# Lignin fractionation strategies in LigninReSurf

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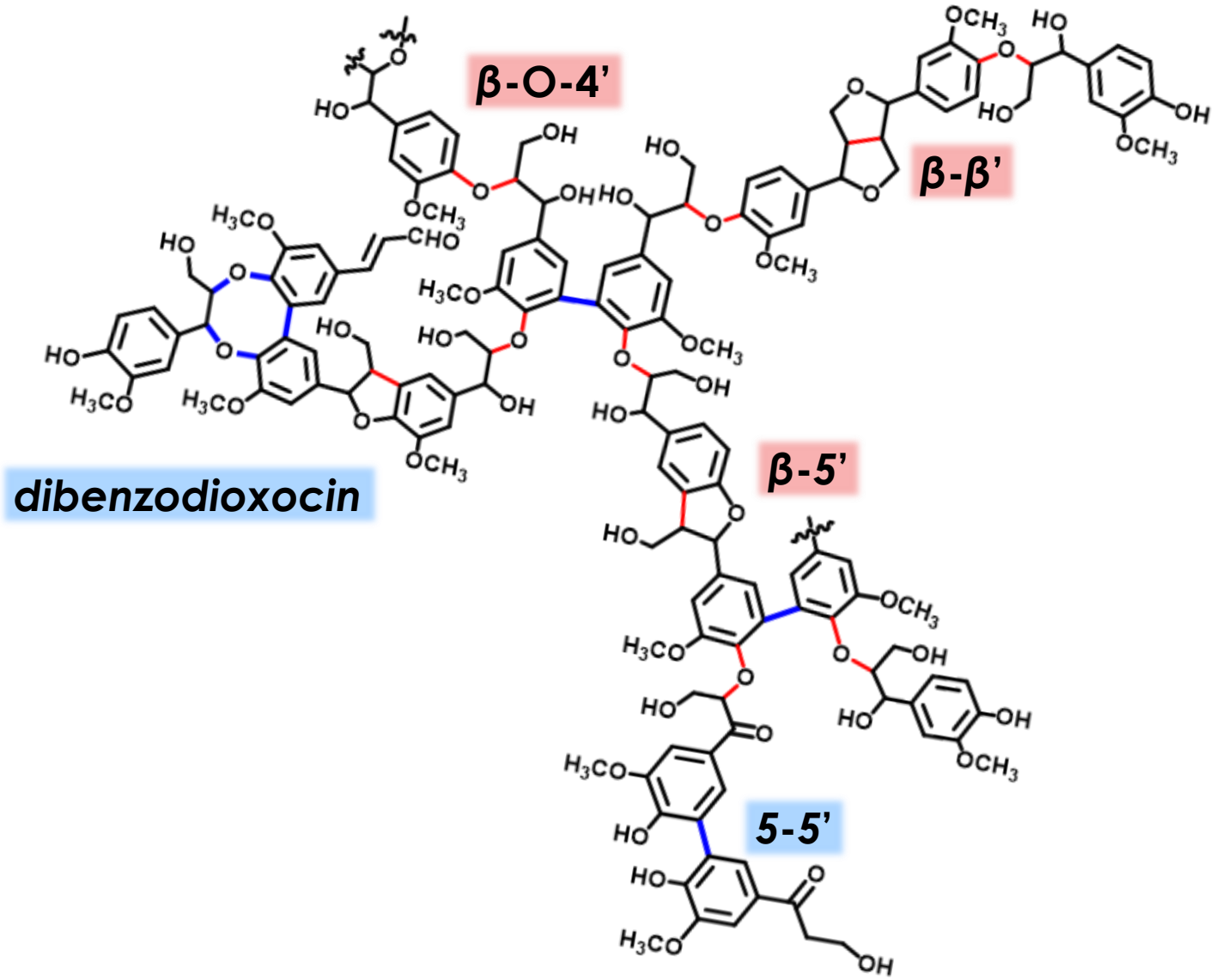
Åbo/Turku, February 6th, 2024

# Content

- Lignin fractionation methodologies applied in LigninReSurf
- Structure-property correlation on solvent-fractionated lignin to functional materials

# Technical lignin is very complex




**Key characteristics**

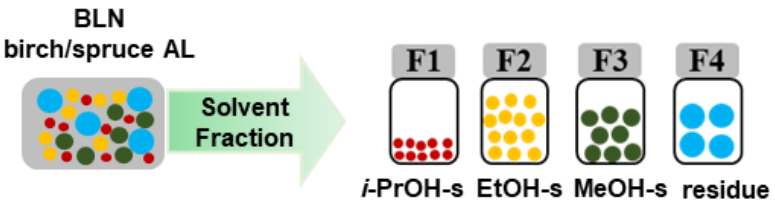
- Heterogeneous
- Polyphenol
- Polyol
- Can be functionalized
- Amorphous
- Cross-linked

Balakshin *et al*. (2020) Green Chemistry, 22, 3985-4001.

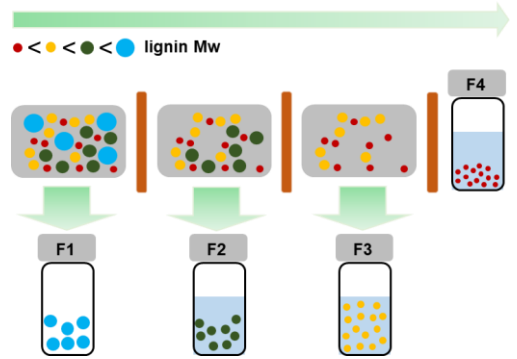
# WP1: Decrease lignin heterogeneity: Lignin fractionation

1) Sequential alcohol extraction
2) Membrane ultrafiltration
3) Simple ethanol/$H_2O$ extraction
4) Gradient acid precipitation

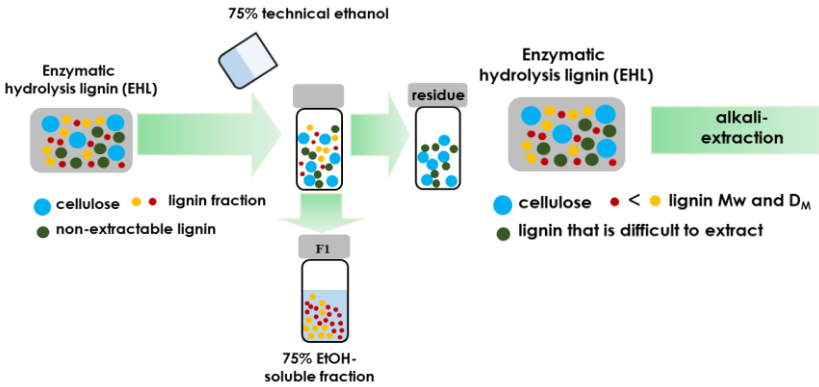
***1) Gradient polarity alcohol extraction***



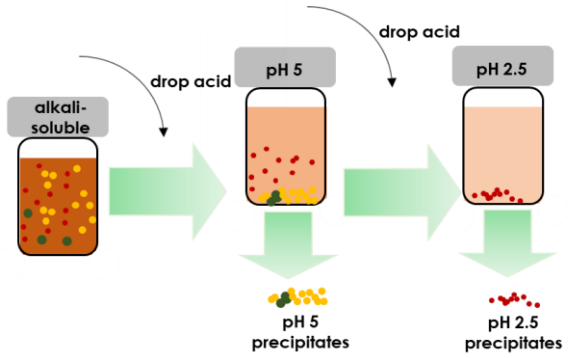

***2) Physical membrane filtration***




***3) Partial solubility in alcohol/water solution***




***4) Changing pH of alkali soluble lignin***





Wang L, Lagerquist L, Zhang Y, et al. Tailored Thermosetting Wood Adhesive Based on Well-Defined Hardwood Lignin Fractions. *ACS Sustainable Chemistry and Engineering*. 2020;8(35):13517-13526.
Liu R, Smeds A, Wang L, et al. Fractionation of Lignin with Decreased Heterogeneity: Based on a Detailed Characteristics Study of Sequentially Extracted Softwood Kraft Lignin. ACS Sustainable Chemistry & Engineering. 2021, 9( 41), 13862–13873.

# 16 lignin fractions was selected in LigninReSurf project

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| Lignins from Finnish biorefinery process | | Lignin fractions |
|---|---|---|
| UPM BioPiva™ 300 and 350 Kraft lignin | | Reference lignins |
| CH-Bioforce alkaline lignin | Birch lignin concentrate[a] (lower NaOH dosage) | • **Membrane ultrafiltration (50, 10, and 3 kDa)** |
| | Spruce lignin concentrate[a] (lower NaOH dosage) | |
| | Birch lignin (dry powder) | • **Sequential alcohol fractionation (*i*-PrOH, EtOH, and MeOH)** |
| | Spruce lignin (dry powder) | |
| | Wheat straw (dry powder) | |
| Enzymatic hydrolysis lignin (softwood lignin, St1) | | • **96% and 75% ethanol/$H_2O$ extraction**<br>• **75% ethanol/$H_2O$ extraction**<br>• **Gradient acid precipitation (pH 5 and pH 2.5 precipitates)** |
| LignoBoost softwood lignin fractions (as received, Metgen) | | • **Membrane ultrafiltration (70 KDa resident, 70-10 kDa, 10 kDa permeate)** |

# Lignin analysis as a key tool for structure-property correlation

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## Compositional analysis

- Lignin content (Klason lignin & UV-vis)
- Sugar content (acid methanolysis & acid hydrolysis)
- Extractive content (GC-FID)

## Structural characteristics

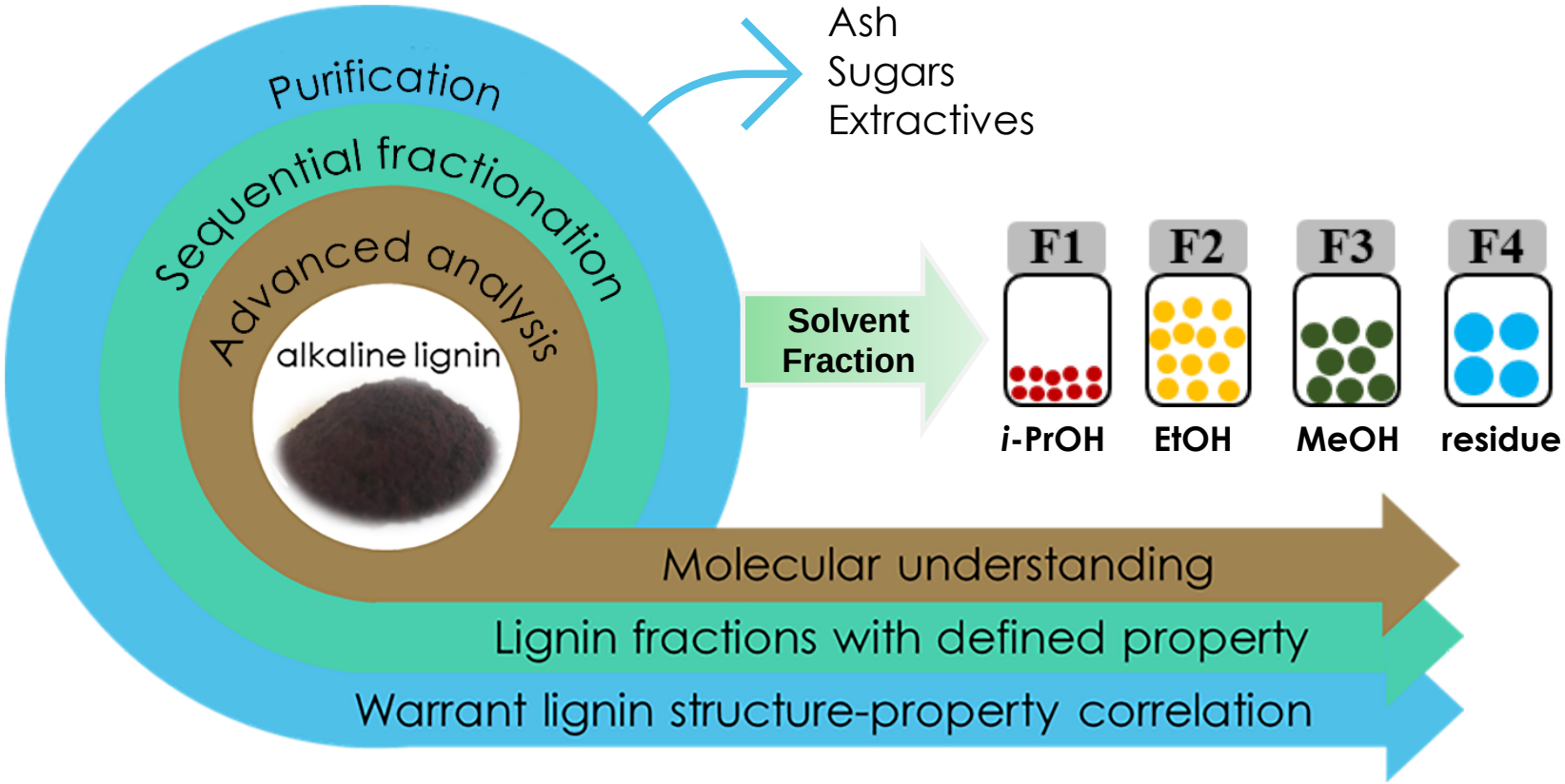
- Molar mass and molar mass dispersity (SEC-MALS-RI)
- Monolignol compositions (S/G unit ratio)
- Interunit linkages (HSQC & $^{13}$C NMR)

## Functionality

- Hydroxyl group distribution ($^{31}$P NMR)
- Methoxyl group ($^{1}$H NMR)
- Carbonyl group (FTIR)
- Condensation degree ($^{13}$C NMR)

# Strategies in the LigninReSurf project

- Decrease lignin heterogeneity
- Establish lignin structure-property correlations in specific applications



# Lignin was refined by solvent fractionation

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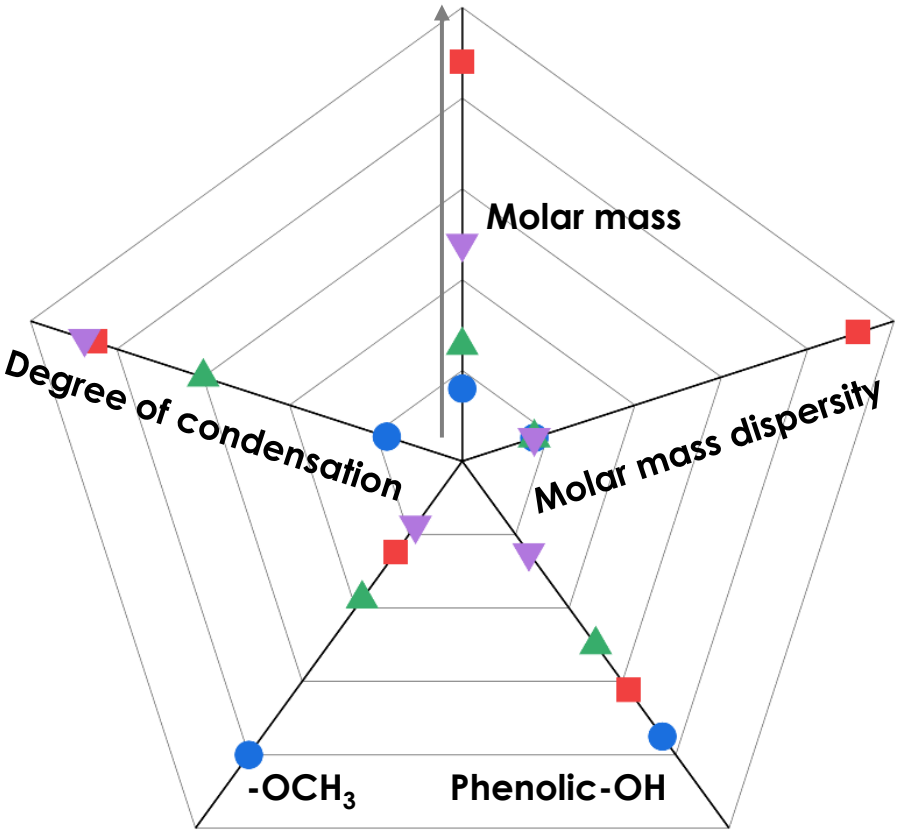
Spruce alkaline lignin



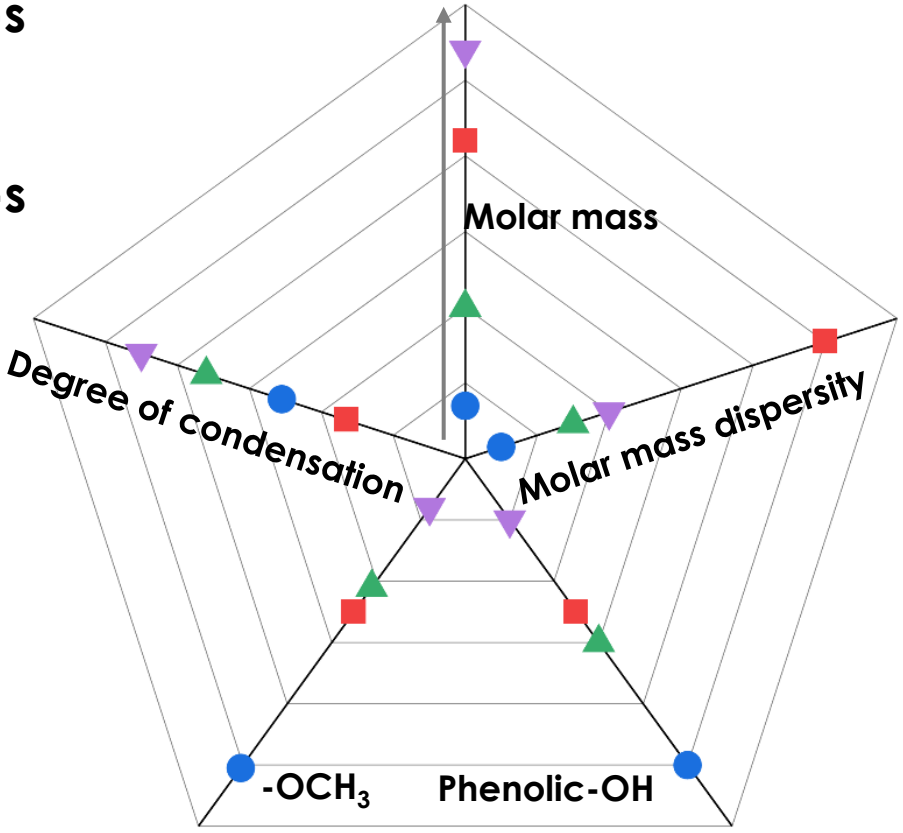

raw

*i*-PrOH-s

EtOH-s

MeOH-s

Birch alkaline lignin



# Pilot-scale lignin black liquor membrane filtration using cross-flow mode

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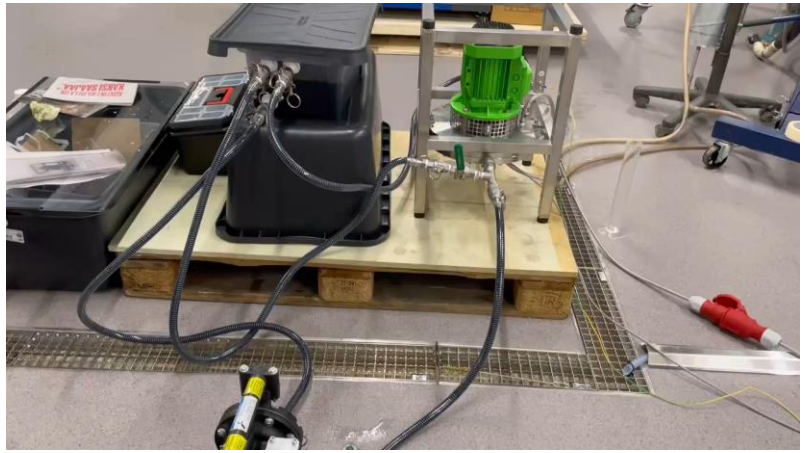

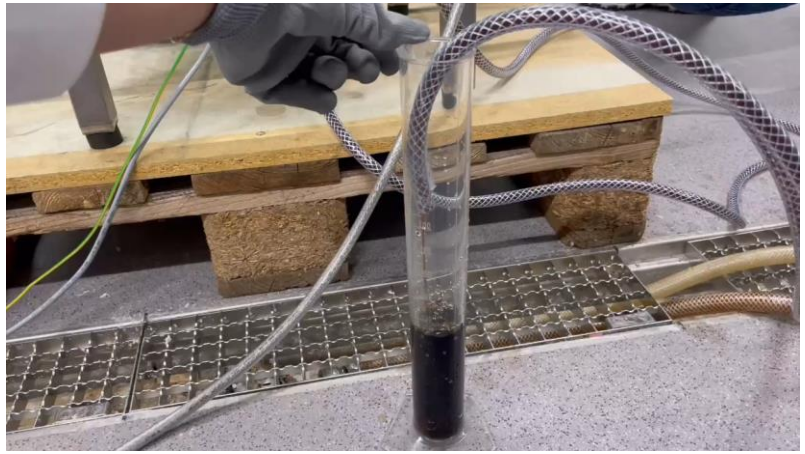

*CH-B birch black liquor 50 KDa PSE permeate*

| | Lab scale | Pilot scale |
|---|---|---|
| **Starting concentration** | 100 g/L | 100 g/L |
| **Volume** | 0.2 L | 8.0 L |
| **Stirring** | rotation 200 rpm | circulation/Rotation |
| **Pressure** | 4.75 bar | 0.8 – 1 bar |
| **Mode** | dead-end | cross-flow |

Polyethersulfone membranes for pilot-scale filtration: 150 kDa, 50 kDa, 30 kDa, 4 kDa

# Lignin structure-property correlation: Case studies

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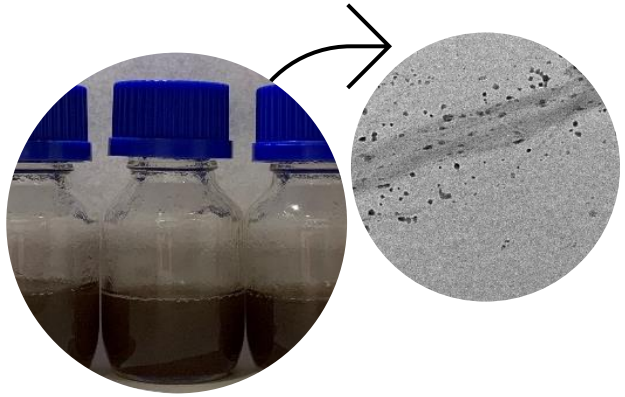
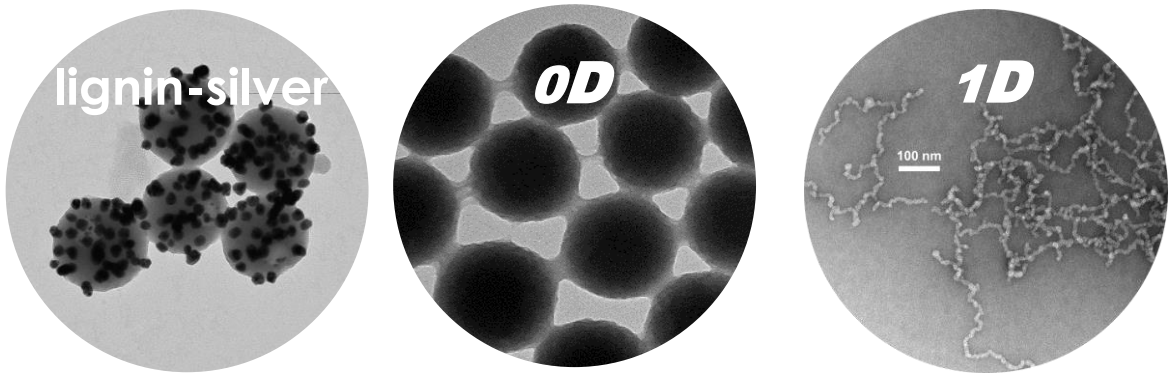


I. Cellulose-lignin dispersion




II. Nanostructured lignin

# Biocatalysis-Laccase

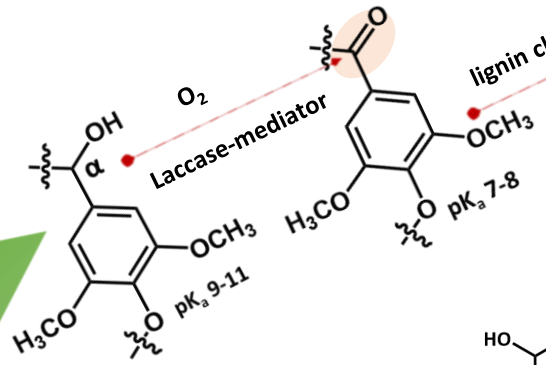
- Operate in mild conditions
- Require less energy
- Use non-toxic chemicals

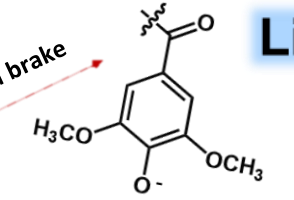
Technical lignin

Laccase

lignin subunits

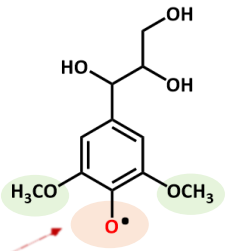
polymeric material



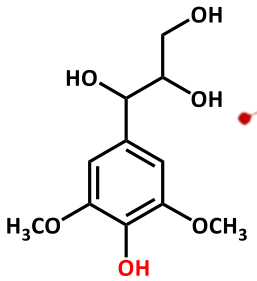




aromatic monomers and oligomers

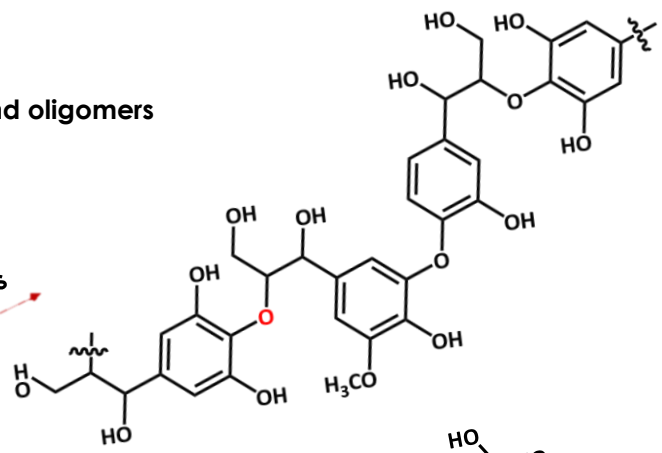
**Lignin depolymerization**



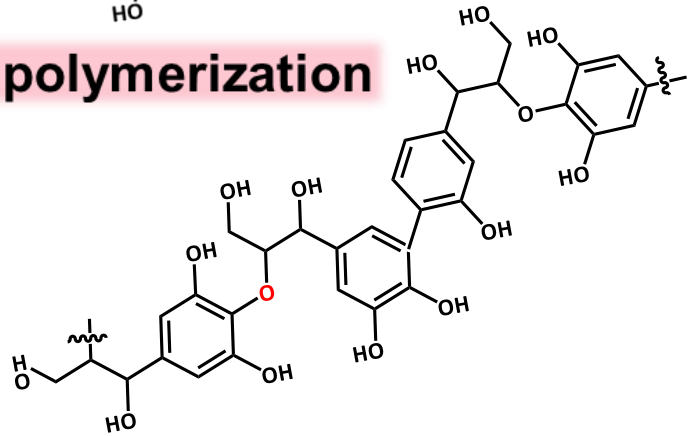

phenoxy radical




macromolecules




**Lignin polymerization**




Hämäläinen et al. (2018) Frontiers in bioengineering and biotechnology, 6, 20.

# WP2: Laccase-catalyzed lignin polymerization

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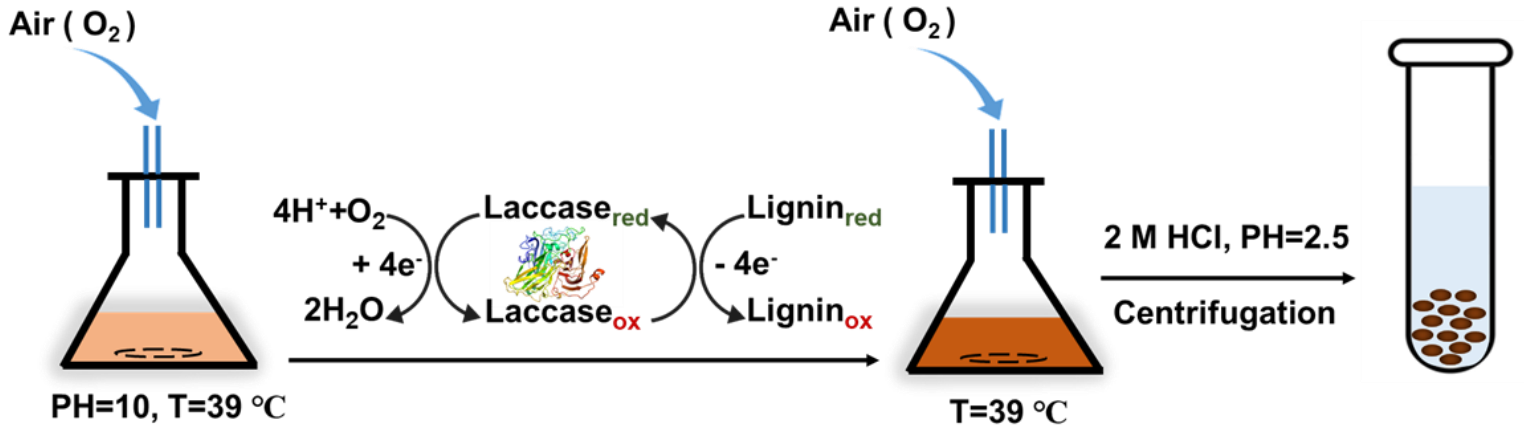


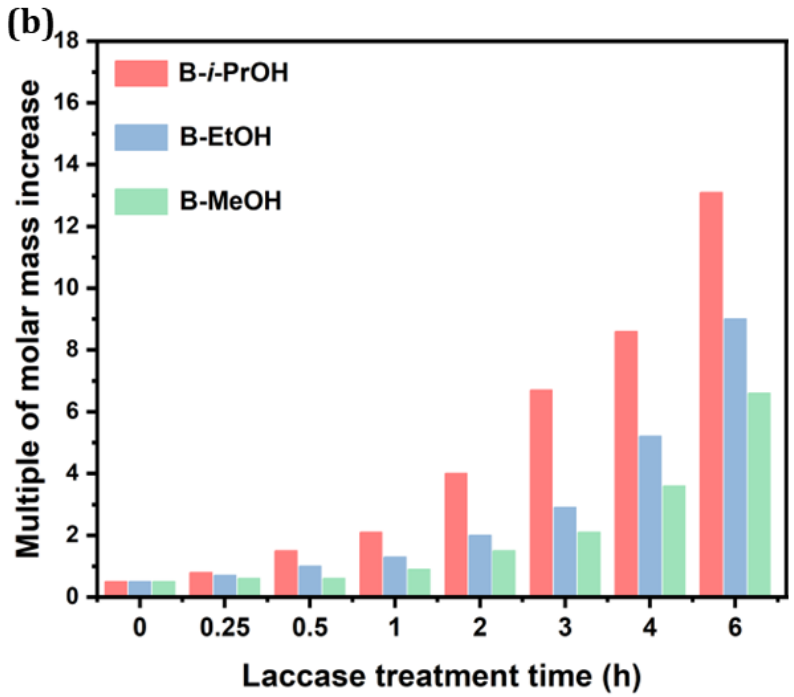


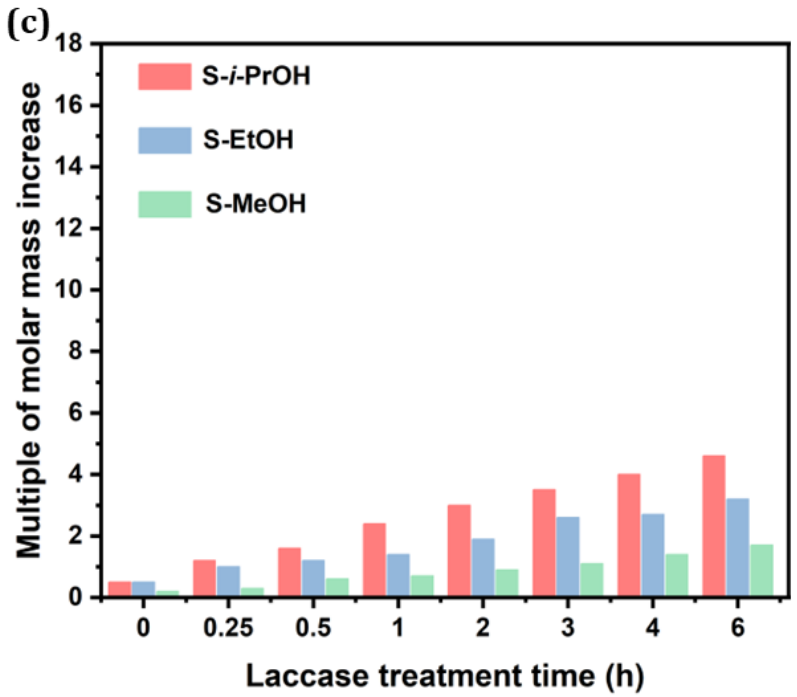

# *In situ* polymerization in fiber suspension for dispersion barrier coating

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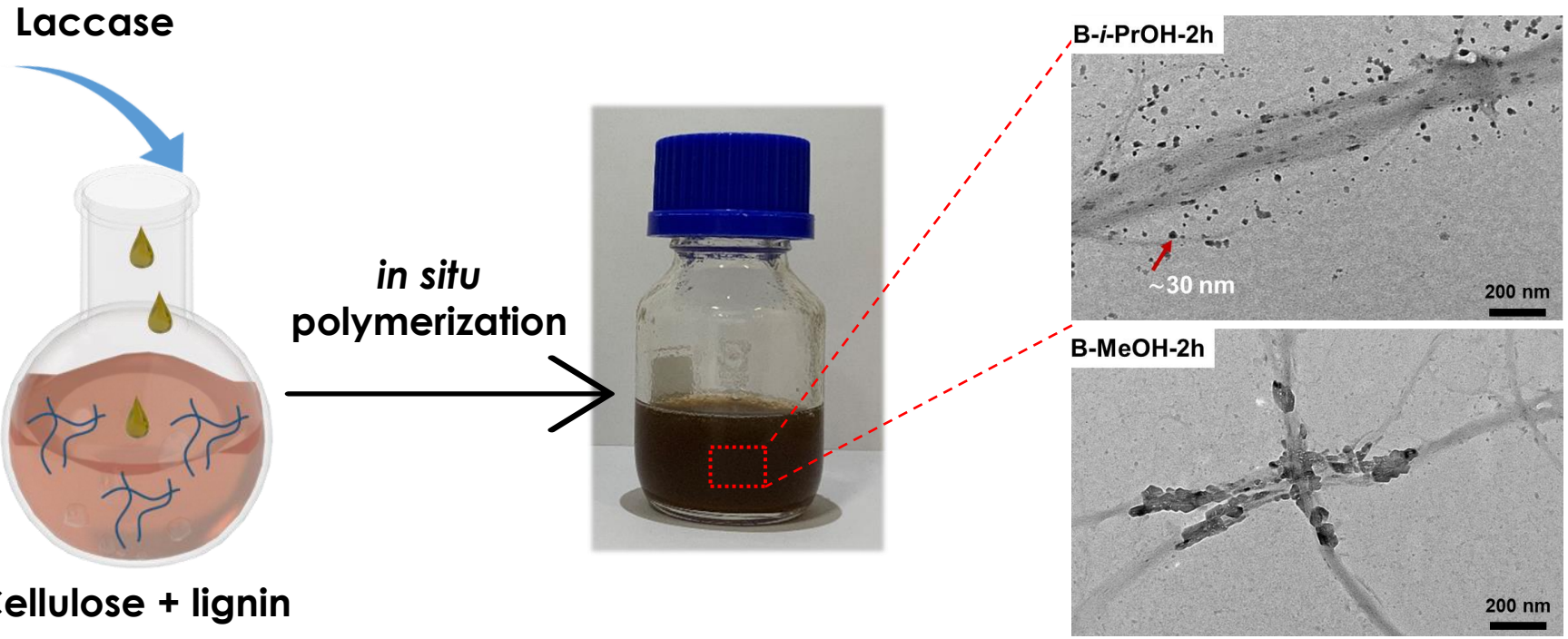
- **A new templating approach to modulate the morphology and dispersibility of lignin in nanocellulose matrix was developed**
  - Lignin fraction with high phenolic-OH content has better interfacial interaction with cellulose fiber



# Alkali-resistant lignin nanoparticles from laccase-treated lignin

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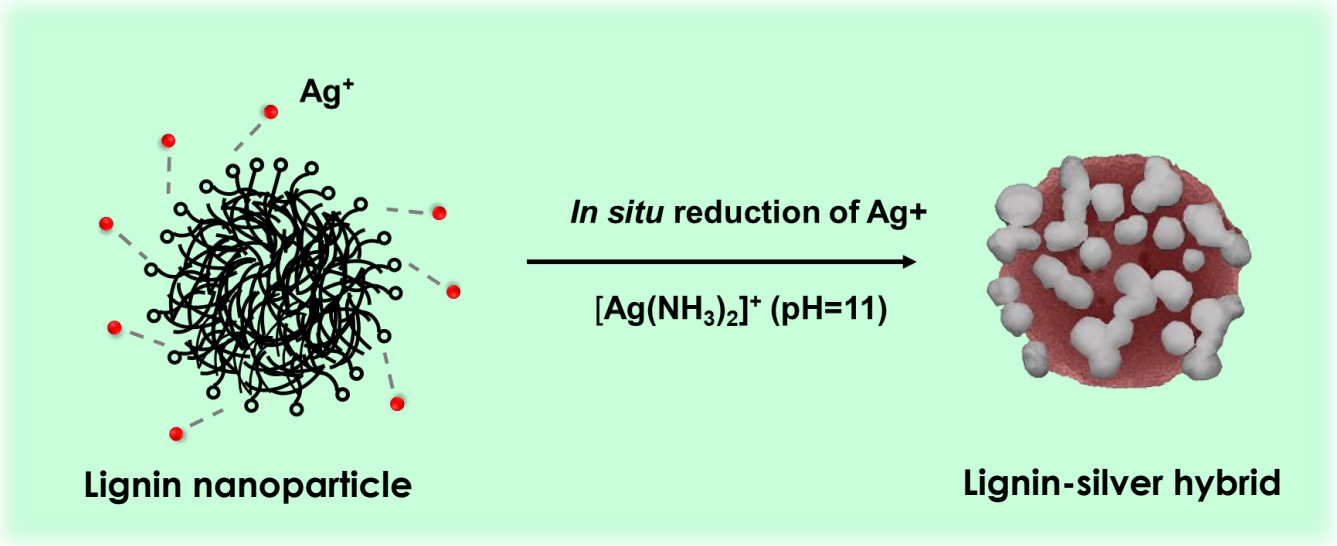
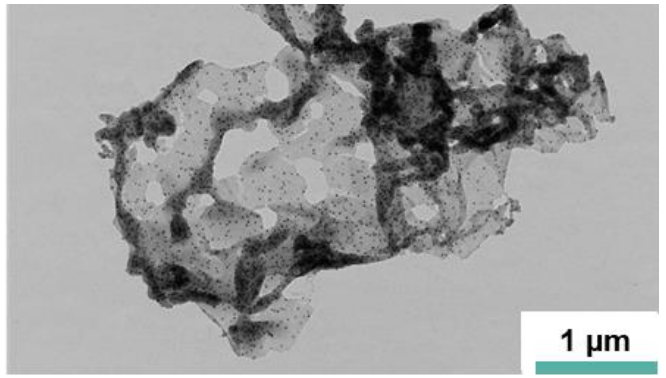
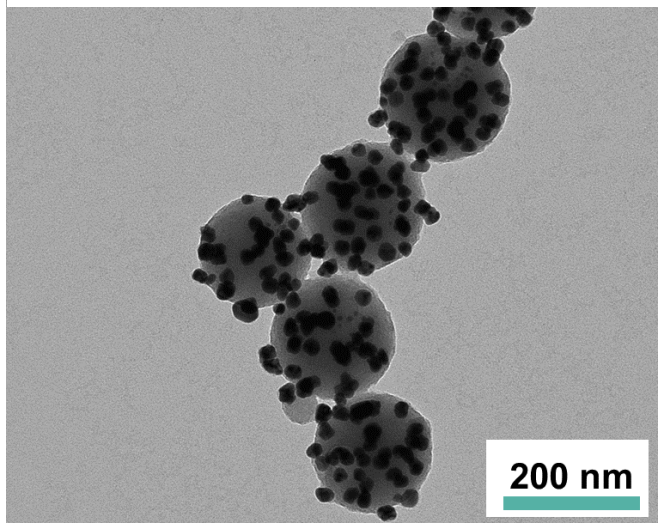



untreated lignin




laccase-polymerized B-MeOH lignin



# Fractionation balances alkali stability and surface reactivities of lignin nanospheres

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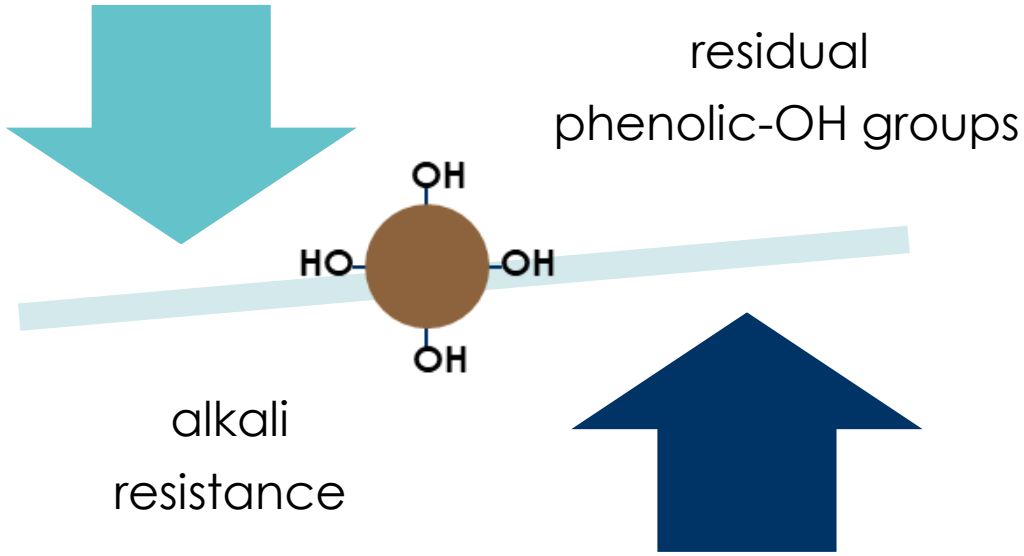
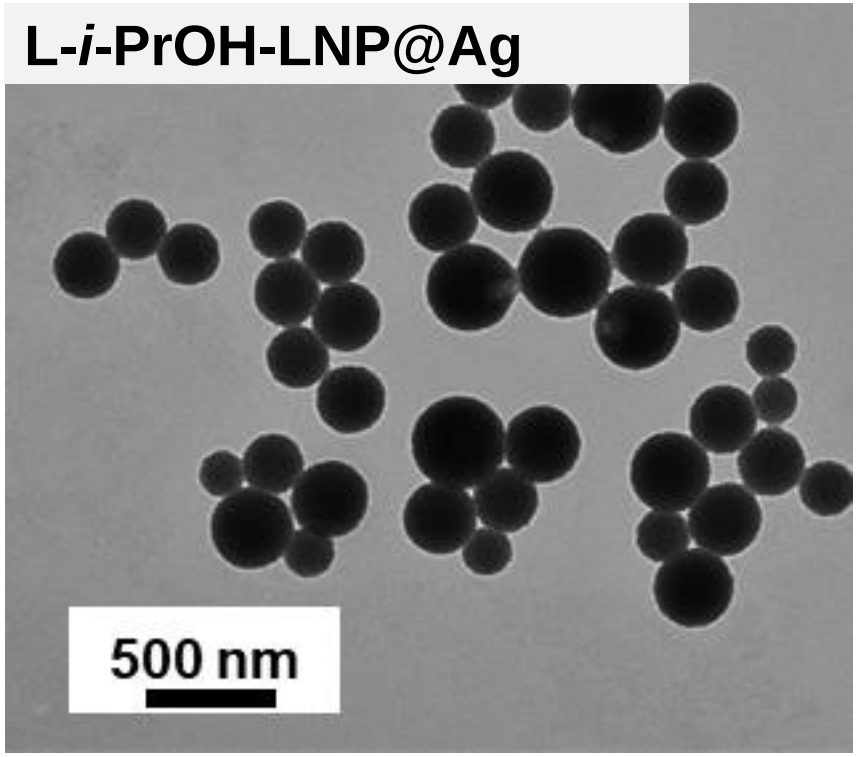
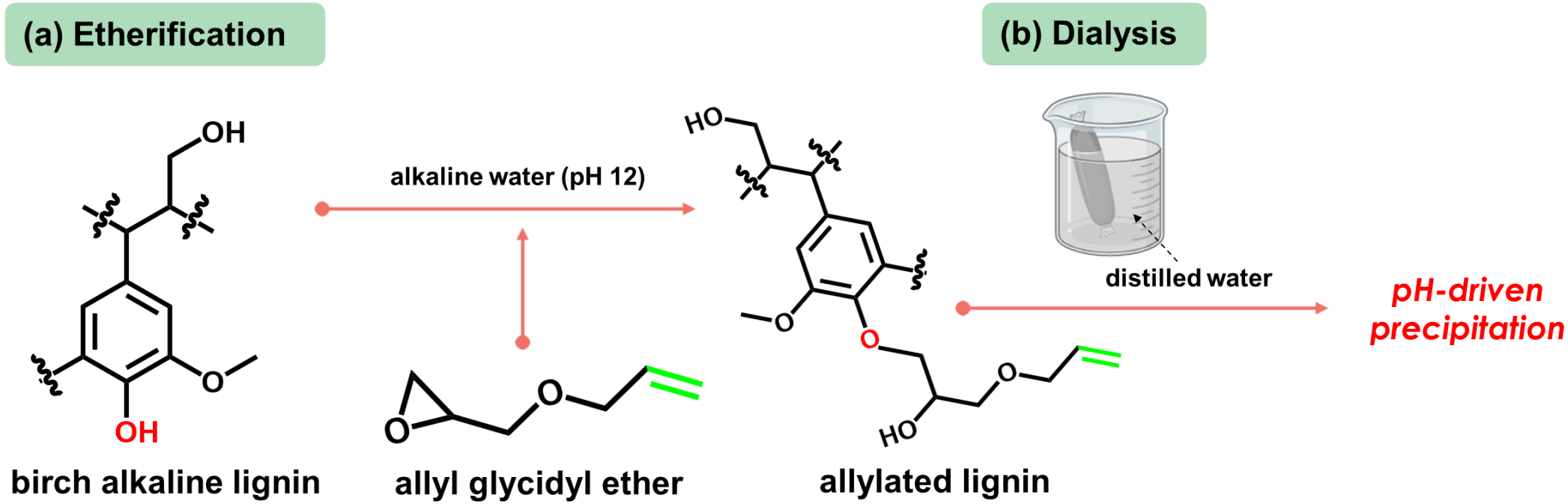


# WP3: Preparation of surface-active lignin nanoparticles

- All industrial lignins from the LigninReSurf project (*e.g.*, kraft lignin, alkaline lignin, enzymatic hydrolysis lignin) and their fractions were subjected to **allylation**



Not published work

# Lignin nanospheres and lignin nanofibers

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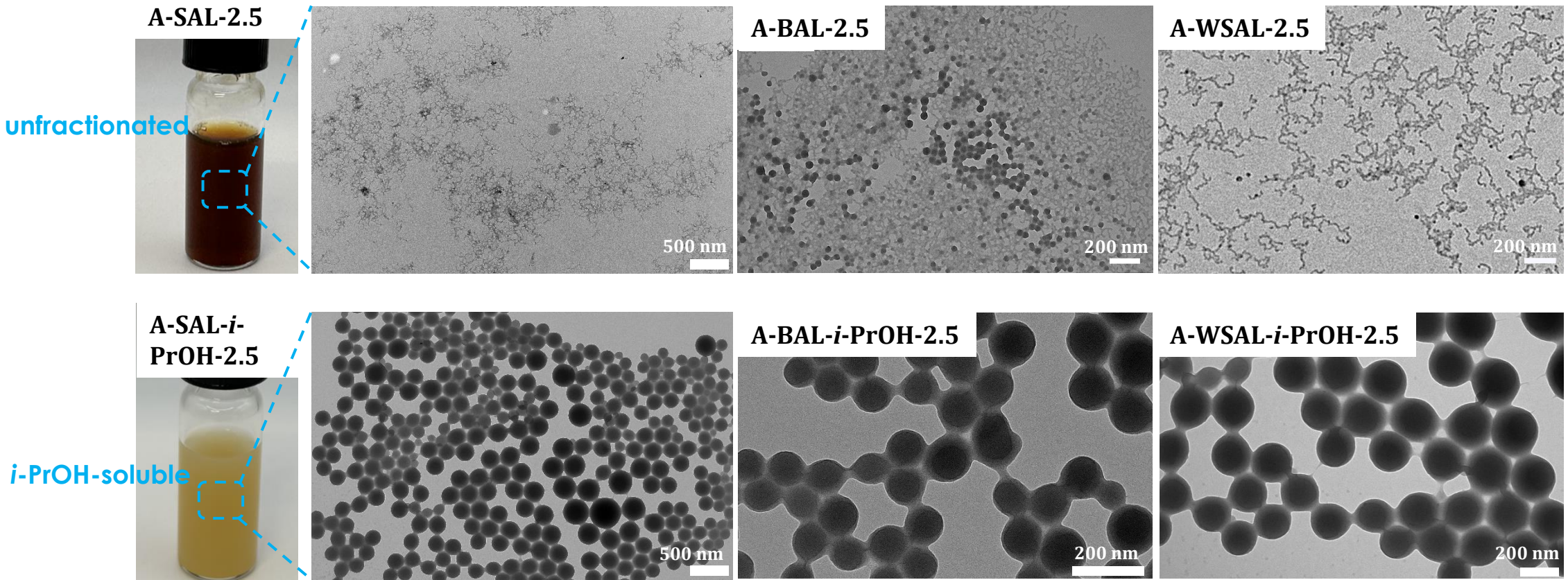
- Two distinct nano/microstructures were observed by lignin solvent fractionation
  - Lignin with a low molar mass and narrow $Đ_M$ favored the formation of lignin nanospheres



# Conclusions

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- Solvent fractionation/membrane filtration are effective strategies to decrease lignin heterogeneity and can be used as a tool to select lignin molecules for certain applications

- Lignin fractions with low degree of condensation and high content of phenolic-OH groups can be effectively integrated to polymeric networks, such as laccase-polymerized lignin

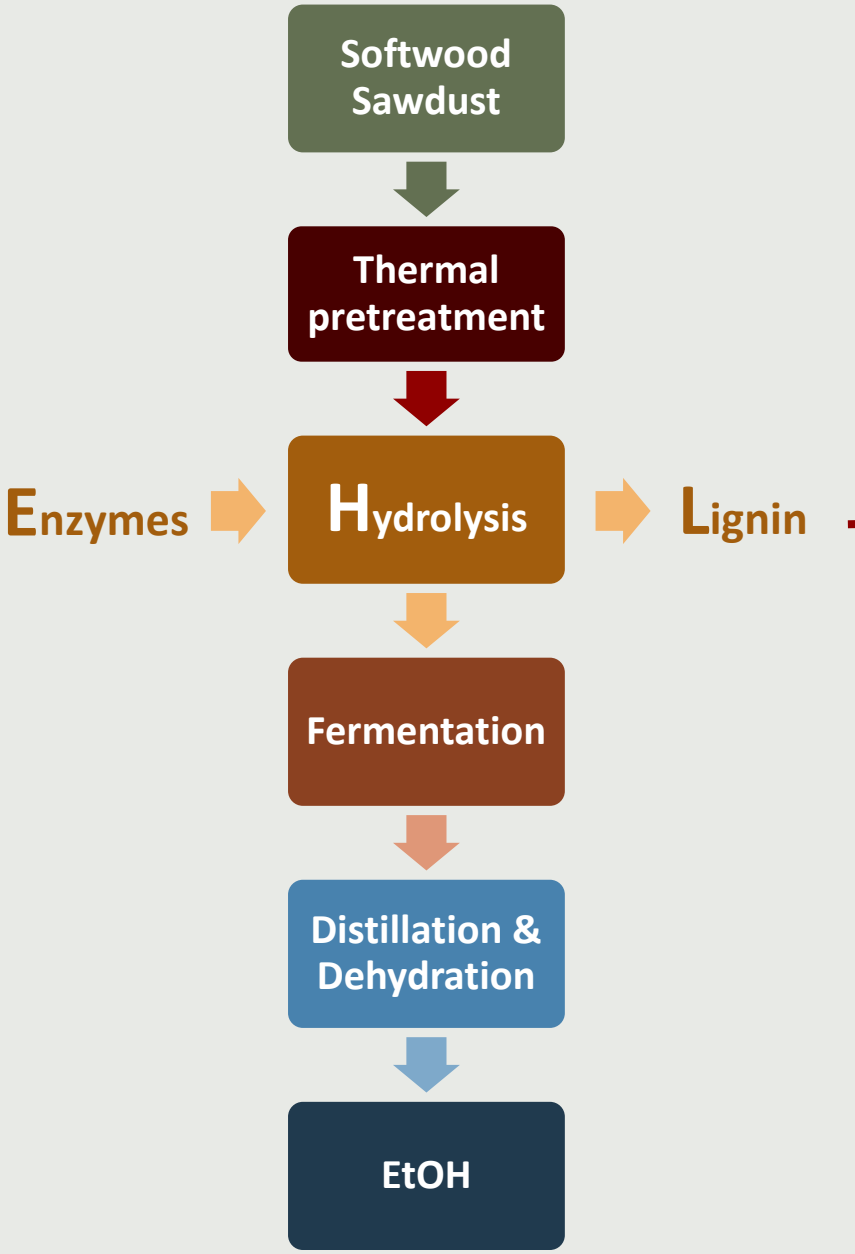
- Lignin fractions with a low molar mass and narrow $Đ_M$ favored the formation of surface-active lignin nanospheres during the pH-driven nanoprecipitation of allylated lignin

# Enzymatic Hydrolysis Lignin Fractionation and Characterisation

Minette Kvikant

Åbo, February 6th, 2024

# Cellunolix®
# Biorefinary concept




# Lignin fractionation
## From Crude and Purified EHL

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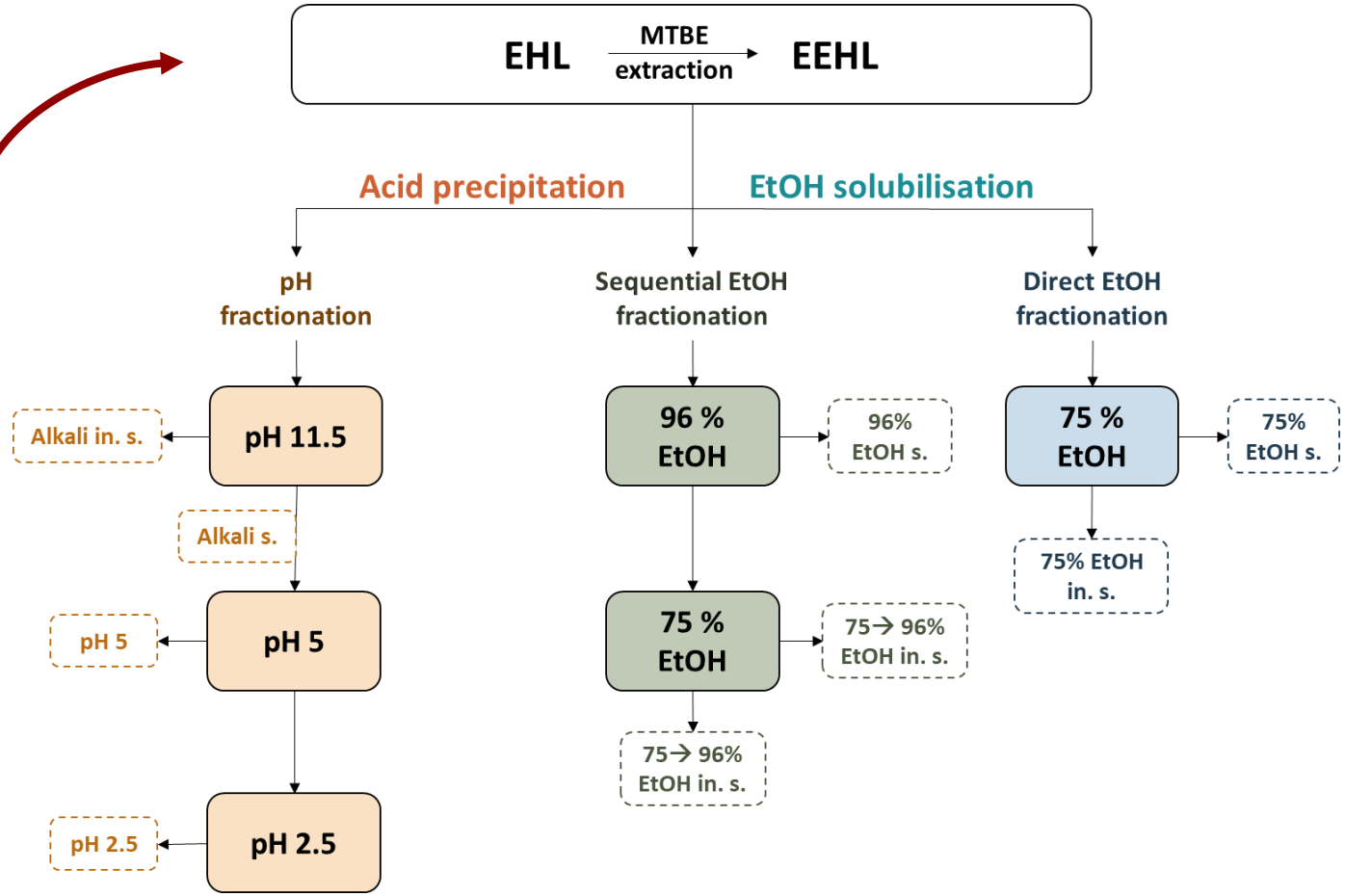
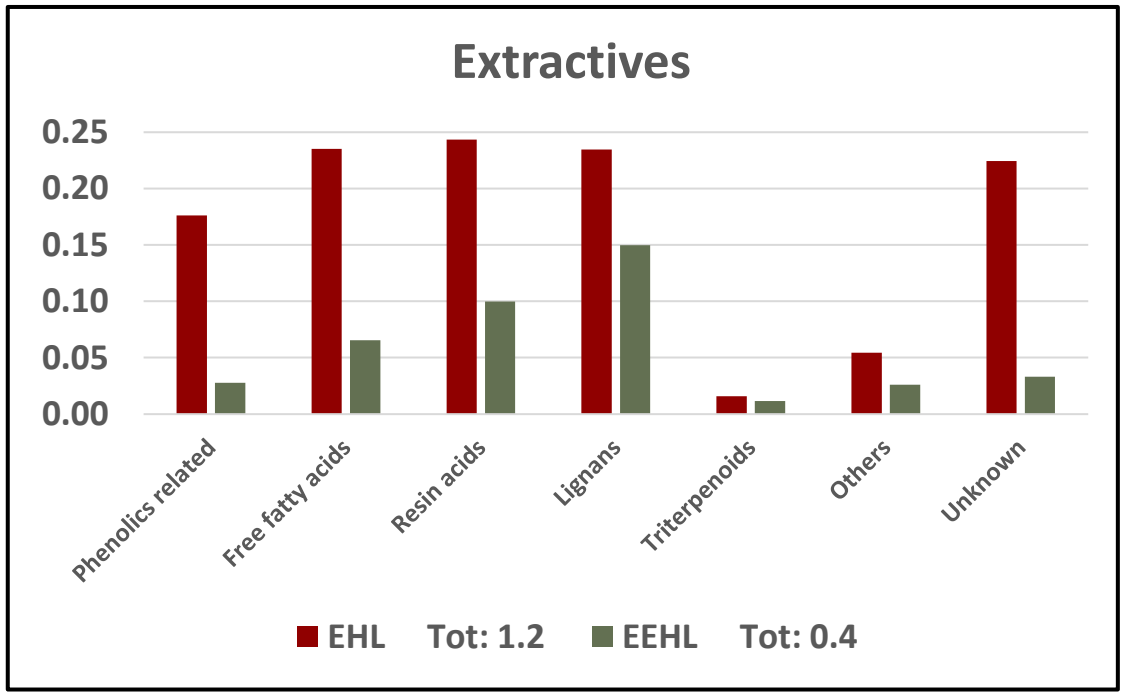


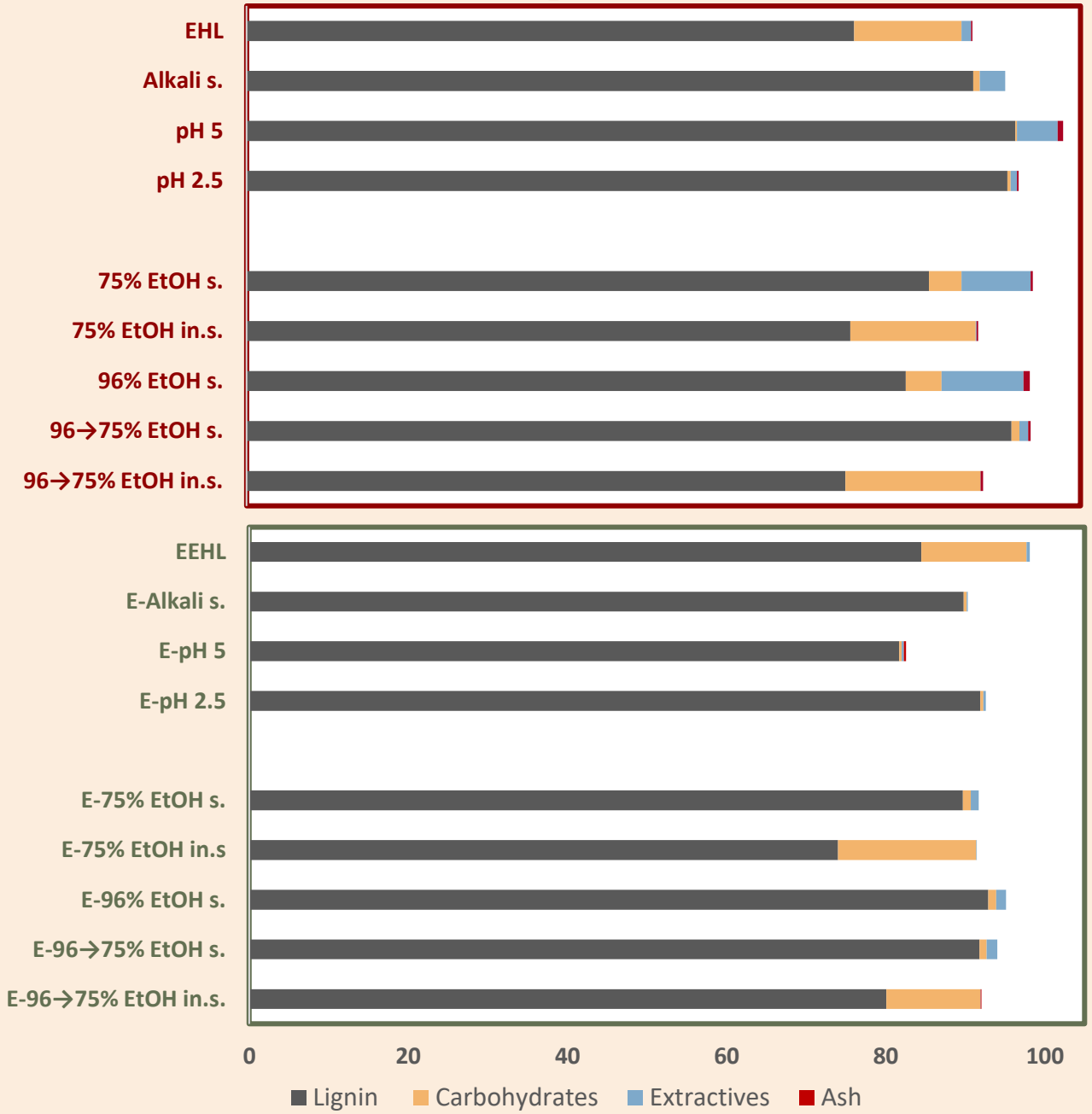
# Purification

**Crude EHL was washed with acid water and extracted with MTBE**

**→ Removing extractives from lignin**




# Composition

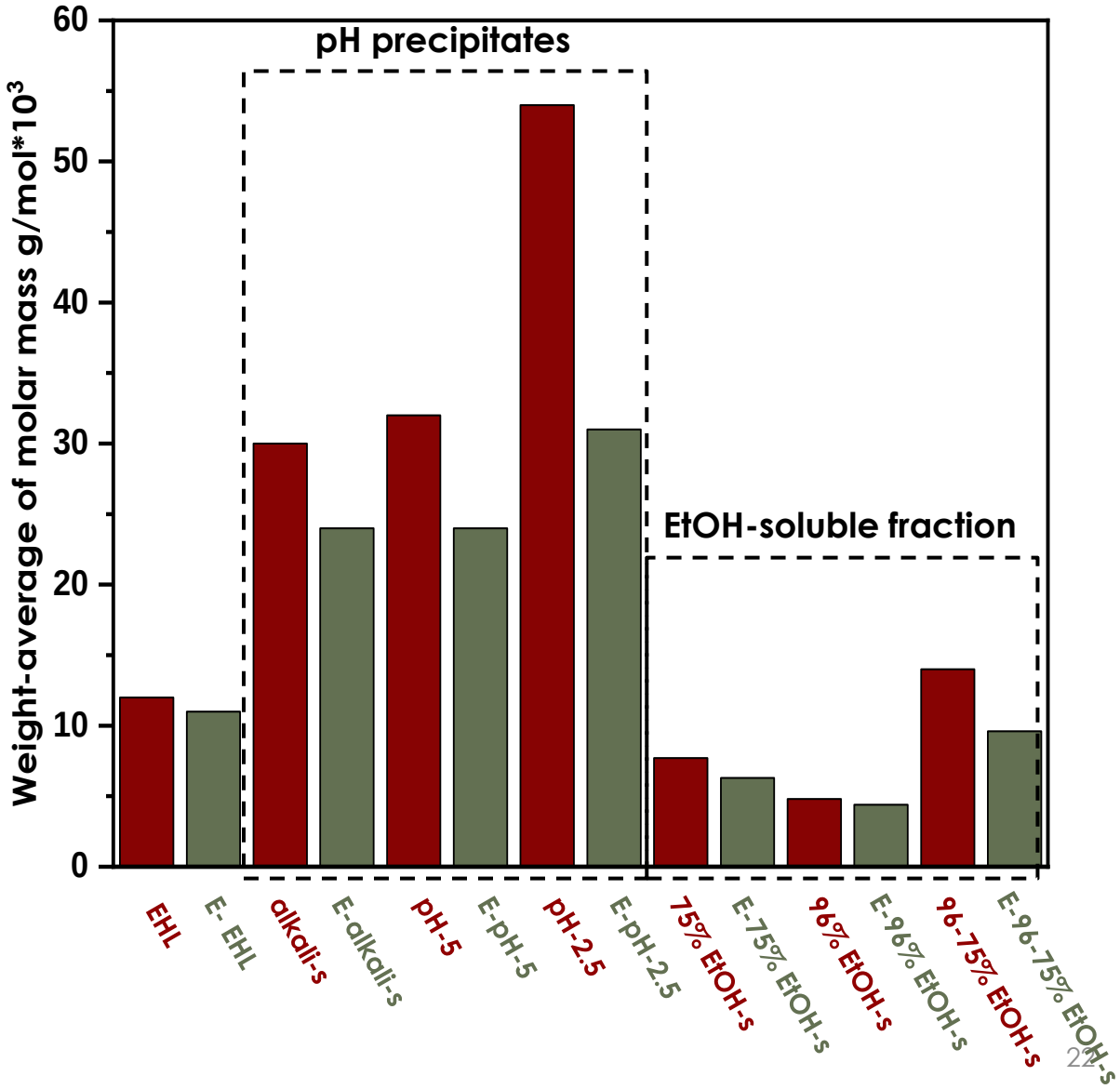


# Molar mass (GPC/MALS)

**Comparison of lignin fractions from EHL and purified EEHL**

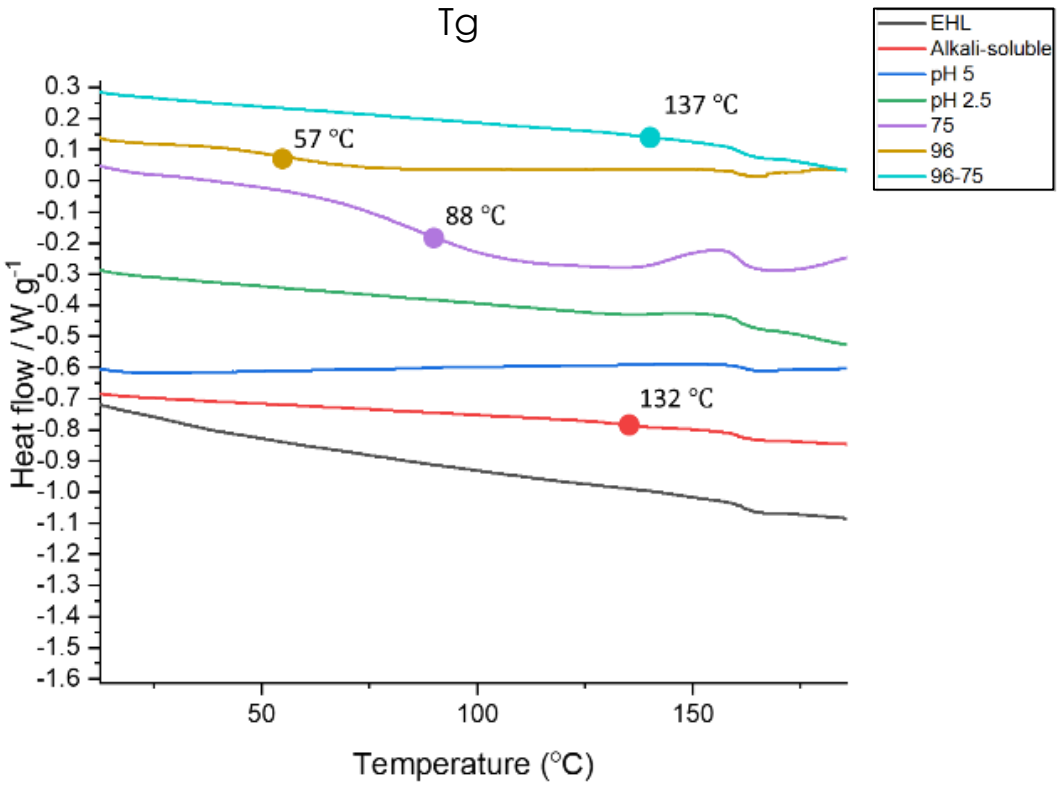
**EEHL exhibited lower molar mass values**

**Molar mass of EtOH-soluble fractions displayed lower values than pH fractions**

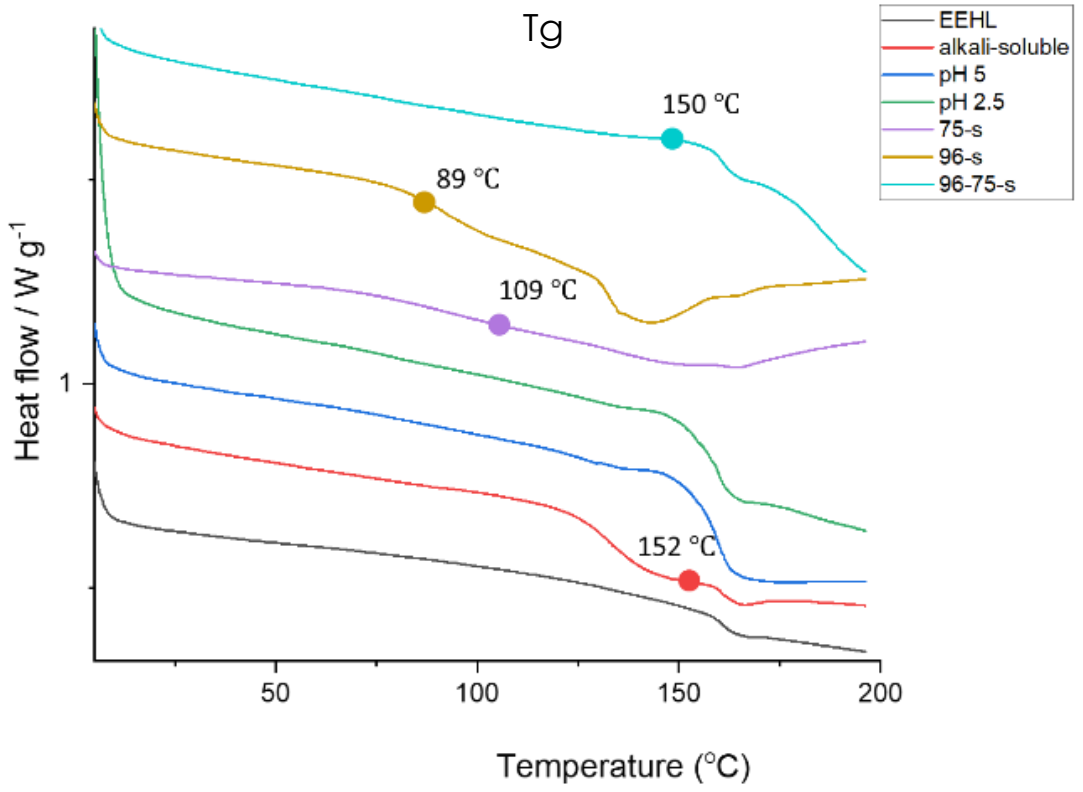


# Thermal properties

## EHL fractions




## EEHL fractions




DSC analysis temperature protocol: 40 to 120 ° C (10 ° C min$^{-1}$, isothermal for 2 min) to 0 to 200 ° C (20 ° C min$^{-1}$) under nitrogen atmosphere (50 mL min$^{-1}$). [c]Not detectable between 0 and 200 ° C. The midpoint of the inflection in the second heating trace is reported as Tg.

# Conclusion

MTBE extraction successfully purified the EHL

→ Revealing lignin structure-property correlation for further modification

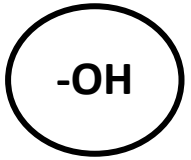
## pH fractions:

Higher Yield

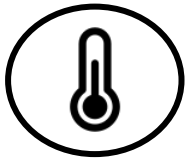
Purer fractions

Higher molar mass

## EtOH fractions:




More hydroxyl groups



Soluble fraction displayed *Tg*

Insoluble fractions show promising mechanical properties

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# Want to learn more?

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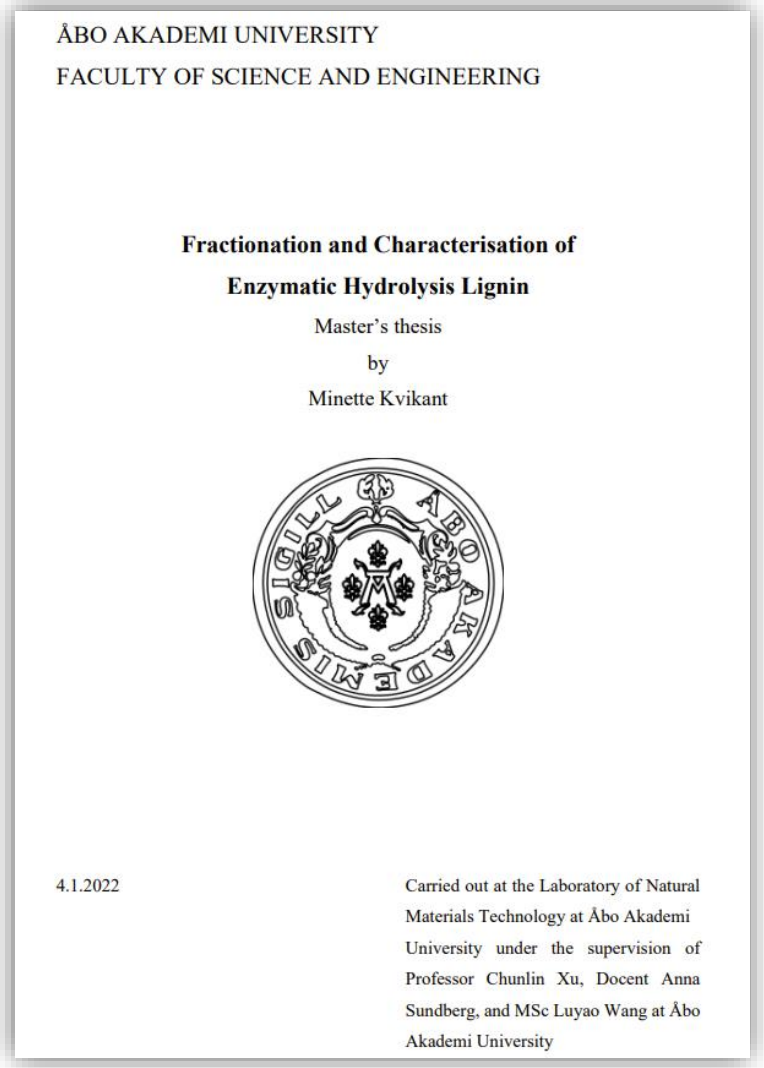
You can continue reading on my MSc Thesis:



ÅBO AKADEMI UNIVERSITY

FACULTY OF SCIENCE AND ENGINEERING

**Fractionation and Characterisation of Enzymatic Hydrolysis Lignin**

Master's thesis

by

Minette Kvikant

4.1.2022

Carried out at the Laboratory of Natural Materials Technology at Åbo Akademi University under the supervision of Professor Chunlin Xu, Docent Anna Sundberg, and MSc Luyao Wang at Åbo Akademi University