attempts.

PREFACE

The experimental work for this thesis was conducted in the frame of a FFG project (Austrian Research Promotion Agency) in cooperation with the company *Annikki GmbH* from Graz, Austria, whose financial support is gratefully acknowledged. Isolation and analysis of the wheat straw lignin fractions was realized to one part in the laboratories of the research group Bioresource Technology at Technische Universität Wien, Austria, and to other parts at the Department for Wood Chemistry at the Royal Institute of Technology in Stockholm, Sweden.

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INTRODUCTION

In a world of ever-growing energy demands, a wide-spread use of plastic goods deriving from mineral oil, and the perspective of depleting fossil energy sources in future, renewable resources are getting more and more important as a feedstock for biofuels and chemicals production (Upton and Kasko, 2016). When considering that the only source of renewable carbon in a large scale on our planet (beside atmospheric carbon dioxide) is found in the plant kingdom, it is obvious that lignocellulosic biomass will be a key feedstock for the chemical industry in a future bio-based economy. However, the development of targeted upgrading processes of biomass components regarding high yields, low costs and energy efficiency will be essential in order to compete with and eventually replace petroleum-based fuels and chemicals (Luterbacher et al., 2014). Further, in order to establish a bio-based economy, large-volume chemicals like monomers for plastics and fibers or industrial solvents need to be targeted first for having a profound influence on the sustainability of chemical production based on renewable resources (Tuck et al., 2012). Since wheat is one of the most grown crops on our planet and the predominant feed crop in Europe (www.faostat.org), the utilization of residual wheat straw as a biorefinery feedstock is favorable. A first commercial production plant for second generation bioethanol from agricultural wastes was established in Crescentino, Italy, in 2013 by the company *Beta Renewables*. As a side-product of a straw biorefinery large amounts of lignin would be produced representing a great potential for further utilization in terms of its caloric value but preferably – in a more sustainable way – regarding the unique chemistry of the lignin macromolecule considering that lignin is the only abundantly available renewable feedstock of aromatics (Tuck et al., 2012). Lignin chemistry has been investigated extensively for decades describing the ways of lignin monomers biosynthesis inside the plant cell (Adler, 1977; Boerjan et al., 2003; Freudenberg, 1957; Freudenberg, 1959; Hatfield et al., 2009; Higuchi, 2006) until the processes of lignin formation in intimate association with cell wall polysaccharides (Crestini et al., 2011; Donaldson, 2001; Iiyama et al., 1994; Ralph et al., 2009) and wood formation in trees (Déjardin et al., 2010; Fengel and Wegener, 1984; Henriksson, 2009). However, the main use of lignin has traditionally been a thermal one after its separation from cellulosic fibers in integrated pulp mills using e.g. Kraft cooking to separate lignin which is in turn burnt in a recovery boiler to generate heat for power production and regenerate pulping chemicals (Lora and Glasser, 2002). Lignin structure and its natural heterogeneity represent a challenge for its utilization by polymer chemistry in the bio-materials field. Nevertheless, substantial research efforts are invested in the use of lignin, modified lignin or monolignols as (macro)monomers

in polymers as reviewed recently by Kai et al. (2016) and Upton and Kasko (2016). Lignin functionalization can either add new functional groups essential for the formation of the desired polymer or assist in the exposure of already available functionalities with reduced accessibility in the native lignin state. The latter strategy was used by Nadji et al. (2005) converting different lignins into polyols via chain extension reaction using propylene oxide. The resulting polyols were in turn used for rigid polyurethane foam formulations showing satisfying thermal properties and dimensional stability. In the case of monocot grasses lignin structures are found that exceed the heterogeneity of wood lignin since *p*-hydroxycinnamic acids (*p*HCA) like ferulic acid (FA) and *p*-coumaric acid (*p*CA) are well integrated in the cell wall compound of lignin and polysaccharides, often forming grass-specific lignin-carbohydrate complexes (LCCs) (Bunzel et al., 2004; Buranov and Mazza, 2008; Crestini and Argyropoulos, 1997; del Río et al., 2008; Hatfield et al., 1999; Martínez et al., 2008; Ralph, 2010; Ralph et al., 2004a; Ralph et al., 2004b). Interestingly, those carboxylic acid equivalents of the regular lignin monomers, *p*-cinnamic alcohols, were recently reported to serve well in the synthesis of polyesters or polyurethanes. Oulame et al. (2015) used ferulic acid together with bio-based diols to synthesize biphenolic monomers which were further reacted with diisocyanates to give a new class of renewable aliphatic-aromatic poly(ester-urethane)s. Further, FA as well as *p*CA were used to synthesize polyesters for topical applications like skin care products intended to release ferulic acid in a controlled manner (Ouimet et al., 2015; Ouimet et al., 2013), illustrating the potential of straw lignin as a source of monomers for functional polymers synthesis. Noel et al. (2014) synthesized polyamides with fluorescence activity by synthesizing the respective monomers by coupling of ferulic acid with amino acids, creating bio-materials potentially applicable for sensing or imaging applications.

Because of the intimate arrangement of the cell wall polymers it is considered as nearly impossible to isolate lignin without polysaccharide impurities. It can cause serious troubles in biorefining processes when the selective and complete separation of biomass fractions is inhibited due to stable covalent bonds between lignin and polysaccharides making it a necessity to decipher the structure of LCCs (Balakshin et al., 2011). Therefore, when studying native lignocelluloses structure, the isolation strategy is focused on leaving the bonds between lignin and polysaccharides intact during isolation for further structural analysis leading to the development of several elaborated LCC isolation protocols by Lawoko (2013) and Lawoko and co-workers (2004; 2005; 2006), Li et al. (2011) and Du et al. (2013) in the recent years. In contrast to their hindrance in biomass fractionation, LCCs as such were

reported to exhibit various pharmacological activities like anti-tumor, anti-microbial, anti-HIV or synergistic action with vitamin C (Sakagami et al., 2010).

As a new component, triclin was detected recently in lignin preparations of monocot grasses (del Río et al., 2012; Li et al., 2016; Zikeli et al., 2014, 2016) and is considered to act as a cross-coupling partner of monolignols based on model compound studies (Lan et al., 2015). Triclin-oligolignol compounds were identified as metabolites in maize lignifying tissue providing further evidence that triclin is naturally cross-coupling with lignin monomers to give flavonolignols which could also act as nucleation sites for lignin polymerization (Lan et al., 2016). Therefore, structure-related lignin studies are exhibited all over the world and even more will be necessary in order to eventually find proper value-added applications for lignin as a functional material. For example, when using lignin as a macromonomer in the synthesis of polyurethanes, polyesters, epoxide and phenolic resins, functional group analysis for determination of aliphatic and phenolic hydroxyl contents is essential for designing lignin-derived macromonomers using those functional groups as linkers (Cateto et al., 2008).

Another challenge when working with lignin is the desired structural preservation during isolation and its recovery in high yield with the latter requiring a mechanical or chemical pretreatment of the lignocellulosic material in order to provide accessibility to the lignin. Its aromatic structure and the presence of numerous functional groups renders lignin sensitive to elevated temperatures or extreme pH values triggering e.g. so-called condensation reactions between aromatic rings and inducing cleavage of alkyl-aryl ether bonds in lignin (Gellerstedt et al., 1994; Ikeda et al., 2001). This problem was addressed by a detailed investigation of ball-milling effects on the resulting lignin structures over a wide range of different milling times. Further, the neutral lignin solvent dioxane was reported to not alter the native lignin structure during isolation (Ben-Ghedalia and Yosef, 1994).

In the present thesis different strategies for wheat straw lignin and LCCs isolation were explored and the specific structural properties of the resulting lignin and LCC fractions were investigated in detail. Wet chemistry as well as one- and two-dimensional NMR spectroscopy methods were used aiming for a more detailed picture of wheat straw lignin and its molecular variation in different cell wall regions and – on a higher level – of the lignocellulosic cell wall architecture.

SUMMARY OF THE RESEARCH ARTICLES

Chapter 1 was dedicated to the development of an isolation protocol providing wheat straw lignin in quantitative yield and separated in several distinct fractions. After the first extraction step for the isolation of “Dioxane Lignin” (DL) from the ball-milled straw by neutral dioxane-water, the major part of the residual lignin in the straw was liberated using a mild and short acid hydrolysis in the same solvent. This newly created sequence of well established lignin isolation methods resulted in a total recovery of 82% of Klason lignin of the original wheat straw under optimized vibratory ball-milling conditions. Comparison of structural details like lignin monomeric composition and contents of *p*-hydroxycinnamic acids of the isolated lignin fractions with literature reports about lignin topochemistry (Donaldson, 2001) suggested either the middle lamella or the secondary wall region as morphological origin of the lignin fractions. Further, a newly described lignin fraction soluble without mechanical or chemical pretreatment of the straw, and therefore denominated as “Free Lignin” (FL), was characterized as a small lignin oligomer with high contents of *p*-hydroxycinnamic acids and tricetin leading to speculations of a free-floating lignin oligomer possibly acting as a transportable UV-protection agent *in planta*.

In the subsequent study, in order to deepen the understanding of wheat straw lignin structure, a fractionation protocol based on the native lignin-carbohydrate associations in the plant cell wall (Chapter 2), which was already established for eucalypt wood and pulp (Li et al., 2011) as well as for spruce wood (Du et al., 2013), was successfully applied to wheat straw. This protocol resulted in two distinct lignin-carbohydrate-complex (LCC) fractions – denominated as “Glucan-Lignin (GluL)” and “Xylan-Lignin (XylL)” respectively – showing preferred association with either glucan or arabinoxylan in the cell wall. The lignin parts of the two LCC fractions showed substantial structural differences suggesting XylL deriving from middle lamella regions while GluL apparently represented the secondary wall layer. So-called ferulic acid ether-ester-bridges were proposed to interconnect the two LCC fractions *in planta*. Application of the protocol to alkaline-pretreated straw led to a much lower recovery of XylL suggesting that ester-bonds between FA and arabinoxylan were the primary target of the alkali attack via hydrolysis.

In a third study (Chapter 3) the wheat straw lignin fraction which was earlier attributed to cell wall middle lamella regions (Chapter 1, Dioxane lignin DL) was re-isolated in a larger scale and subjected to preparative flash chromatography providing several distinct molar mass fractions for further structural characterization. Molar mass dependent structural differences were observed regarding lignin monomeric ratio and contents of *p*-hydroxycinnamic acids.

One fraction showed special properties with a high ratio of dibenzodioxocin (DBDO) bonds and high content of FA while the content of free carboxylic hydroxyl groups was low. These findings gave rise to the idea that this fraction was enriched in lignin nucleation sites consisting of arabinoxylan chains cross-linked by dimerization of the attached FA molecules. This structural motif could serve as the starting point of lignin growth through formation of a DBDO-type linkage using the two free phenolic hydroxyl groups of the FA dimer. In the two lower mass lignin fractions higher ratios of guaiacyl units and triclin as well as glucan contributions were found providing support for the presence of FL inside the raw DL fraction, since the same characteristics were determined for FL in Chapter 1.

In summary, Chapter 1 showed that ball-milling physically opened the straw fiber structure and thus provided access to the lignin-rich middle lamella for neutral solvent extraction of DL. The lignin in the more rigid secondary cell wall region, where lignin content is lower and polysaccharides are more abundant, however requires acid-catalyzed depolymerization of cell wall polysaccharides in order to be isolated as AL. In Chapter 2, a picture of higher wheat straw cell wall organization was projected where Xylan-Lignin is located in the middle lamella regions and interconnects with Glucan-Lignin in the secondary wall regions via FA ether-ester bridges. In Chapter 3 one special wheat straw lignin molar-mass-fraction was identified with a high abundance of DBDO bonds, FA and almost exclusively arabinoxylan as polysaccharide contribution. From this data the model of a structural motif was developed which represents lignin nucleation sites on cross-linked arabinoxylan chains. Further, the presence of a newly described FL fraction (Chapter 1) was confirmed inside DL in the lower-molar-mass fractions. The collected data provided new insights regarding wheat straw lignin variation in structure and composition and its organization together with cell wall polysaccharides. The results presented in this work will be an invaluable guide for the planning of future wheat straw lignin application approaches, as a whole or fraction-wise, aiming for more sophisticated applications as a chemical precursor for macromonomers or as a component in functional materials.

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CHAPTER 1

Successive and quantitative fractionation and extensive structural characterization of lignin from wheat straw

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DISCLAIMER:

During the submission process of the respective manuscript it happened that wrong values for total phenolic hydroxyl groups of Dioxan lignin and Acidolysis lignin arrived in Table 5 and it was not noticed until the final publication of the article. For this reason I would like to provide a corrected Table 5 as supplement material at the end of the chapter.

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SUPPLEMENT MATERIAL

Corrected Table 5:

Table 5. Content of free aliphatic, phenolic (total phenolic, condensed/uncondensed ratio and S/G/H ratio of phenolic hydroxyls) and carboxylic hydroxyls in the isolated Dioxane lignin (DL) and Acidolysis lignin (AL) fractions at different ball-milling times; NetDL: after subtraction of free lignin (FL).

Ball-milling [h]	Dioxane lignin (DL)					Acidolysis lignin (AL)				
	aliphatic OH [mmol/g lignin]	phenolic OH			carboxylic OH [mmol/g lignin]	aliphatic OH [mmol/g lignin]	phenolic OH			carboxylic OH [mmol/g lignin]
		total [mmol/g lignin]	S/G/H type ratio [%]	cond./uncond. [%]			total [mmol/g lignin]	S/G/H type ratio [%]	cond./uncond. [%]	
0	5.44	2.02	10/56/34	28/72	1.04	4.35	1.55	10/54/36	14/86	0.23
4	5.47	1.93	12/54/34	24/76	0.55	3.42	1.58	17/49/34	13/87	0.38
8	6.29	1.46	12/55/33	16/84	0.36	3.29	1.51	16/51/33	13/87	0.28
8 (Net)	7.20	0.86		3/97	0.00					
12	6.41	1.91	13/53/34	18/82	0.23	3.07	0.00	16/50/34	18/82	0.43
16	6.63	1.76	14/53/33	16/84	0.30	2.70	0.00	17/49/34	27/73	0.39

CHAPTER 2

Wheat straw lignin fractionation and characterization as lignin-carbohydrate complexes

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CHAPTER 3

Fractionation of wheat straw Dioxane lignin reveals molar mass dependent structural differences

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LIST OF PUBLICATIONS

Peer reviewed research articles used as chapters in this thesis:

Zikeli, F., Ters, T., Fackler, K., Srebotnik, E., Li, J., 2016. Fractionation of wheat straw dioxane lignin reveals molar mass dependent structural differences. Accepted for publication in the peer reviewed journal *Industrial Crops and Products* on July 12, 2016.

DOI: 10.1016/j.indcrop.2016.07.014

Contribution of Florian Zikeli:

Design of experiments, isolation and analysis of samples, interpretation of data, literature research, writing and editing of the manuscript

Zikeli, F., Ters, T., Fackler, K., Srebotnik, E., Li, J., 2016. Wheat straw lignin fractionation and characterization as lignin-carbohydrate complexes. *Ind. Crop. Prod.* 85, 309-317

Contribution of Florian Zikeli:

Design of experiments, isolation and analysis of samples, Interpretation of data, literature research, writing and editing of the manuscript

Zikeli, F., Ters, T., Fackler, K., Srebotnik, E., Li, J., 2014. Successive and quantitative fractionation and extensive structural characterization of lignin from wheat straw. *Ind. Crop. Prod.* 61, 249-257.

Contribution of Florian Zikeli:

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Further thesis-related publications:

Zikeli, F., 2015. Wheat straw lignin – Successive fractionation and structural characterization;
In: *Cost Action FP1105 : Understanding wood cell wall structure, biopolymer interaction and composition : implications for current products and new materials : Sixth workshop : Scientific Program and Book of Abstracts*, San Sebastian, Basque Country, Spain, May 26-27, 2015.

Zikeli, F., Ters, T., Fackler, K., Srebotnik, E., Li, J., 2012. Structural characterization of lignin from ball-milled wheat straw;
In: *Proceedings of the 12th European Workshop on Lignocellulosics and Pulp*, Espoo, Finland; August 27-30, 2012.

Publications not related to this thesis:

Zikeli, F., Ters, T., Fackler, K., Srebotnik, E., 2011. Analysis of lignins from various sources using alkaline HP-SEC and NMR;
In: *Proceedings of the 19th Annual Meeting of the BioEnvironmental Polymer Society*, Vienna, Austria; September 28-30, 2011.

Zikeli, F., Ters, T., Fackler, K., Srebotnik, E., 2011. Aspects of alkaline HP-SEC for molar mass distribution analysis of lignin;
In: *Proceedings book of the Italian meeting on lignocellulosic chemistry italic 6 - Science & Technology of Biomasses: Advances and Challenges*, Viterbo, Italy; September 5-8, 2011.

Fuqaha, A., Zikeli, F., Ters, T., Friedl, A., 2011. Sustainable Lignin Fractionation with Ultrafiltration;
In: *Proceedings book of the Italian meeting on lignocellulosic chemistry italic 6 - Science & Technology of Biomasses: Advances and Challenges*, Viterbo, Italy; September 5-8, 2011.

Fuqaha, A., Zikeli, F., Ters, T., Friedl, A., 2011. Evaluation of various ultrafiltration membranes for the treatment of solutions derived from alkali treatment of straw;
In: *International Congress on Membranes and Membrane Processes 2011 - Book of Abstracts*, Amsterdam, Netherlands; July 23-29, 2011.

APPENDIX: Curriculum vitae Florian Zikeli

DI Florian Zikeli

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Educational experience:

- 09/1991 – 06/1999 High school BG/BRG Kufstein, Austria, general qualification for university entrance
- 11/1999 – 06/2000 Military services as a medic at TÜPI Walchen/Wattener Lizum, Tirol, Austria
- 09/2000 – 01/2001 Master studies in *Chemical Engineering* at Vienna University of Technology, Austria; not continued
- 03/2001 – 08/2010 Master studies in *Technical Chemistry* at Vienna University of Technology, Austria; specialization in Technical Biochemistry and Technology of Natural Resources;
- 09/2009 – 08/2010 Master thesis at Vienna University of Technology, Austria; research group Bioresources Technology
Supervisors: Ao.Univ.Prof. Ewald Srebotnik
Docent Karin Fackler
- 10/2010 – 08/2016 Combined Doctorate studies at Vienna University of Technology, Austria, research group Bioresources Technology and at Royal Institute of Technology (KTH), Stockholm, Sweden, Division of Wood Chemistry and Pulping Technology
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Professional experience:

07– 08/2005 and 07– 08/2006 07– 08/2007	Internships as a workshop assistant at Freudenberg Sealing Technologies, Weinheim, Germany; Internship as a laboratory assistant at Freudenberg Simrit, Kufstein, Austria
09/2007 and 08-09/2008 03/2009	Internships as a laboratory assistant at Vienna University of Technology, Austria, Research group Bioresource Technology; Visiting researcher at KTH Stockholm, Division of Wood Chemistry and Pulping Technology, Sweden; Supervision: Docent Jiebing Li
10/2010 – 10/2012	Project Assistant at Institute of Chemical Engineering, Vienna University of Technology, Austria, research group Bioresource Technology;
11/2012 – 10/2013	Research Assistant at the Laboratory of Cellulose and Paper Technology at Federal University of Viçosa, MG-Brazil; Supervisor: Prof. Jorge L. Colodette
12/2013– 02/2015 04/2015	Career breaks for health reasons; Mechanic and sales assistant at Bike the Lake GmbH, Weiden am See, Austria;
05/2015	Visiting researcher in a Short Term Scientific Mission in the frame of EU COST Action FP1105 at Polyphenols Chemistry Laboratory, Tor Vergata University Rome, Italy; Supervisors: Prof. Claudia Crestini, Dr. Heiko Lange
06-07/2015	Mechanic and sales assistant at Bike the Lake GmbH, Weiden am See, Austria;
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03-06/2016	Laboratory assistant in the Division of Chemistry of Renewable Resources at the University of Natural Resources and Life Sciences, Tulln, Austria.