KTH ROYAL INSTITUTE
OF TECHNOLOGY

Licentiate Thesis in Fiber and Polymer Science

Bark Biorefinery

Isolation, Characterization and Application

ISABELLA KWAN

Stockholm, Sweden 2023

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Academic Dissertation which, with due permission of the KTH Royal Institute of Technology, is submitted for public defence for the Degree of Licentiate of Engineering on Friday the 20th October 2023, at 1:00 p.m. in Treesearch conference room, Teknikringen 38, Stockholm.

Licentiate Thesis in Fiber and Polymer Science
KTH Royal Institute of Technology
Stockholm, Sweden 2023

TRITA-CBH-FOU-2023:44
ISBN 978-91-8040-704-5

Printed by: Universitetsservice US-AB, Sweden 2023

To my dear family

Abstract

To fulfill the United Nations' 17 sustainable development goals, there is a need to transition from a petroleum-based society toward a more sustainable one which requires new solutions and the production of materials, chemicals, and energy from renewable resources. Using side-stream products from industries to produce value-added products will be economically beneficial. Furthermore, finding more environmentally friendly process routes will aid industries in achieving their goal of reducing carbon dioxide emissions and contributing to a more sustainable society.

Tree bark is the outer protective layer of a tree and today, mills and factories incinerate the bark to produce energy. Norway spruce (*Picea abies*) bark consists of cellulose, lignin, hemicellulose, and extractives. Applying the biorefinery concept makes it possible to extract and isolate these compounds in the bark and utilize them to produce high-value materials and chemicals.

This thesis applies the biorefinery concept to isolate cellulose and lignin from spruce bark by using mild extraction processes and more eco-friendly chemicals.

Cellulose is the most abundant compound in spruce bark and has great potential to be used in various applications. The isolation of cellulose fibers from bark was in the present work carried out by first removing the extractives and non-cellulosic polysaccharides via sequential extractions using acetone and subcritical water. Nanocellulose was isolated from the bark cellulose and used to produce Pickering emulsions successfully. This proves that using side-stream products such as bark is feasible to produce high-value products like emulsions.

Lignin is the second most abundant compound in the spruce bark. Following the acetone and subcritical water extractions, a mild cyclic organosolv extraction sequence was applied to be able to recover spruce bark lignin. The lignin extracted was comparable to lignin extracted from wood. Stilbene glucosides incorporated in the bark lignin provide the lignin with additional beneficial properties, i.e., antimicrobial and antioxidative. This elevates the value of the lignin further and makes it suitable for a variety of different applications.

Keywords: sustainability, bark, biorefinery, Norway spruce, nanocellulose, Pickering emulsion, organosolv, extraction, lignin.

Sammanfattning

För att kunna uppnå Förenta Nationernas 17 globala hållbarhetsmål behöver det ske en förändring från ett oljebaserat samhälle till ett mer hållbart samhälle. Detta innebär att det finns ett behov för nya hållbara lösningar och material, kemikalier och energi som produceras med förnybara råvaror. Att utnyttja sidoströmmar från industrier för att producera högvärdiga produkter kommer vara ekonomiskt fördelaktigt. Dessutom, att finna mer miljövänliga processer kommer att bidra till att hjälpa industrier att uppnå deras mål med att minska koldioxid-utsläppen och bidra till ett mer hållbart samhälle.

Trädbark är det yttersta skyddande lagret på ett träd och idag förbränner bruk och fabriker den för att skapa energi. Granbarken består av cellulosa, lignin, hemicellulosa, och extraktivämnen. Att använda bioraffinaderikonceptet gör det möjligt att extrahera och isolera komponenterna i barken och vidare producera högvärdiga material och kemikalier.

Denna avhandling applicerar bioraffinaderikonceptet för att isolera cellulosa och lignin från den inre granbarken genom att använda milda extraktions-processer och mer miljövänliga kemikalier.

Cellulosa är den vanligaste förekommande komponenten i bark och har stor potential att användas för olika tillämpningar. Isoleringen av cellulosafibrer från bark genomfördes i detta arbete först genom att avlägsna extraktivämnen och icke-cellulosahaltiga polysackarider via sekventiella extraktioner med aceton och subkritiskt vatten. Nanocellulosa isolerades från barkcellulosan och användes för att producera Pickering emulsioner med framgång. Detta visar att det är möjligt att utnyttja en produkt såsom bark från en sidoström till att skapa högvärdiga produkter som emulsioner.

Lignin är den näst mest vanligt förekommande komponenten i bark. Efter extraktion med aceton och subkritiskt vatten genomgick barkfibrerna en sekventiell cyklisk organosolv-extraktion för att erhålla gransbarkslignin. Det extraherade ligninet var jämförbart med lignin som extraherats från ved. Stilbene glykosider som är inkorporerade i barkligninet ger ligninet ytterligare fördelaktiga egenskaper, exempelvis antimikrobiella och antioxidativa. Detta förhöjer värdet ännu mer och gör den lämplig för flera olika tillämpningar.

Nyckelord: hållbarhet, bark, bioraffinaderi, gran, nanocellulosa, Pickering emulsioner, organosolv, extraktion, lignin.

List of appended papers

This thesis is a summary of the following two appended papers.

Paper I

Emulsions of cellulose oxalate from Norway spruce (*Picea abies*) bark and dissolving pulp
Isabella Kwan, Barbara Rietzler, Monica Ek
Holzforschung, 2023, 77 (7), 554-565

Paper II

Fundamental Insights on the Physical and Chemical Properties of Organosolv Lignin from Norway Spruce Bark
Barbara Rietzler, Maria Karlsson, Isabella Kwan, Martin Lawoko, Monica Ek
Biomacromolecules, 2022, 23 (8), 3349-3358

Contributions to the appended papers

The author's contributions to the appended papers are as follows:

Paper I. Lead author. Major part of the planning, designing, and performing the experimental work, analyzing all data, and writing/editing the manuscript.

Paper II. Third author. Performed part of the experimental work (extractions, THF-SEC, carbohydrate analysis, and NMR), analyzed part of the data (yield, chemical composition, and THF-SEC), and edited a major part of the submitted manuscript.

Publications not included in the thesis

Bark from Nordic tree species – a sustainable source for amphiphilic polymers and surfactants
Isabella Kwan, Tianxiao Huang, Monica Ek, Rauni Sepppänen, Peter Skagerlind
Nordic Pulp & Paper Research Journal, 2022, 37 (4), 566-575

Effect of cellulose oxalate as cellulosic reinforcement in ternary composites of polypropylene/maleated polypropylene/cellulose
Tianxiao Huang, Isabella Kwan, Kloce Dongfang Li, Monica Ek
Composites Part A: Applied Science and Manufacturing, 2020, 134, 105894

Betulin from outer birch bark as a potential white pigment in substitution of titanium dioxide
Isabella Kwan, Antonia von Schreeb, Monica Ek
Manuscript in preparation

Suberin from birch bark as potential cationic surfactant for capillary electrophoresis
Isabella Kwan, Parisa Askarisarvestani, Monica Ek, Åsa Emmer
Manuscript in preparation

Author's conference participation

Treesearch Progress Conference, 2022, 11th – 12th May, Norrköping, Sweden
Bark biorefinery; fundamental insights on the properties of a novel organosolv lignin from Norway spruce bark
Oral presentation (together with Maria Karlsson)

16th European Workshop on Lignocellulosics and Pulp (EWLP), 2022, 28th June – 1st July, Gothenburg, Sweden
Emulsifying properties of cellulose oxalate and pectin from Norway spruce bark
Pitch and poster presentation

Berzeliusdagarna, 2023, 20th – 21st January, Stockholm, Sweden
Trädbark – en biprodukt från skogen som råvara för nya hållbara material
Oral presentation

TAPPI International Conference on Nanotechnology for Renewable Materials, 2023, 12th – 16th June, Vancouver, Canada
Pickering emulsions from Norway spruce bark
Oral presentation

WWSC Workshops, 2021-2023, Norrköping, Gothenburg, Stockholm, Sweden
Pitch and poster presentations

List of abbreviations

AEB	Acetone extracted bark
AFM	Atomic force microscopy
ASE	Accelerated solvent extractor
ASL	Acid soluble lignin
$CDCl_3$	Deuterated chloroform
CNC	Cellulose nanocrystal
CNF	Cellulose nanofibril
COX	Cellulose oxalate
CTAB	Cetrimonium bromide
DB	Delignified bark
DMSO	Deuterated dimethyl sulfoxide
EI	Emulsion index
FCC	Free carboxyl content
FTIR	Fourier transform infrared
HPAEC	High-performance anion-exchange chromatography
HLB	Hydrophilic-lipophilic balance
HSQC	Heteronuclear single-quantum coherence
HW	Hardwood
IEC	Ion exchange chromatography
KL	Klason lignin
MBL	Milled bark lignin
MRL	Milled residue lignin
M_n	Number average molecular weight
M_w	Weight average molecular weight
NMR	Nuclear magnetic resonance
OSE	Organosolv extract
OSL	Organosolv lignin
OSR	Organosolv residue
O/W	Oil-in-water emulsion
PAA	Peracetic acid

PAD	Pulsed-amperometric detection
PD	Polydispersity
PEI	Polyethyleneimine
PFAS	Polyfluoroalkyl substances
SDG	Sustainable development goals
SDS	Sodium dodecyl sulfate
SEC	Size exclusion chromatography
SW	Softwood
SWB	Subcritical water-extracted bark
SWE	Subcritical water extracts
TEMPO	2,2,6,6-tetramethylpiperidin-1-yl)oxyl
THF	Tetrahydrofuran
UN	United Nations
W/O	Water-in-oil emulsion

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1. Introduction

Mankind has for generations used coal and petroleum resources to produce energy and materials. However, considering the worsened environmental situation over the past decades regarding carbon dioxide emissions, extreme climates, and depletion of fossil-based resources, the mindset of people worldwide has changed, and their concern for the environment has increased drastically. Actions are much needed to find new solutions and precursors to preclude the worsening environmental situation.

In 2015, the United Nations (UN) adopted “The 2030 Agenda for Sustainable Development” where the 17 Sustainable Development Goals (SDGs) are at the center. These goals include among others eliminating poverty, improving gender equality, health and education, promoting economic growth, protecting the planet, and tackling climate change.[1, 2] Three of the SDGs have been prioritized in this thesis: number 9 (Industry, innovation, and infrastructure) by utilizing a side-stream product, and by the development of facile and scalable greener process routes, number 12 (Responsible consumption and production) by using renewable resources to produce value-added products which may replace fossil-based materials, and number 13 (Climate action) by using green chemicals and solvent recovery via low-temperature distillation to decrease carbon dioxide emissions. To transition from a fossil-based society to a bio-based one, new solutions together with renewable and sustainable alternatives are needed. Lignocellulosic biomass from plants is an excellent alternative as it originates from a renewable and sustainable resource.

Lignocellulosic biomass is available in large quantities and is used in many various types of products such as paper, packaging material, hygiene products, pharmaceuticals, cosmetics, and textiles. The pulp and paper industries are the largest producers and consumers of lignocellulosic biomass. However, as society is moving forward, there is a need for new innovative strategies to utilize the complete biomass, including the side-stream products. One such product is the bark.

Previous studies have investigated the bark's structure and composition of different tree species and found that it is a source filled with unique compounds, which may have a wide range of applications. Tree bark is generated in large amounts. Thus, globally the amount was approximately 359 million m^3 in 2015[3] if assuming that the bark represents approximately 10% of the tree. Due to the high availability of tree bark, it is suitable to be utilized as a renewable raw material for sustainable solutions to replace petroleum-based products.

1.1. Thesis Objectives

In this thesis, bark from Norway spruce (*Picea abies*) is used as raw material, and different compounds are isolated via the bark biorefinery concept. The research objectives are 1) to find suitable applications for the extracted compounds from spruce bark, and 2) to extract these compounds with as high purity as possible. In addition to these two objectives, the thesis also focuses on using as green chemicals and processes as possible when obtaining the desired compounds.

In paper I, objective number 1 is in focus where nanocellulose is isolated from the spruce bark and used to produce Pickering emulsions. Paper II focuses on objective number 2, thus, using the cyclic ethanol organosolv process to extract lignin from the bark.

2. Background

2.1. Hierarchical structure of wood

Wood is an anisotropic material that has hierarchical structural levels organized in specific orientations, and the dimensions of wood extend from the nanoscale to the macroscale.[4, 5] Due to the hierarchical structure with different orientations and dimensions, wood has great mechanical strength and stiffness which supports the tree's growth and resistance toward environmental stress[6]. The structural composition is different between different tree species, softwood or hardwood, but also within species. Nevertheless, the main compounds wood is comprised of are cellulose, hemicellulose, lignin, and extractives.[7] To access these compounds individually, the wood structure must be disintegrated with mechanical and chemical treatments, also known as pulping.[8] Depending on which chemicals are used and the ratio of these chemicals, one may control the amount of lignin and hemicellulose left in the pulp, i.e., the quality of the pulp.

2.1.1. Cellulose

Cellulose is the most dominant (40-50%) and important polysaccharide in wood and consists of β-(1→4) linkages (**Figure 1a**) forming linear D-glucose chains.[7, 9, 10] Each glucose unit has three hydroxyl groups, hence, secondary hydroxyl groups at the C2 and C3 position and primary hydroxyl groups at the C6 position. The hydroxyl groups are bound together via inter- and intra-hydrogen bonds between the chains, and along with van der Waals interactions, nanofibril structures are created with high aspect ratios.[9, 11] These fibrils have both disordered and crystalline domains which are affected by the molecular orientation of the chains. The orientation also determines the characteristic properties of the cellulose.[12, 13] The fibrils aggregate into fibers (20-40 μm in diameter and >2000 μm long), embedded with hemicellulose and lignin, forming the wood fiber wall.[14]

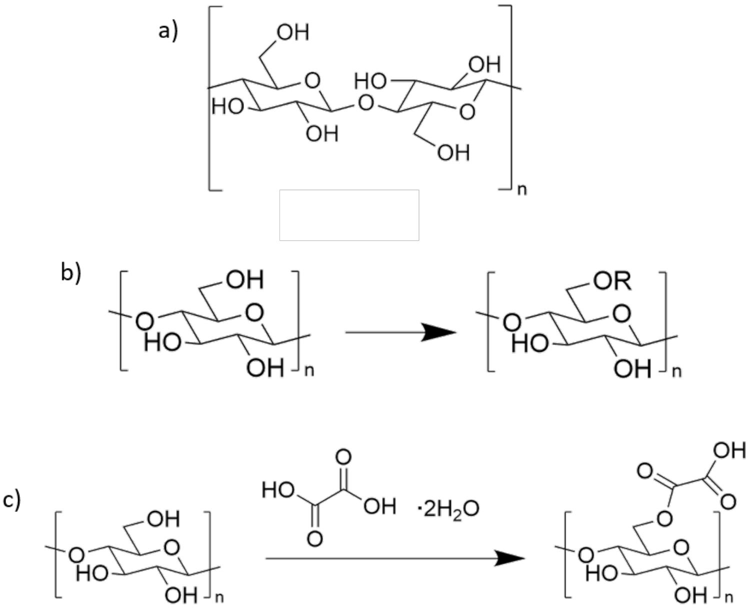


Figure 1 – a) cellulose, b) cellulose derivative where R represents various groups that can be attached during modifications, and c) simplified reaction of cellulose oxalate.

Chemical modification of cellulose fibers

Once pulp has been obtained, the cellulosic material is often further treated with different chemical modifications. The chemicals will activate the cellulose by interfering with inter- and intra-interactions between the chains and making the hydroxyl groups more accessible for reactions. Substituting one or several of the hydroxyl groups will form cellulose derivatives, and depending on which chemicals are used, different derivatives will be provided (**Figure 1b**). The possibility of tailoring the modifications will have a positive impact on the use of cellulosic materials. For example, the properties of materials may be improved, the cellulose interactions with other materials may be improved, but further processing will also be facilitated.

The chemical modification used in this thesis (Paper I) is a simple hydrolysis and esterification reaction between cellulosic material and oxalic acid dihydrate where the hydroxyl group at C6 is substituted with an oxalic acid group (**Figure 1c**). The obtained product is called cellulose oxalate (COX) and appears in powder form.[15] Oxalic acid is an organic compound that can be produced from plant-based material and is therefore a sustainable reagent. The reaction is carried out under relatively mild conditions and in the absence of solvent, which is possible because the oxalic acid dihydrate contains 29% crystallized water. Overall, the reaction is therefore considered more eco-friendly than for example 2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) mediated oxidation of the fibers. Most of the chemical modifications only take place on the surface of the cellulose fibrils, which will preserve the cellulose structure and properties, as in the case of producing COX. Furthermore, crosslinking does not happen, unlike some other chemical modifications, due to that the esterification reaction does not proceed through an anhydride mechanism which requires two adjacent carboxylic acids[16].

Nanocellulose

Research on cellulose in nanoscale, i.e., nanocellulose, has increased over the past decades. Interesting properties such as high stiffness and strength, specific surface area, biocompatibility, and high aspect ratio make nanocellulose suitable for a variety of applications.[17, 18]

Nanocellulose from wood and plants is commonly divided into two types; cellulose nanofibrils (CNFs) and cellulose nanocrystals (CNCs) where the main difference is the morphology. CNFs are thin, long fibrils with twists and kinks, and have a high aspect ratio (4-20 nm wide and 0.5-2 µm long[19]). CNFs contain both disordered and crystalline domains which provide great strength and stiffness. CNCs are stiff, rod-shaped, and short particles with a low aspect ratio (3-5 nm wide and 0.05-0.5 µm long[19]). The crystalline domain is the most abundant since the chemical pretreatment includes acid hydrolysis which removes the disordered regions of the cellulose.[20]

The cellulose fibers are separated into individual nanofibrils by applying shear forces. However, the processing of nanocellulose is energy-demanding, especially if the fibers are only separated mechanically[11]. Mechanical shearing methods include microfluidization, high-pressure homogenization, and friction grinding (Masuko grinder). To lower energy consumption and increase fibrillation efficiency, the cellulose fibers are usually pretreated chemically (or enzymatically).[11, 17, 20] These pretreatments include among others: TEMPO-mediated oxidation[21], carboxymethylation[22], enzymatic hydrolysis[23], and esterification with oxalic acid[15]. Most treatments include adding a charged group (cation or anion) to the cellulose which makes the fibrils repulse each other and facilitates fibrillation[24]. The choice of pretreatment will affect the properties of the nanocellulose, and therefore it is important to choose the treatment according to the desired application. Due to the high surface area, aspect ratio, and cellulose's high affinity to water, hydrogels with high viscosity may be obtained. As CNFs have a higher aspect ratio, viscous hydrogels are easily formed even at low concentrations. In contrast, CNCs have a lower aspect ratio and form less viscous hydrogels at low concentrations.

2.1.2. Lignin

Lignin is the second building block of wood and comprises 20-30% of the biomass (depending on species)[25]. Lignin has a complex three-dimensional molecular structure, and even until today, researchers have not been able to fully determine the structure. Although, lignin has an important role in the cell wall; it provides stiffness, keeps the cell together, hinders swelling, and is resistant to microbial attacks. The three lignin monomers (monolignols) are p-coumaryl alcohol (hydroxy phenol (H) units, **Figure 2a**), coniferyl alcohol (guaiacyl (G) units, **Figure 2b**), sinapyl alcohol (syringyl (S) units, **Figure 2c**). These monolignos are covalently bonded to each other via ether and carbon-carbon bonds, where the β-aryl ether (β-O-4') is the most common. The monolignols are also covalently bound to other polysaccharides available in the cell wall.[26] A purposed general lignin structure (softwood) is illustrated in **Figure 2d**.

Lignin is commonly divided into softwood (SW) lignin, hardwood (HW) lignin, and grass lignin where the content of the H-, G-, and S-units varies between each type. SW lignin consists mainly of G-units with small traces of H-units. HW lignin consists of an even mixture of S- and G-units with small traces of H-units. Grass lignin consists of all three units but with a higher amount of H-units than the other two.[26]

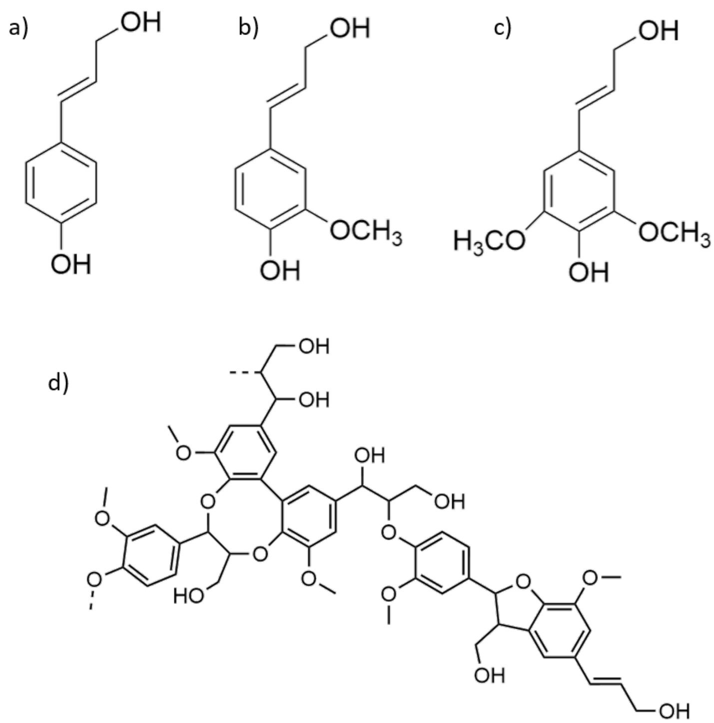


Figure 2 – Lignin monolignols where a) p-coumaryl alcohol (H-unit), b) coniferyl alcohol (G-unit), and c) sinapyl alcohol (S-unit). d) General chemical structure of lignin from softwood.

Most pulping methods produce wastes such as black liquor that contains a relatively high amount of lignin and hemicellulose. The black liquor is combusted in the recovery boiler in the processing system to recover cooking liquors. However, lignin is an interesting compound and can be used for value-added applications. Therefore, lignin is extracted from the liquor, and technical lignins are produced. The more traditional technical lignins include kraft lignin (from kraft pulping), soda lignin (from soda pulping), and lignosulfonate (from sulfite pulping). Drawbacks with these traditional pulping processes include the use of hazardous chemicals, sulfur emissions into the air, polluted wastewater streams, and most importantly degraded lignin. To overcome these drawbacks, a greener processing method is desired, i.e., the organosolv process. Instead of cooking liquor, the process uses organic solvents (e.g., ethanol) mixed with water with or without a catalyst to extract the lignin.[27] The Canadian pulp and paper mill factory Repap

Enterprises Inc. developed the alcohol cellulose (Alcell) process which is based on the organosolv process[28]. The extractions are commonly carried out with 20-80 wt% alcohol, at 180-210 °C, and 20-35 atm[29]. The catalysts used can vary between acid, alkali, and inorganic salts, but also other pretreatment methods such as biological (bio-organosolv), steam explosion, extraction in the presence of sub-/supercritical CO_2, and oxygen (oxiorganosolv) can be used[27]. The original Alcell process did not include the use of a catalyst[28]. However, adding a catalyst, usually a mineral acid (sulfuric (H_2SO_4) or hydrochloric (HCl)), at low concentrations (0.1-2.5 wt%) will improve the efficiency of the delignification[27]. The obtained organosolv lignins are of high purity, have low glass transition temperature, and have low-molecular weight and are therefore beneficial in consideration to certain applications[30]. This thesis focused on applying a cyclic ethanol organosolv process with an acid catalyst to extract bark lignin (Paper II).

2.1.3. Hemicellulose

Hemicellulose is the third building block in wood and it is carbohydrate polymers of relatively low molecular weight. The carbohydrate sugars are arranged in different configurations forming long polymer chains with the two dominant compounds being (gluco-)mannans (5-10%[25]) and xylans (6-10%[25]).[31] A purposed general hemicellulose structure is illustrated in **Figure 3**.

During the cooking and bleaching process of wood fibers, the hemicelluloses are broken down into their monosaccharides in the black liquor (along with degraded lignin)[32] which means that the recovery of hemicellulose is approximately zero. The hemicellulose that is not removed during cooking is strongly bound to the surface of cellulose fibrils, and this may affect the properties of cellulose such as mechanical strength and viscosity[31, 33].

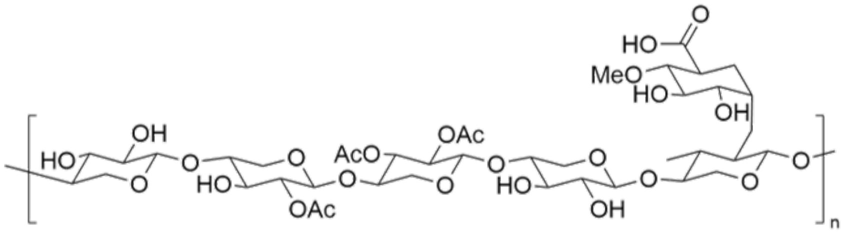


Figure 3 – Example of a purposed general structure of hemicellulose.

2.1.4. Other components – extractives

The extractives in wood only represent a few percent (5-10%) depending on species and geographical location[34]. Moreover, the amount of extractives also varies significantly within different parts of the tree. Wood extractives influence several characteristics of the tree, i.e., color, fragrance, and microbial resistance[35]. Due to its varying properties, commonly comprising a mixture of both low- and high-molecular-weight compounds, the chemical composition of extractives is complex[36].

Wood extractives are commonly extracted using hot water or organic solvents. The type of solvent should be chosen with care as different solvents extract different extractives. In certain cases, sequential extraction with different solvents is required to remove as many extractives as possible. In general, the extractives are divided into lipophilic and hydrophilic extractives. Lipophilic extractives include aliphatic compounds and terpenes and hydrophilic extractives include phenols.[36, 37]

2.2. Tree bark

In Scandinavia, Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*), and birch (*Betula pendula/nana/pubescens*) are the most common tree species. Due to the high quantity of extractives in the bark, it may cause problems during the pulping process. Therefore, sawmills and pulp and paper mills remove the bark from the logs before chipping and cooking. Instead, the bark is incinerated to produce heat, electricity, and steam for the mills. The bark represents approximately 10% of the tree, and considering the annual wood pulp produced in Sweden year 2021 of ca 11,700 tons[38], 1170 tons of industrial bark was generated.

Since the bark is a side-stream product from mills, it is cheap, has high caloric value, and is economically beneficial to incinerate to produce energy. This is also valuable for industries to reduce their dependence on fossil-based fuels and carbon footprint by utilizing the whole biomass. However, there are drawbacks to burning bark. It has a low sintering point and high ash content which may cause agglomeration problems in the incineration hatch[39]. Bark and other biomass fuels contain compounds such as chlorine and alkali metals that will also corrode and damage the combustor[40]. In addition, the bark incinerated at the mills is wet (ca. 40% moisture content) and this lowers the heating value of bark, meaning that less energy is produced as if the bark was dry or if the wood was burnt[41, 42].

Other than incinerating the bark for energy production, there have been studies on alternative uses for the whole bark, not to mention that tree bark has been used since ancient times in medicine, agriculture, and food[3]. Bark-based wooden panels, bark as a polyol feedstock to produce polyurethane (PU) foams, and thermal insulation panels are only a few examples of the use researchers have investigated[39, 43-46].

Previous research has proven that bark contains unique chemical compounds such as tannins, non-cellulosic polysaccharides (e.g., pectin and starch), stilbene glucosides, suberin, and betulin[47-49]. The bark is divided into outer and inner bark (**Figure 4**), and some compounds are only found in either the outer or the inner bark, and others are found in both. With the transition to more sustainable materials, bark has been used as a resource to produce value-added products such as ethanol, pulp, nanocellulose, adhesives, and insulation material[39, 50-53].

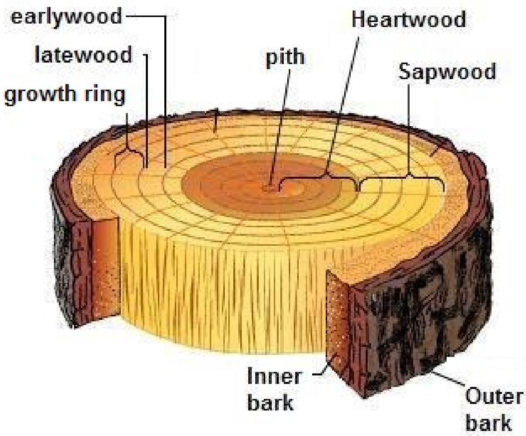


Figure 4 – Cross-section of wood (© Merriam Webster).

2.2.1. Norway spruce bark

Norway spruce (*Picea abies*) is the most common tree species in Sweden, together with Scots pine (*Pinus sylvestris*), and represents approximately 40% of the Swedish forests[54]. In general, tree bark contains the same building blocks as wood with a difference in chemical distribution. For example, bark from spruce contains a higher amount of extractives (32%) than wood but a lower amount of cellulose (27%), hemicellulose (9%), and lignin (12%)[55]. Much of the published research on spruce bark has focused on isolating individual compounds from the whole bark assuming the chemical composition of the outer and inner bark was the same[56, 57]. However, Krogell et al.[48] proved that there is a difference in the composition of the outer and inner spruce bark, especially the chemical distribution (**Figure 5**). By selecting the solvent with care in combination with the extraction method, the extractives may be separated into individual compounds with only small traces of other extractives.

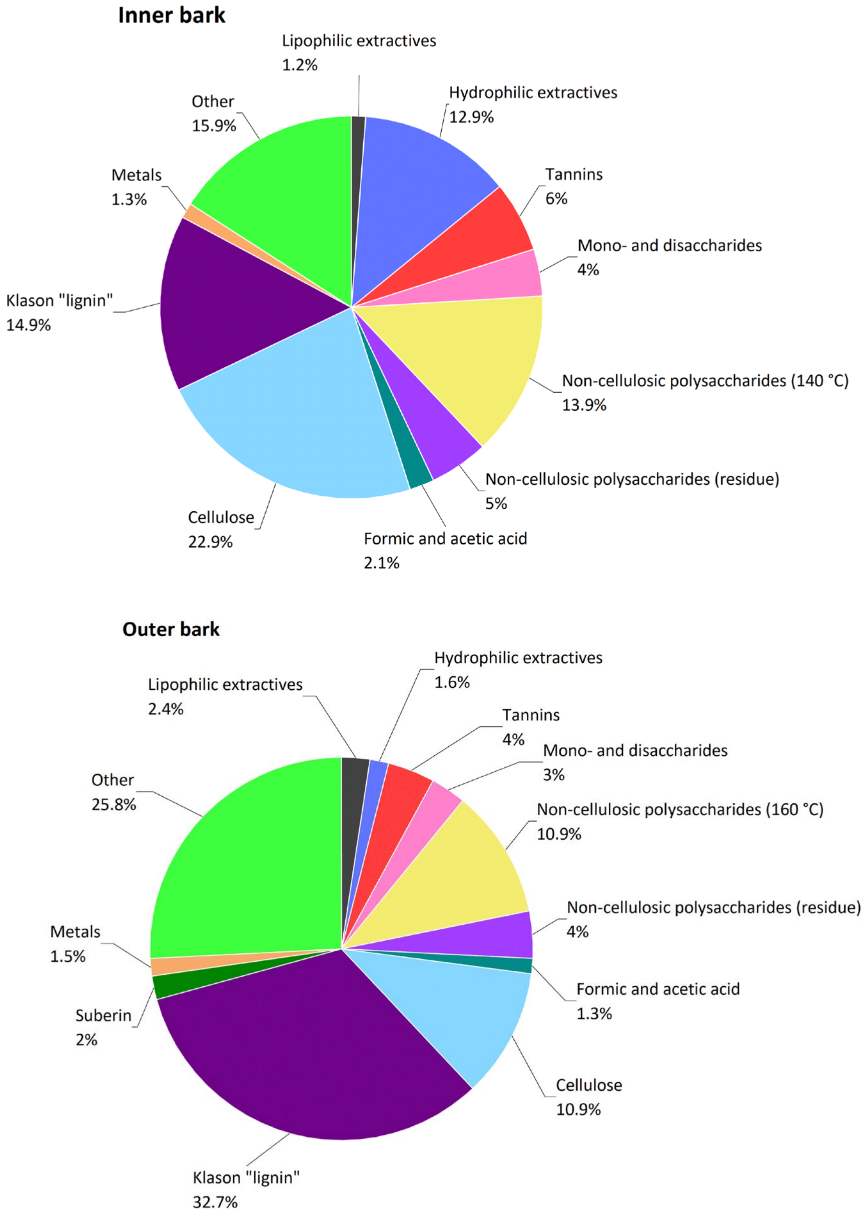


Figure 5 – Chemical composition of Norway spruce bark. Figure reproduced with permission from J. Krogell[48].

2.3. Biorefinery concept

The more conventional oil refinery implies separating crude oil from water and impurities before fractionating (via distillation) the crude oil into among others gas, naphtha, gasoline, kerosene, diesel, lubricant oils, and bitumen before further processing and utilization[58, 59]. The biorefinery concept is similar as it also implies separating the raw material (biomass) into its constituents (cellulose, hemicellulose, lignin, and extractives) that may be used to produce value-added products such as energy, chemicals, and fuels[59, 60]. Biomass is seen as a carbon-neutral resource as it commonly originates from renewable resources (e.g., agriculture and forestry) which if combusted produces the same quantity of carbon dioxide emissions as plants adsorbs during photosynthesis[61, 62]. Using biomass instead of petroleum as a raw material to produce materials and chemicals promotes the circular bio-economy and a sustainable society.

2.3.1. Bark biorefinery concept

Using tree bark as a raw material and isolating its constituents applies the bark biorefinery concept (**Figure 6**). To fully apply the concept, the whole biomass feedstock should be used efficiently, i.e., the whole tree should be used to produce energy, materials, and chemicals. As the separation of the outer and inner bark is tedious, and if the bark biorefinery is to be applied by large industries, the whole bark should ideally be used as is from the debarking process, which means that it will have a certain moisture content. As aforementioned, the moisture content, along with the high ash content, lowers the thermal efficiency[41, 42, 63] which raises the debate on the economic efficiency of direct incineration of bark[39]. There have been bioeconomic studies on factories that pass the bark through the steamer before reaching the incineration hatch to increase the heating value.

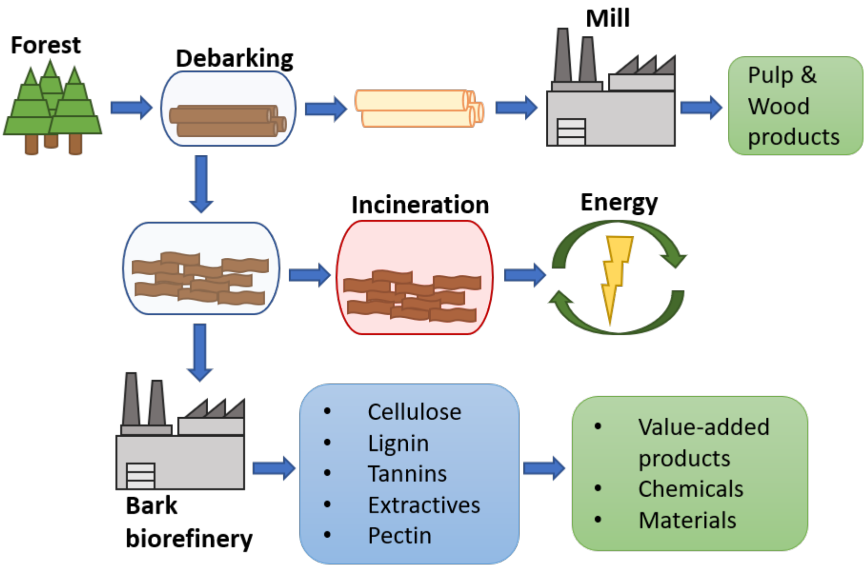


Figure 6 – Schematic illustration of bark biorefinery.

The vision of this thesis is not solely to apply the concept but also to use as many green chemicals as possible to reduce any hazardous residues and reduce environmental impact. For example, peracetic acid (PAA) is used as a delignification solvent as it is more sustainable than the common chlorine-containing solvents and does not produce hazardous residues containing chlorites[64]. The organosolv process uses milder chemicals (ethanol, water, and mild acid catalyst) than the sulfur-containing cooking liquors used during the pulping processes and is considered a greener process[65]. Furthermore, the hazardous emissions formed during conventional kraft and sulfite pulping and bleaching are avoided with the organosolv process[66] and the organic solvents used are usually easily recovered[27].

2.4. Emulsions

Emulsion systems are formed when two immiscible liquid ("oil" and "water") phases are mixed constituting kinetically stable droplets in the presence of surface-active agents, i.e., surfactants[67-69]. Without the surface-active agent, the formed emulsion droplets are unstable and phase separate. There are two main types of emulsions, oil-in-water (O/W) and water-in-oil (W/O) depending on which phase the surfactant is soluble in. Thus, O/W is obtained if the surfactant is soluble in the water phase and W/O is obtained if the surfactant is soluble in the oil phase.

Emulsions are used by many industries in products such as food, pharmaceuticals, and cosmetics[70-72], and it is necessary to use surfactants in these products to prolong their shelf lives. A surfactant is an amphiphilic molecule which implies a hydrophilic head group and a hydrophobic tail (**Figure 7a**). This head group can be non-ionic, cationic, anionic, or zwitterionic (**Figure 7b**). Due to the characteristics of surfactants, an oil droplet in water is surrounded by the surfactant (**Figure 7c**). This lowers interfacial tension at the liquid-liquid interface which will prevent or slow down the emulsion from creaming, flocculation, coalescence, and/or Ostwald ripening.[69]

Several factors affect the properties of emulsions. Production process, oil purity, pH, ionic strength, and water-to-oil ratio are some factors that affect the emulsion. Other important factors are the type and concentration of the surface-active or emulsifying agent used.[73, 74]

One method commonly used by industries is to investigate or calculate the hydrophilic-lipophilic balance (HLB) number to select the right emulsifying agent for the intended application[75]. HLB was first developed in 1949 by Griffin[76] and is a semiempirical scale based on the hydrophilic and lipophilic ratio in non-ionic surfactants. This was further developed by Davies in 1957[77] to include different types and more complex surfactants.

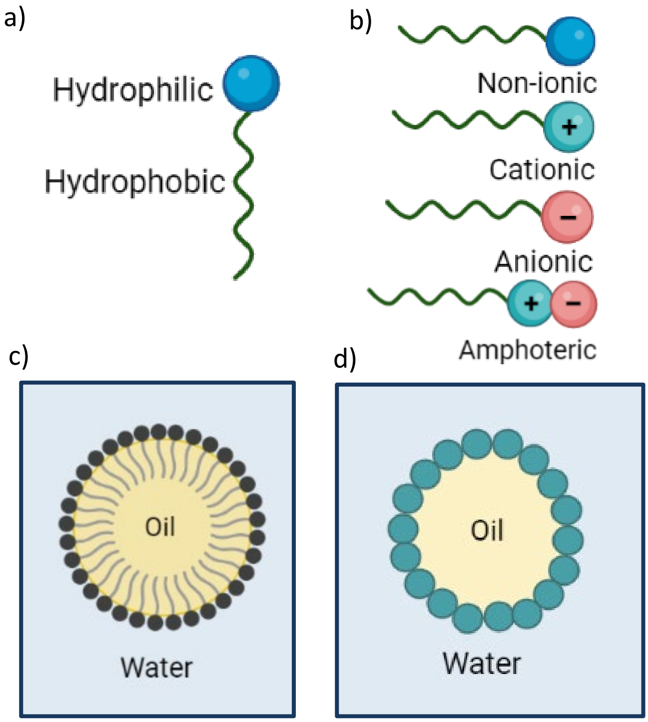


Figure 7 – Illustrations of a) amphiphilic molecule with a hydrophilic head group and hydrophobic tail group, b) different variations of headgroups of an amphiphilic molecule, c) "traditional" emulsion (O/W) where surfactant surrounds an oil droplet, and d) Pickering emulsion where solid particles surround the oil droplet.

Many surfactants used today in the industries (e.g., sodium dodecyl sulfate (SDS), per- and polyfluoroalkyl substances (PFAS), and cetrimonium bromide (CTAB)) are fossil-based. In high concentrations, these are toxic both for humans and the environment. It is, therefore, necessary to find more sustainable compounds that originate from renewable sources, non-toxic, yet surface-active[78-80]. Bio-based and naturally occurring amphiphiles are for example pectin (usually from fruits and vegetables), saponin (from soapbark tree (*Quillaja Saponaria*) bark)[81], and coco betaine (from coconut oil)[82]. Other compounds such as tannins and betulin can be modified into amphiphilic molecules[83].

2.4.1. Pickering emulsions

Emulsions that contain solid particles instead of surfactants to increase stability are called Pickering emulsions (**Figure 7d**). Ramsden[84] and Pickering[85] first discovered the use of solid particles in the early 20th century, and it has since then been studied by different researchers who have used various types of solid particles to stabilize emulsions. One benefit of solid particles is that they can be modified and tuned to the requested application[70]. Particles such as clay, silica, and metals may be good stabilizers, but for some applications (biomedical, food, and cosmetics) it may be troublesome regarding the toxicity and bio-degradability.[86] Therefore, more sustainable alternatives are desired. Starch, proteins, lignin, and cellulose are non-toxic, sustainable, and bio-degradable alternatives for fossil-based particles.[87]

Cellulosic material, and especially nanocellulose, has caught researchers' interest in producing Pickering emulsions. An important parameter that the particles should have is wettability, i.e., the particle should be able to be wetted by both the continuous and the dispersed phase[70]. Nanocellulose fulfills this requirement as cellulose is both lipophilic and hydrophilic. Already in 1986, Oza and Frank[88] demonstrated the possibility of using microcrystalline cellulose to stabilize emulsions. The first work to use nanocellulose (bacterial nanocrystals) to prepare Pickering emulsions was presented by Kalashnikova et al.[89] in 2011, and since then the investigation of using nanocellulose from different sources has taken off. Cranston and co-workers[90] summarized the use of various nanocelluloses to produce Pickering emulsions, and the study on using CNC for emulsions is dominant. However, there is a debate on whether CNF or CNC is the better choice as the aspect ratio is a parameter that affects the droplet size formed which in turn affects the stability. Jiménez Saelices and Capron[91] compared Pickering emulsions produced from CNC and CNF and found that due to the larger aspect ratio of CNF, the emulsion droplets formed were larger than the ones formed with CNC. They also saw that the CNF emulsions formed networks between the droplets while the CNC emulsions formed individual droplets. This may be explained by the morphology of the nanocelluloses. The long fibrils of CNF provide large interfacial coverage of emulsion droplets and facilitate fibril entanglements between the emulsion droplets via electrostatic interactions. This forms stable emulsions (emulsion gels) which are not prone to coalesce. The shorter fibrils of CNC are not able to form entanglements. Instead, a dense interfacial coverage of the droplets is formed that stabilizes the emulsion. However, this coverage by CNC is not as stable against coalescence as CNF, and over time the emulsion will phase separate. Thus, a low concentration of salt is necessary to screen the surface charge to allow the particles to pack at the droplet surface and hinder electrostatic repulsion.[92-96] Bai et al.[93] used a mixture of CNC and CNF to stabilize Pickering emulsions and discovered that there is an optimal concentration of CNFs to stabilize emulsions. Kalashnikova et al.[96] investigated the effect of the aspect ratio of rod-shaped nanocellulose (i.e., CNC from various sources) and found that the aspect ratio had a considerable influence on the surface coverage density. Mixing different aspect ratios and combining CNCs and CNFs will be beneficial when producing Pickering emulsions as advantageous properties from both nanocellulose types will be applied.

3. Materials and Methods

In this chapter, a brief description of the experimental methods and characterization techniques are presented. More details are found in the appended papers.

3.1. Materials

Bark from Norway spruce (*Picea abies*) was used as raw material and gathered in the Stockholm area, Sweden. The bark was harvested manually using a scalpel and stored in a freezer at -20 °C until use.

All purchased chemicals were used as received unless stated otherwise.

3.2. Experimental Methods

The spruce bark was dried in an oven at 70 °C for at least 48h before manually separating the outer and inner bark, and the inner bark was selected to be in focus to reduce the complexity of the spruce bark. The inner bark was cut into matchsticks before Wiley milled (3383 L70, Thomas Scientific, New Jersey, USA) to 20 or 40 mesh. The chosen extraction sequences were based on previous studies.[53, 97]

3.2.1. Preparation of spruce bark cellulose fibers

Soxhlet extraction with acetone (90%) for 40 cycles was performed to remove extractives from the raw inner bark (40 mesh). A second extraction of the acetone-extracted bark (AEB) using subcritical water was performed using an Accelerated Solvent Extractor ASE350 (Dionex, California, USA). The extraction was carried out at a pressure of 100 bar and temperatures 100, 140, and 160 °C with 3 cycles of 20 min for each temperature. The subcritical water-extracted bark (SWB) and subcritical water extractives (SWE) were freeze-dried before further use.

Delignification of the SWB was performed using peracetic acid (PAA) solution (10 wt%, pH 4.5). The delignification was carried out at 65-70 °C for 4-5h until the bark became white. After the reaction, the delignified bark (DB) was washed thoroughly with deionized water until the filtrate was neutral (ca. pH 6-7) to remove excess PAA and then freeze-dried before further modifications.

3.2.2. Preparation of cellulose oxalate and nanocellulose

Cellulose oxalate (COX) was prepared by mixing molten oxalic acid dihydrate with DB (acid:bark ratio of 4:1) at 110 °C for 5 min under reflux. The product slurry was washed with ethanol (96%) via vacuum filtration to remove excess acid. When the conductivity of the filtrate was below 10 μS cm^{-1}, the washing was terminated and the product was left to air dry at room temperature.

A nanocellulose suspension was prepared by mechanical disintegration via homogenization in a microfluidizer (M-110EH, Microfluidics Corp, USA) of the COX dispersion (10 g L^{-1}). The samples were passed through the chambers (400/200 μm and 200/100 μm, respectively) a total of six times. The nanocellulose suspension obtained was stored at +4 °C.

A homogeneous nanocellulose dispersion was prepared via high-shear mixing (UltraTurrax, IKA, Germany) for 20 min, probe sonication (Vibra-Cell VC 750, Sonics, USA) for 10 min at 75% amplitude, and lastly centrifugation (Rotina 420, Hettich Zentrifugen, Tuttlingen, Germany) for 2h at 4688 G. The dispersion was stored at +4 °C until further use.

3.2.3. Pickering emulsion (Paper I)

There are many different process routes to produce Pickering emulsions, i.e., different research groups use different procedures.[70, 90] The following procedure and concentrations were selected for the current project.

Three nanocellulose concentrations, i.e., 0.1, 0.5, and 1 wt%, were tested to investigate how the emulsion stability was affected by nanoparticle concentration. Two types of oils were tested, i.e., almond oil and hexadecane, to investigate how the oil purity affected the emulsion stability.

Oil-in-water (O/W) Pickering emulsions were produced using high-shear mixing (T18 UltraTurrax, IKA, Germany) for 5 min at 13,000 rpm. The water-to-oil ratio was selected to 14 to 6 v/v, and the nanocellulose was present in the water phase. Carboxymethyl cellulose nanofibrils (CNFs) were used as reference nanocellulose. The produced emulsions were stored in a ventilated cabinet at room temperature.

3.2.4. Lignin extraction of spruce bark (Paper II)

Soxhlet extraction and ASE were performed on the raw inner bark (20 mesh) as mentioned previously (section 3.2.1). For the lignin extraction, the SWB was not freeze-dried but extracted again in the ASE for 15 cycles for 5 min each at 160 °C and 100 bar using a solvent mixture of ethanol/water (70:30 v/v, 1.5 wt% sulfuric acid (H_2SO_4)). Lignin was precipitated by adding MilliQ water to the extracts and ethanol was removed via rotary evaporation under reduced pressure. The remaining aqueous solution was centrifuged for 30 min at 4688 G, and the precipitate was washed twice with MilliQ water. The obtained organosolv lignin (OSL) was freeze-dried before further analysis. The collected supernatant from the precipitation and centrifugation was neutralized, rotary evaporated, and oven-dried. The obtained organosolv bark residue (OSR) was freeze-dried.

Acetone extracted bark (AEB) and OSR were ball milled (Retsch PM400, Dusseldorf, Germany) for 24h at 300 rpm in 1h intervals with 30 min breaks. The ball-milled bark was dioxane extracted (25 mL solvent/g ball-milled bark) for 72h at room temperature under constant mixing[98]. The milled bark lignin (MBL) and milled residue lignin (MRL) were centrifuged to remove the supernatant before freeze-drying. No further purification was performed on the lignin after the drying.

3.3. Characterization Techniques

Carbohydrate analysis. The chemical composition, including Klason lignin (KL) and acid-soluble lignin (ASL), of the samples was determined according to the standard TAPPI T222 om-06[99]. Carbohydrate analysis was performed using high-performance anion-exchange chromatography (HPAEC) connected with pulsed-amperometric detection (PAD) (Dionex ICS 300, Thermo Scientific, USA). KL was determined gravimetrically and ASL was determined with an ultraviolet (UV) spectrophotometer (Shimadzu, Japan) at the wavelength 205 nm.

Conductometric titration. The free carboxyl content (FCC) or total fiber charge was determined using conductometric titration according to SCAN-CM 65:02[100] with slight adjustments. Titration curves were obtained using a conductometer (856, Metrohm, USA) and evaluated using the software tiamo (Metrohm, USA). The total fiber charge was calculated according to **equation (1)**:

$$Total\ charge\ [mmol\ g^{-1}] = \frac{c_{NaOH} \times V_{NaOH}}{m} \qquad \textbf{(1)}$$

where V_{NaOH} is the consumed volume (L) of NaOH, c_{NaOH} is the NaOH concentration (mol L^{-1}) and m is the dry weight (g) of the sample.

Fourier Transform Infrared (FTIR) spectroscopy. FTIR spectra were recorded using a Spectrum 100 FT-IR Spectrometer (Perkin Elmer, Connecticut, USA) equipped with a Golden Gate single attenuated total reflection (ATR) diamond (Gaseby Specac Ltd., Kent, UK). Number of scans per sample was 32 at a resolution of 4 cm^{-1} between wavelengths 4000 and 600 cm^{-1}.

Atomic Force Microscopy (AFM). AFM images were obtained using a MultiMode 8 AFM (Bruker, Santa Barbara, California, USA) with ScanAsyst in air mode with ScanAsyst-Air HR cantilevers with a resonance frequency of 130 kHz, spring constant of 0.4 N m^{-1} and tip radius of 2 nm. The scan size of the images was 2 x 2 µm and the nanocellulose suspension was adhered to a mica sheet using polyethyleneimine (PEI). The images were analyzed with NanoScope Analysis (Bruker, Santa Barbara, California, USA) and ImageJ.

Viscosmetry. The viscosity was measured using a cone-and-plate viscometer (CP1, TQC Sheen B.V., Netherlands) equipped with a 0-10 poise cone. The measurements were carried out for 30 seconds at 20 °C and 750 rpm.

Optical microscopy. The emulsion droplets (nanocellulose concentration of 1 wt%) were studied using a Standard 25 ICS optical microscope (Zeiss, Germany). For comparison, all images were captured using x10 magnification with a Nikon D5000 (Nikon Corp., Japan) and analyzed with the software ImageJ.

Emulsion index (EI). Emulsion stability was studied in two ways, 1) via centrifugation and 2) over time via gravitation. The emulsions were centrifuged at 4688 G for 30 min and digital images were captured before and after the centrifugation. Over time stability was studied by capturing digital images of the emulsions shortly after produced (day 0) and on days 1, 7, and 27. For both cases, the emulsion index (EI) was calculated according to **equation (2)**:

$$EI\ [\%] = \frac{Hc}{Ht} \times 100 \qquad \textbf{(2)}$$

where Hc is the height of the cream (emulsion) layer and Ht is the total height of the emulsion.

Size Exclusion Chromatography (SEC). The molecular weight of the different lignin samples was analyzed using SEC, with tetrahydrofuran (THF, HPLC grade) as eluent. The separation was carried out on a Waters system (Waters Milford, Massachusetts, USA) using Waters Ultrastyragel HR4, HR2, and HR0.5 (4.6 x 300 mm) solvent-efficient analytical columns coupled in series with a Styragel guard column (4.6 x 300 mm). The detectors used were a photodiode array detector (PAD, Waters-2998) operating at 254 and 280 nm. For the calibration, polystyrene standard solutions with molecular weights between 176 kDa and 266 Da were used along with a UV detector at 254 nm. The samples were acetylated[101], dissolved in THF, and filtered before being injected into the SEC system.

Nuclear Magnetic Resonance (NMR) spectroscopy. NMR analyses were performed with a Bruker Avance III HD 400 MHz instrument (Bruker Corporation, Massachusetts, USA) at 300 K equipped with a 5 mm Z-gradient BBFO broadband smart probe. The gradient (e/a TAPPI) was used to improve the sensitivity. Heteronuclear single quantum coherence (HSQC) experiments were performed using the “hsqcetgpsi” pulse program with a minimum of 80 scans and the solvent used was deuterated dimethyl sulfoxide (DMSO). For the semiquantification, the aromatic C2 signal in the lignin was selected as the internal standard[102].

^{31}P NMR was carried out using the “zgig30” pulse program with a minimum of 256 scans. Lignin samples were prepared according to Argyropoulos[103] where the hydroxyl groups in lignin are phosphorylated with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane. The solvent used was deuterated chloroform ($CDCl_3$) and the internal standard selected was endo-N-hydroxy-5-norborene-2,3-dicarboximide.

All obtained spectra were processed and analyzed with the software MestReNova (version 9.0.0, Mestrelab Research, A Coruña, Spain)). All spectra were Fourier transformed, baseline corrected, and manually phase corrected.

4. Results and Discussion

4.1. Nanocellulose of cellulose oxalate from spruce bark (Paper I)

The isolation of cellulose from bark was performed via acetone extraction, subcritical water extraction, and peracetic acid (PAA) delignification. The extractions are important to remove extractives (stilbene glucosides, tannins, etc.) and non-cellulosic polysaccharides (pectin and starch) that can be used for other applications. The delignification was performed to remove lignin, and by using PAA the process is more sustainable as it is chlorine-free and the residue product is non-toxic[64]. The delignification may be optimized to control the remaining lignin content, but this was not within the scope of this thesis.

The process of producing nanocellulose is energy-demanding. However, a chemical pretreatment, i.e., attaching a charged group on the surface of the cellulose fiber, lowers the energy demand. Therefore, cellulose from spruce bark was modified with molten oxalic acid where an oxalic acid group is attached to the C6 position to produce cellulose oxalate (COX). COX from dissolving pulp was also analyzed and used to prepare nanocellulose. The free carboxyl content (FCC), i.e., fiber charge, and esterification were determined via conductometric titration (**Table 1**) and FTIR (**Figure 8**).

The FCC of COX bark was measured to 1.6 mmol g^{-1}, and of COX dissolving pulp to 0.7 mmol g^{-1}. This is a rather large difference between the samples. Moreover, the charge for COX dissolving pulp was lower than what has been reported previously[104]. The charge of COX bark was in alignment with what had been reported previously[53]. Two reasons why the charge may differ are the esterification process and the washing of the product. According to Benselfelt et al.[105], the optimal fiber charge is approximately 0.6 mmol g^{-1} to yield a good nanocellulose dispersion that is colloidally stable.

Table 1 – Free carboxyl content (FCC) of cellulose oxalate (COX) and viscosity of organic phases (pure solutions) and nanocelluloses (1 wt%). The values are an average of two measurements for FCC and ten measurements for viscosity, with the corresponding standard deviation. Table reproduced from Paper I[106].

	FCC [mmol g^{-1}]	Viscosity [mPa.s]
COX bark	1.6±0.004	10.2±0.9
COX dissolving pulp	0.7±0.005	16.1±1.2
CNF	n.a.	54.3±2.4
Almond oil	n.a.	80.1±1.3
Hexadecane	n.a.	10.9±2.3

n.a. – no analyzed.

The viscosity of the nanocellulose and oil phase was analyzed and the result is shown in **Table 1**. When comparing the two COX samples, dissolving pulp had a slightly higher viscosity, i.e., 16 mPa·s, while bark had a viscosity of 10 mPa·s. A contributing factor to this is the hemicellulose content (**Table 2**). Thus, the higher the hemicellulose content, the nanocellulose becomes more aggregated, which results in stiffer nanocellulose hydrogels. The aggregation is due to that hemicellulose is able to form hydrogen bonds with the cellulose, which will facilitate the network-forming of the fibrils.[33] Another contributing factor to the higher viscosity may be the carboxyl content. With higher fiber charge, stronger electrostatic interactions occur, which gives more stable gels. For COX bark, the viscosity is lower than for dissolving pulp despite the charge, and this may be due to the fiber charge being too high which results in the fibers repulse each other. For COX dissolving pulp, the hydrogen bonding between hemicellulose and cellulose had an impact on the slightly higher viscosity. For CNF, the gel was very viscous, i.e., 54 mPa·s, which was due to the long fibrils forming stable entanglements and networks[107].

From the FTIR spectra (**Figure 8**), the marked band at 1730 cm^{-1} indicates the stretching vibration of the carbonyl (C=O) group in esters (–COO) and of carboxyl groups (–COOH). The small peak is visible for both COX samples which indicates that the reaction was successful. The peak shown in raw bark at the marked wavelength is due to non-separated compounds such as extractives, pectins, and lignin.

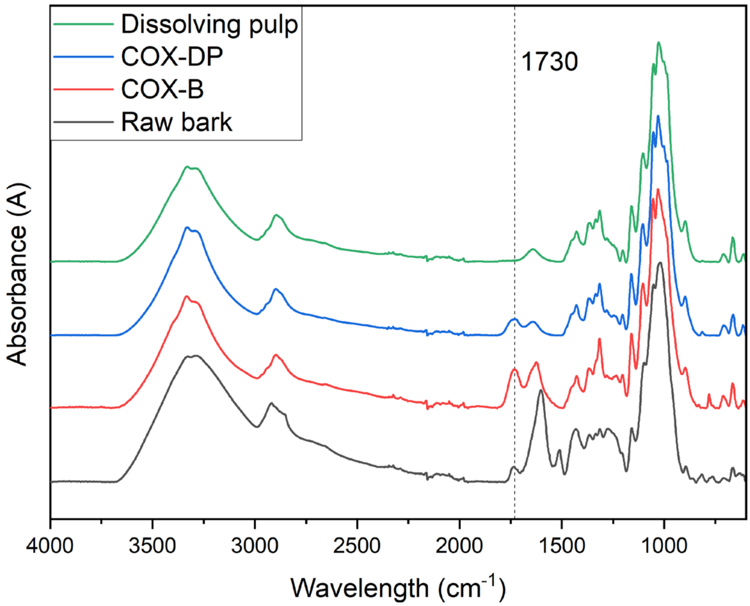


Figure 8 – FTIR spectra of unmodified dissolving pulp (green), non-extracted raw bark (black), and COX from dissolving pulp (DP, blue) and bark (B, red). The indicated peak at 1730 cm^{-1} corresponds to the stretching vibration of a carbonyl (C=O) group. Figure obtained from Paper I[106].

The COX's chemical composition was analyzed via carbohydrate analysis. **Table 2** shows the chemical composition of the two COX samples where it is assumed that the glucose content equals the cellulose content, xylose and mannose equals the hemicellulose content, and the Klason lignin together with the acid-soluble lignin (ASL) represents the lignin content. COX dissolving pulp is composed of 82% cellulose, 8% hemicellulose, and 5% lignin. In comparison, COX bark is composed of 68% cellulose, 5% hemicellulose, and 7% lignin. The total amount analyzed for both samples was not 100% and this is because not all components were analyzed with this analysis method. Additional analyses such as ash content and methanolysis would increase the total percentage of sample analyzed.

Table 2 – Chemical composition of cellulose oxalate samples analyzed via carbohydrate analysis. The shown values are averages of 3 measurements with the corresponding standard deviation. Table obtained from Paper I[106].

	COX dissolving pulp [% w/w]	COX bark [% w/w]
Arabinose	0.0±0	0.08±0.05
Rhamnose	0.0±0	0.0±0
Galactose	0.0±0	0.08±0.1
Glucose	81.5±1.5	68.1±0.07
Xylose	4.9±0.3	4.1±0.3
Mannose	3.6±0.3	1.0±0.05
Klason lignin	4.2±1.2	5.0±1.5
ASL	1.3±0.06	1.6±0.2
Total	95.5±1.2	80.0±1.4

The morphology of the nanocelluloses was analyzed by capturing height images with AFM (**Figure 9**) and studying the size distribution of the fibrils (**Table 3**). For COX dissolving pulp (**Figure 9a**), the fibrils were thin and long with kinks and splits which indicates CNF. However, fibrils that were rod-shaped and shorter were also present which indicates CNC. This yielded a large size distribution with an aspect ratio of 68. In comparison, in COX bark (**Figure 9b**), the fibrils were rod-shaped and short but also thin and long fibrils were visible, and the aspect ratio was 66. The aspect ratio of CNF is between 50-100 or above and for CNC it is between 6-70[19, 108]. In conclusion, the COX samples contain a mixture of both CNF and CNC. The carboxymethylated CNF (**Figure 9c**) had an aspect ratio of 230 which is within the range of what is reported in the literature. It should be noted that the length of the fibrils may be underestimated due to fiber entanglements and the limiting scan size of the AFM images.

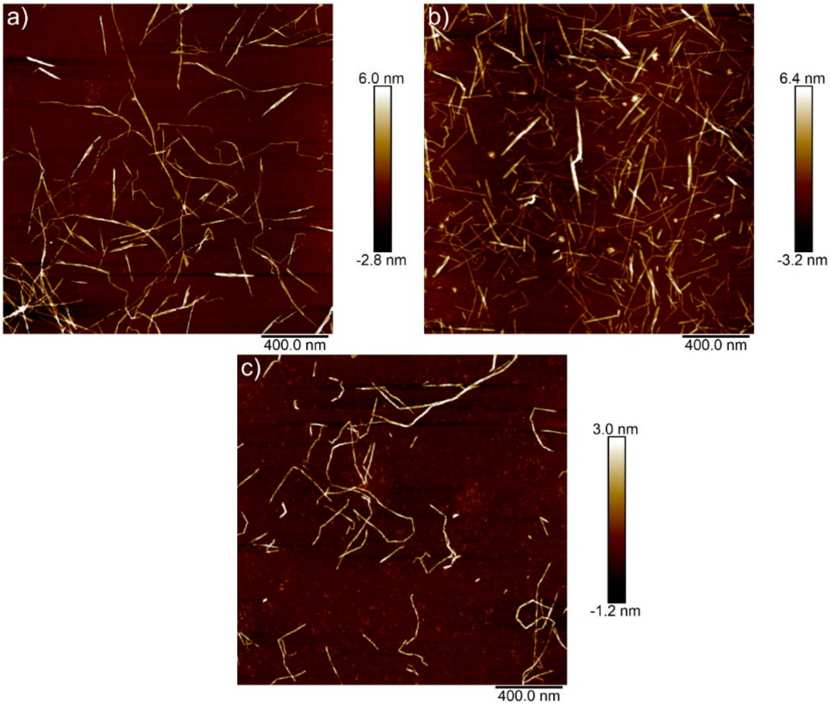


Figure 9 – AFM images of a) COX dissolving pulp, b) COX bark, and c) CNF. The nanocellulose concentration was 0.01 g L^{-1} and the scan size was 2 x 2 µm. Figure obtained from Paper I[106].

Table 3 – Average length and width distribution (in nm) and the according standard deviation of the nanocellulose particles. Over 100 particles were measured for each nanocellulose. Table obtained from Paper I[106].

	Length [nm]	Width [nm]	Aspect ratio
COX bark	257±212	4±2	66
COX dissolving pulp	372±277	6±2	68
CNF	564±287	3±1	226

4.2. Pickering emulsion with nanocellulose from spruce bark (Paper I)

Pickering emulsions were produced with three nanocellulose concentrations, 0.1, 0.5, and 1 wt%, and two oil phases, almond oil and hexadecane. The chosen concentrations were chosen because according to the literature, the higher the particle concentration the more stable emulsion[93]. Almond oil was used as the oil phase as it is a more sustainable choice while hexadecane is one of the most used in the literature to produce emulsions due to its purity. After producing the emulsions, droplet size was studied and emulsion stability was analyzed by calculating the emulsion index (EI).

The droplet size for the different emulsions varied significantly for the two oil phases (**Figure 10**). The droplets formed with almond oil were larger than those formed with hexadecane and this may be due to the purity and polarity of the oil phases. Almond oil consists of a mixture of fatty acids while hexadecane is a homogeneous carbon chain. The purer the oil used, the more stable are the emulsions which slows down the susceptibility of Ostwald ripening[70, 74, 109-111].

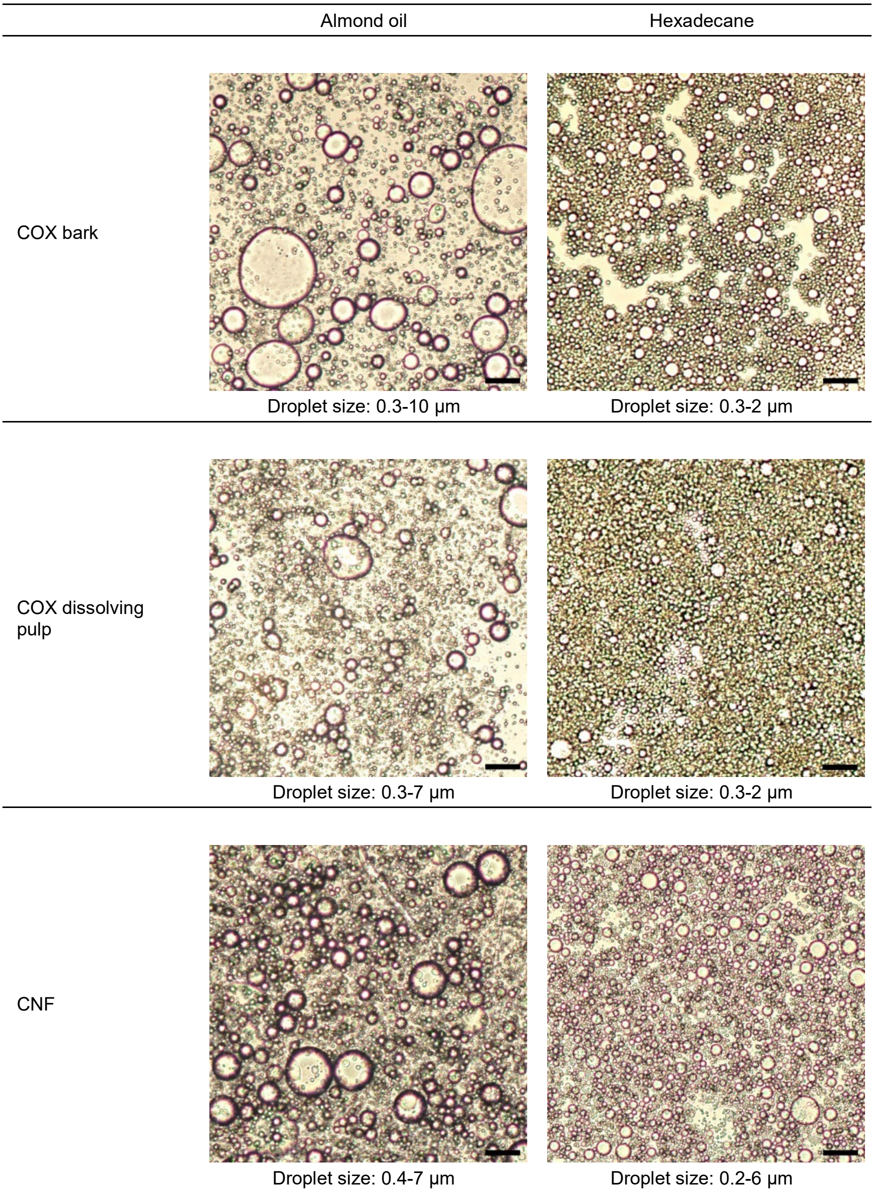
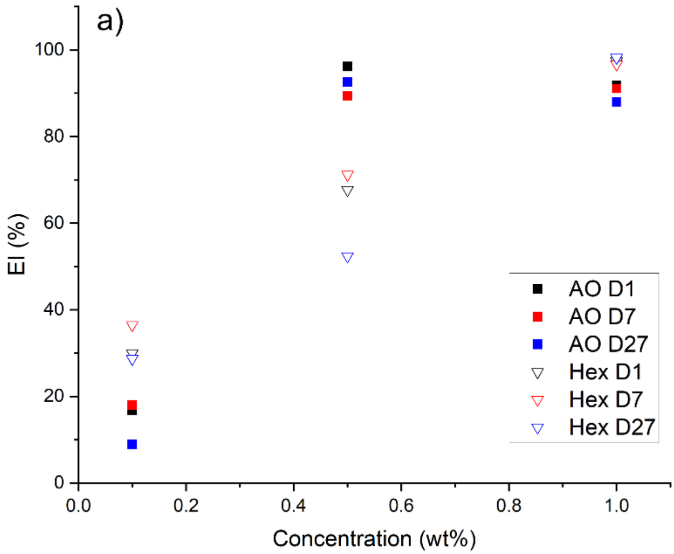
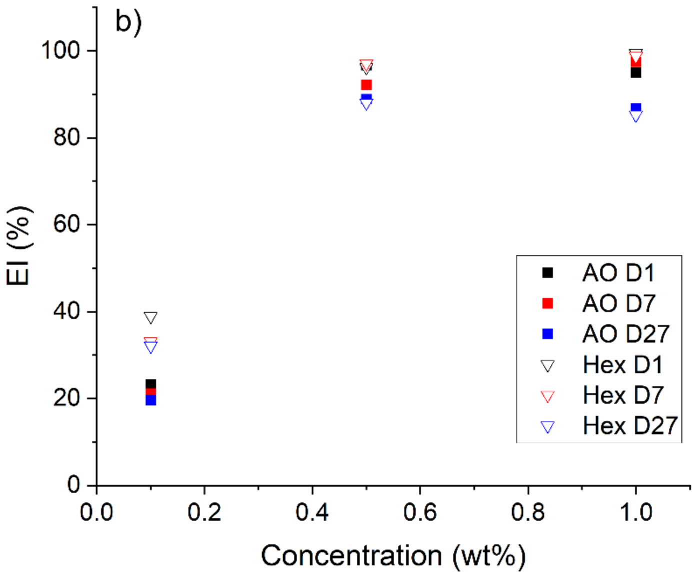


Figure 10 – Microscopy images of emulsions with a nanocellulose concentration of 1 wt%. The images are captured with x10 magnification and the scale bar is 5 µm. Figure reproduced from Paper I[106].

The emulsion stability was analyzed with gravitational testing over time (**Figure 11**) and via centrifugation (**Figure 12**). The emulsion index (EI) is a ratio that indicates the emulsion's ability to resist structural changes over time. Thus, the higher the EI value, the more resistant to changes is the emulsion. The EI was affected by the concentration and aspect ratio of nanocellulose, but also the oil phase used. The concentration of nanoparticles indicated that a higher concentration, i.e., 1 wt%, produced more stable emulsions regardless of oil phase, which is consistent with what has been reported in the literature. The low concentration of nanocellulose did not yield stable emulsions due to insufficient surface coverage of the droplets[112]. Nanocellulose with a high aspect ratio will be able to entangle with neighboring droplets via electrostatic interactions[92] which creates more stable emulsions, i.e., high EI value, that hinders the droplets from coalescence/Ostwald ripening. The long fibrils are also beneficial as they are able to bend and surround the droplet surface yielding efficient interfacial coverage[95]. When particles with a high aspect ratio, e.g. CNF, are used to produce emulsions, so-called emulsion gels are easily formed[93]. Once emulsion gels are formed, they are very stable which is beneficial for products that require a long shelf life. The emulsions produced with CNF (**Figure 11c**, blue symbols) at high particle concentration, irrespective of the oil phase used were very stable which indicates that emulsion gels were formed. The COX produced from bark and dissolving pulp had characteristics of both CNF and CNC which created sufficient interfacial coverage to yield moderately stable emulsions (**Figures 11a-b**), especially for 1 wt% nanocellulose. However, as no emulsion gel was formed for the COX emulsions, these were not stable against centrifugal forces (**Figure 12**, black – bark and red – dissolving pulp). It should be noted that other factors also affect emulsion stability such as water/oil phase ratio and salt concentration, but these were not within the scope of the study.


a)
100
80
60
40
20
0
EI (%)
0.0
0.2
0.4
0.6
0.8
1.0
Concentration (wt%)
AO D1
AO D7
AO D27
Hex D1
Hex D7
Hex D27


b)
100
80
60
40
20
0
EI (%)
0.0
0.2
0.4
0.6
0.8
1.0
Concentration (wt%)
AO D1
AO D7
AO D27
Hex D1
Hex D7
Hex D27

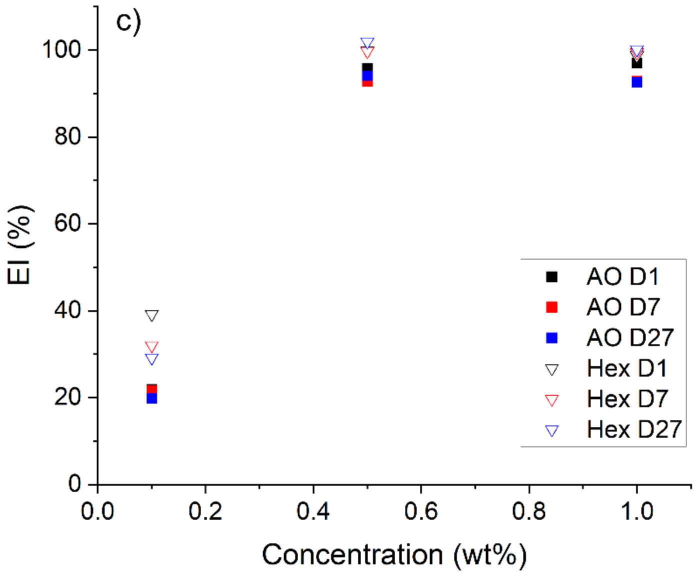


Figure 11 – Emulsion index (EI) over time for a) COX bark, b) COX dissolving pulp, and c) CNF. The result is an average value of two duplicates. Almond oil (AO), hexadecane (Hex), D# represent the day after the emulsion was produced. The displayed results are the average values of two duplicates of each sample. Figure obtained from Paper I[106].

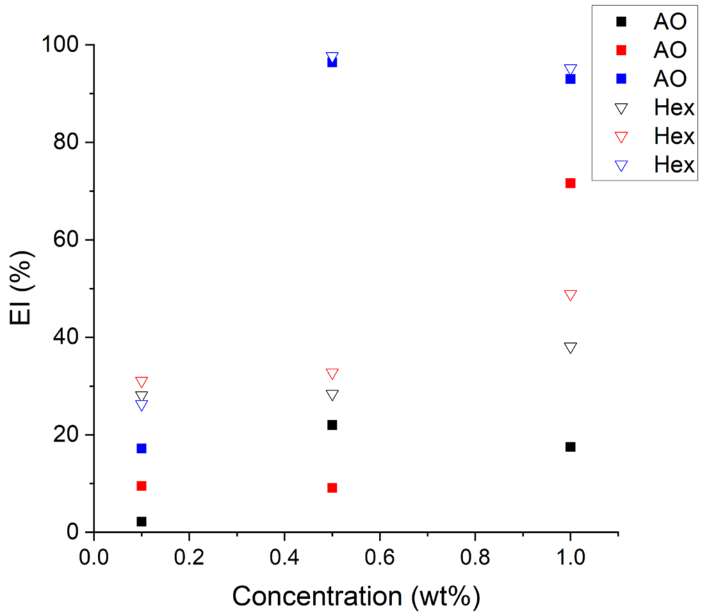


Figure 12 – Emulsion index (EI) after centrifugation where the result is an average of two duplicates. Black – COX bark, red – COX dissolving pulp, blue – CNF, almond oil (AO), hexadecane (Hex). Figure obtained from Paper I[106].

In summary, using cellulose extracted from spruce bark, an industry side-stream product to produce Pickering emulsions, is a promising substitute for fossil-based surfactants and emulsifiers. Moreover, utilizing the bark to produce value-added materials and products will be economically beneficial for industries that strive to lower their carbon footprint and produce climate-neutral products.

4.3. Physical and chemical properties of Norway spruce bark lignin (Paper II)

Extracting lignin from wood biomass by using a mild, cyclic extraction method has been developed by Karlsson et al.[97]. However, investigations on lignin from spruce bark are limited, especially using mild methods that preserve the lignin structure.

A mild extraction sequence was carried out to extract high-quality lignin from spruce bark which can be used in value-added products (**Figure 13**). The whole bark was separated into outer and inner bark in order to reduce the complexity of the investigation which focuses on the inner bark. The inner bark was milled before the first extraction step, Soxhlet extraction, which was used to remove hydro- and lipophilic extractives (e.g., fatty acids, stilbene glucosides, tannins, etc.). Acetone is a good choice of solvent as it is not only eco-friendly but also removes both types of extractives at the same time. The benefits of using a Soxhlet apparatus are the low amount of solvent used due to the cyclic extraction process and low temperature which yields a mild extraction. The second extraction step was subcritical water extraction at three temperatures (100, 140, and 160 °C) with constant pressure to recover different polysaccharides such as oligosaccharides, pectic polysaccharides, and hemicellulose with low degradation and good yield.

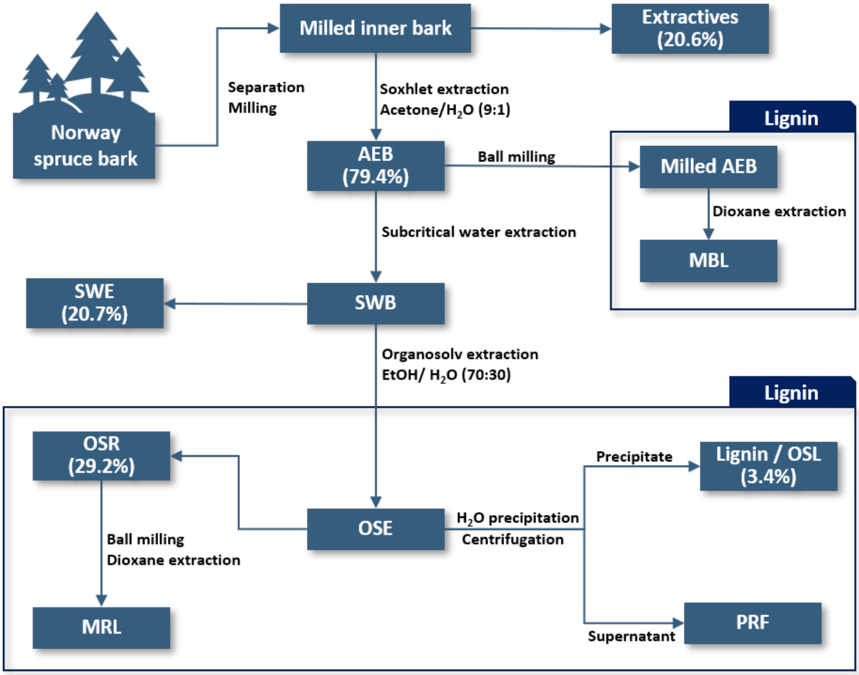


Figure 13 – Schematic illustration of the extraction sequence of Norway spruce bark. The percentage displayed represents the yield of each fraction in wt% (based on the initial mass of raw bark). Acetone extracted bark (AEB), subcritical-water extracted bark (SWB), subcritical-water extracts (SWE), milled bark lignin (MBL), organosolv extract (OSE), organosolv residue (OSR), milled residue lignin (MRL), organosolv lignin (OSL), polysaccharide-rich fraction (PRF). Figure reproduced from Paper II[113].

The third extraction step was organosolv extraction to recover the lignin in the bark via precipitation. Performing the extraction in an additive-free, dynamic cyclic extraction process with ASE made it possible to keep the extraction mild and preserve the sensitive lignin bonds. From the organosolv extraction, 3.4% lignin (OSL) was recovered based on the initial mass of the raw bark.

In order to investigate the lignin in the acetone extracted bark (AEB) and organosolv extracted fiber residue, ball milling was performed followed by dioxane extraction without further treatment. The ball milling is performed with the assumption of increasing the yield of lignin; however, the yield is also dependent on the milling time and conditions[98].

The efficiency of the extraction sequence performed was investigated with carbohydrate analysis on the raw bark and organosolv residue (**Figure 14a**, more information is found in the supporting information of the appended paper). After the extractions, 70.8% of the bark is extracted based on the initial mass of the raw bark, and the residue contained cellulose (18.7%), lignin (7.9%), non-cellulosic polysaccharides (0.6%), and other minor components (2.0%). While the cellulose content is preserved, approximately 63% of the lignin was removed from the raw bark which corresponds to 16.1% of the total lignin that was possible to be recovered from the organosolv extraction.

The lignin yield of each extraction step is shown in **Figure 14b** which also includes the fraction of Klason lignin (KL) and acid-soluble lignin (ASL). The lignin content of the extracts after the subcritical water extraction was determined to be 17.2%. The total lignin recovered after the subcritical water and organosolv extractions was 33.3%. The remaining lignin in the organosolv residue (OSR) was found to be 37.4% which leaves 29.3% that was not possible to separate. One explanation is that the lignin still remains in the polysaccharide-rich fraction (PRF) which is confirmed by further analysis of the PRF via 2D HSQC NMR (Figure S3 in the appended paper) and UV/Vis (Figure S4 in the appended paper). Another explanation could be that the total lignin content was overestimated in the bark due to that other extractives such as stilbene glucoside and tannins were condensed during the acid pretreatment and ended up in the Klason lignin, influencing the mass balance.

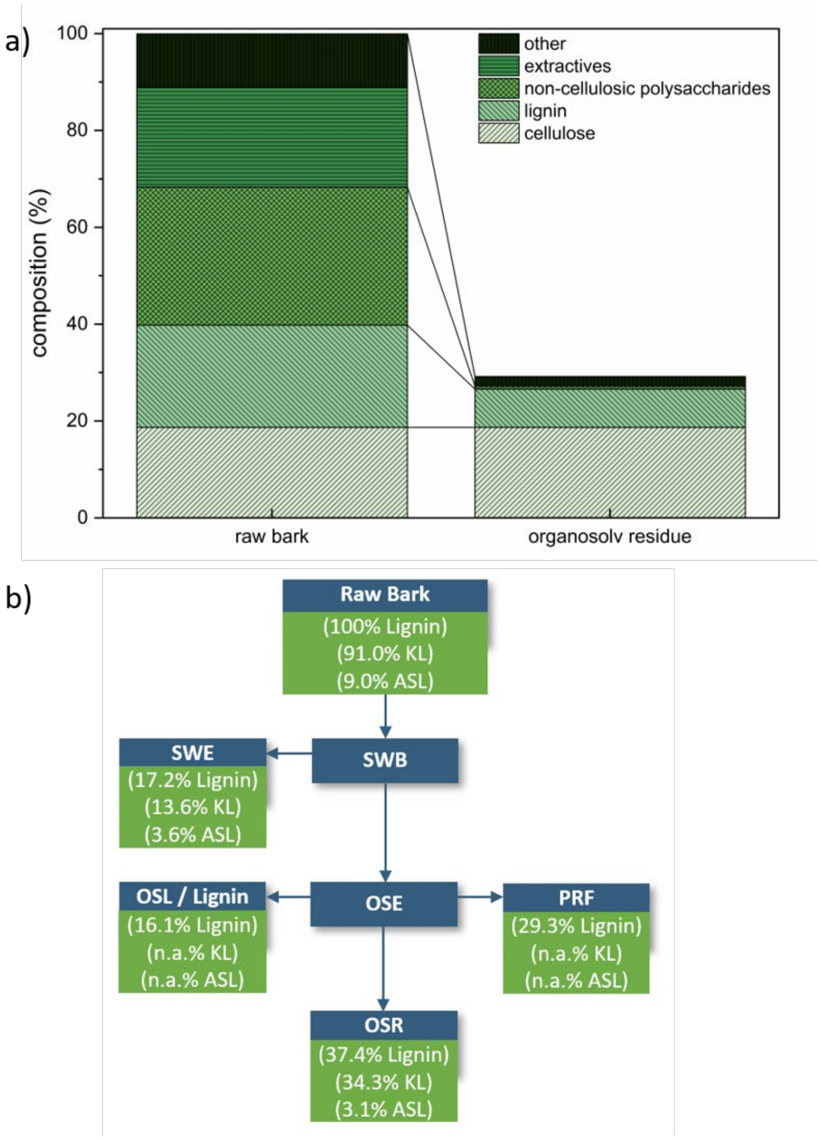


Figure 14 – a) Chemical composition of Norway spruce bark before (raw bark) and after (organosolv residue) organosolv extraction. b) Illustration of the amount of lignin extracted in each extraction step. Subcritical-water extracted bark (SWB), subcritical-water extract (SWE), organosolv extract (OSE), organosolv lignin (OSL), organosolv residue (OSR), polysaccharide-rich fraction (PRF). The percentage represents the total amount of lignin recovered in each fraction, with the representative amount of Klason lignin (KL) and acid-soluble lignin (ASL). Figure reproduced from Paper II[113].

The mildness of the extraction sequence and lignin degradation was determined by analyzing the content of native linkages, especially the β-aryl ether (β-O-4') linkage, contents in all three types of lignin (organosolv, milled bark, and milled residue).

The interunit linkages content of the three lignin types is shown in **Table 4** where the values were obtained via 2D HSQC NMR (**Figure 15** for OSL. MRL and MBL are found in the appended paper (Figure 7) and supporting information (Figure S1)), i.e., semiquantifying the interunit linkage per 100 aromatic units with the C2 lignin signal as internal standard. The β-O-4' content is an indication of mild extraction and an amount of 30% of β-O-4' aryl ethers were determined for the OSL. This is consistent with what has been reported for wood lignin extracted via the cyclic organosolv process[97]. It is commonly known that the β-ether linkage is easily cleaved during mild acidic extraction processes (e.g., the organosolv process with an acidic catalyst)[27, 114, 115]. The organosolv extraction process degrades the lignin which gives a lower β-O-4' content and simultaneously, new phenolic -OH groups are formed. However, the β-O-4' interunit linkage content is affected by the acid catalyst used and the extraction conditions. By using a cyclic organosolv extraction process, with the removal of hemicelluloses and extractives beforehand, it is assumed that a milder route is obtained where the β-O-4' bonds are more protected and therefore not cleaved to as high extent as in a one-batch process.

The amount of β-O-4' aryl ethers for MBL was estimated to 31%. This is in correlation to what was determined for the OSL, which also proves the mildness of the organosolv process and low lignin degradation. For MRL, the β-O-4' aryl ether linkage content was significantly lower, i.e., 17%, than what was found for MBL and OSL. This suggests that the lignin remaining in the residue was more degraded, and hence contained fewer β-O-4' bonds.

Table 4 – Content of lignin interunit linkages from the integration of 2D HSQC NMR spectra of lignin extracted from the spruce inner bark. The result values of the organosolv lignin (OSL) linkages are the mean value of 4 measurements along with the corresponding standard deviation. The standard deviation also applies to the measurement of milled bark lignin (MBL) and milled residue lignin (MRL). The interunit linkages were semi-quantified per 100 Ar units, using the C2 region in the aromatic region on lignin as internal standard. Table obtained from Paper II[113].

	OSL	MBL	MRL
β-O-4' aryl ethers	30±2	31	17
β-5' phenylcoumarans	12±1	12	6.2
β-β' resinols	1.9±0.2	2.2	1.9
5-5' dibenzodioxocins (DBDO)	0.5±0.2	3.8	0.1
Enolether	1.3±0.1	1.0	n.d.
Coumaraldehyde	2.3±0.2	0.8	n.d.
Hibbert's ketone	2.0±0.1	1.2	2.1
Stilbene-β1'	3.5±0.5	1.4	1.1
Stilbene-β5'	3.0±0.3	0.4	1.5

n.d. – no data

From the HSQC NMR of organosolv lignin (**Figure 15**), the β-O-4' Cα position of the extracted lignin (δ_C/δ_H 79.7/4.5 ppm) is both hydroxylated and etherified. When integrating the signal, it is estimated that 57% of the β-O-4' linkages were etherified. This agrees with previously reported results[97]. During acidic conditions, the β-O-4' bond is cleaved and forms a benzylic carbocation that further hydrolyzes into a Hibbert's ketone substructure[115, 116]. Another cleavage route, especially in the presence of ethanol, is a nucleophilic attack at the benzylic Cα position[116, 117]. The etherification increases the lignin solubility in ethanol, but it does not prevent the cleavage of the β-O-4' bond. The cleavage is mainly avoided via cyclic extraction, hence the lignin extracted is more preserved and less degraded.

The lignin extracted via organosolv had a high purity because the polysaccharide signals were low. However, stilbene glucoside signals were observed (stilbene-β1': δ_C/δ_H 127/7, stilbene-β5' δ_C/δ_H 120-130/7.2-7.5) suggesting there were traces of hydroxy stilbene glucosides incorporated in the lignin structure[57]. The stilbene glucoside has properties such as antioxidative and antibacterial which will elevate the lignin's properties and make it suitable for more potential applications.

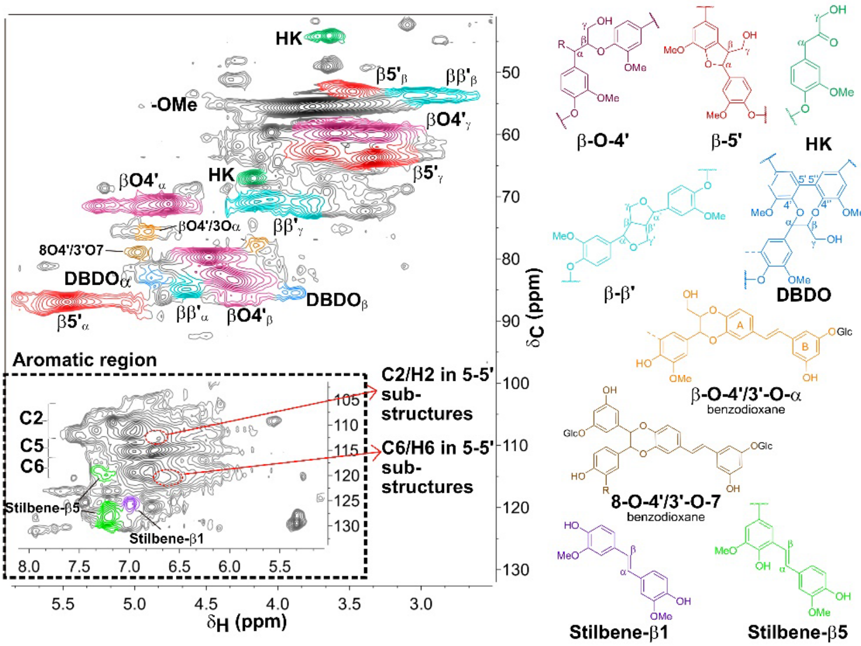


Figure 15 – HSQC NMR of lignin extracted by cyclic organosolv process. The main lignin inter unit linkages are depicted to the right of the spectrum: β-O-4' aryl ethers; β-5' phenylcoumarans; β-β' resinols, 5-5' dibenzodioxocins (DBDO); Hibbert's ketone (HK); β-O-4'/3-O-α benzodioxane structure formed through coupling of coniferyl alcohol and astringin; 8-O-4'/3'-O-7 benzodioxane structure formed by stilbene glucoside units; Stilbene-β1'; Stilbene-β5'. The glucoside units of the hydroxystilbene glucosides were cleaved under the organosolv extraction. Figure obtained from Paper II[113].

^{31}P NMR is a quantitative analysis that was performed to investigate the hydroxy functionalities of the three lignin samples (**Figure 16a**). Generally, after the ethanol organosolv extraction, the total lignin content decreases along with the aliphatic OH content (due to cleavage of β-O-4') while phenolic OH (in **Figure 16a**: guiacyl OH and *p*-hydroxy phenyl OH), phenolic condensed linkages (in **Figure 16a**: C5 substituted OH), and carboxyl functionalities (in **Figure 16a**: carboxylic acid) increases[116]. The results show that the organosolv extracted lignin (OSL) followed this trend. An interesting note is that the C5 substituted OH content of OSL was significantly higher than the milled bark lignin (MBL). This can be explained with the assumption that when the β-O-4' bond is cleaved, β– and phenoxy radicals are formed, and from the phenoxy radicals, 5-5' interunit linkages are formed leading to a higher amount of C5 substituted OH.

The molecular weight and polydispersity (PD) were analyzed with SEC using THF as the solvent and the result is shown in **Figure 16b**. The weight-average molecular weight (M_w) showed a significant difference between the lignins while the number-average molecular weight (M_n) was approximately the same. It was expected to find more low-molecular-weight lignin in MBL and MRL since the ball milling not only increases the lignin yield but also cleaves interunit bonds and degrades the lignin. This will also affect the polydispersity results. In conclusion, the obtained SEC result is therefore inconclusive. Solely SEC analysis, together with 2D HSQC NMR, on lignin is commonly not enough as characterization methods. Karlsson et al.[118] successfully used matrix-assisted laser desorption/ionization with time-of-flight mass spectroscopy (MALDI-TOF MS) to study the lignin structure, proving that the selected analytical method (along with HSQC NMR and density functional theory (DFT)) is suitable for characterizing lignin.

In summary, by applying the bark biorefinery concept, lignin from spruce bark was successfully recovered using the cyclic ethanol organosolv process. The obtained lignin was of high purity, comparable to lignin extracted from spruce wood using the same method, and with additional antioxidative and antibacterial properties due to the incorporation of stilbene glucosides. This opens up a large variety of applications where the lignin can decrease the use of fossil-based resources and make the production of more eco-friendly materials possible.

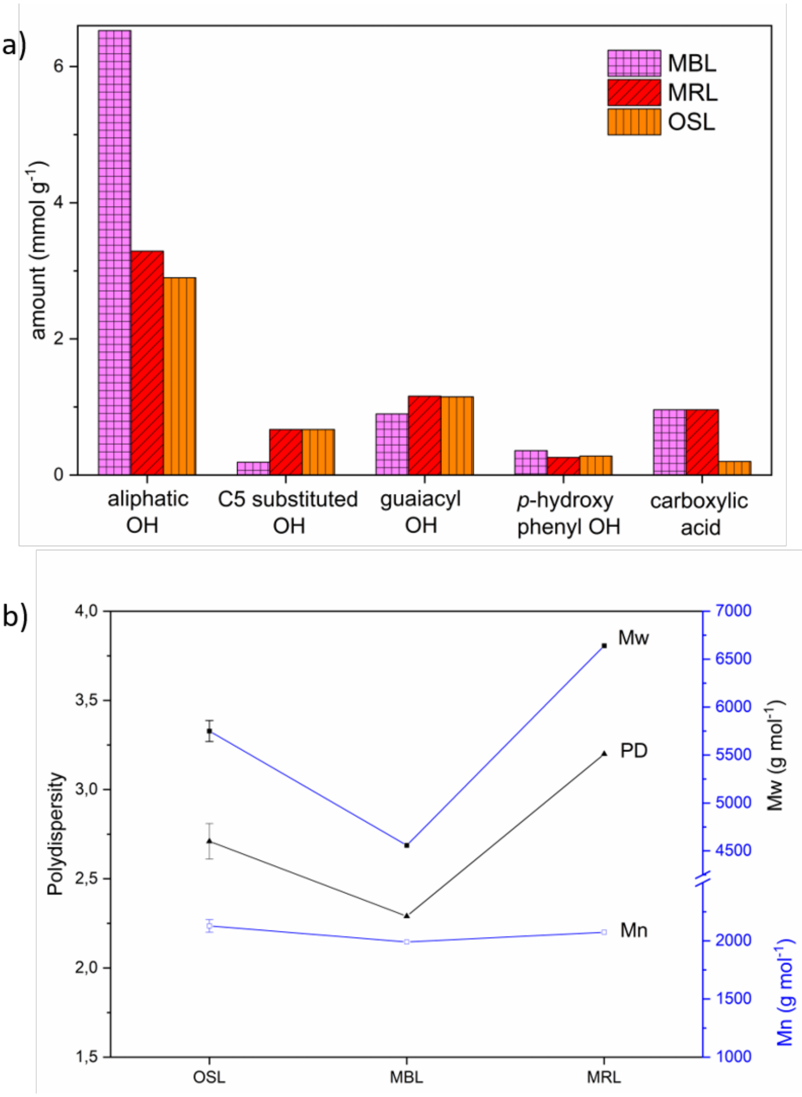


Figure 16 – a) Comparison of hydroxy functionalities determined by ^{31}P NMR between organosolv lignin (OSL), milled bark lignin (MBL), and milled residue lignin (MRL). The values are the mean of at least 2 measurements. b) The weight-average (M_w) and number-average (M_n) molecular weight and corresponding polydispersity of the OSL, MBL, and MRL. The values are the mean of at least 2 measurements. The standard deviation was only determined for OSL. Figure reproduced from Paper II[113].

5. Conclusion

Tree bark is generated in large quantities and is a great source for producing energy. However, unique compounds are found in the bark that can be used to produce value-added materials and chemicals. In this thesis, bark biorefinery was applied to separate cellulose and lignin from the inner bark of Norway spruce.

The cellulose fibers from the bark were liberated via acetone and subcritical water extractions and delignification with peracetic acid. The cellulose was modified with oxalic acid dihydrate prior to the production of nanocellulose. Pickering emulsions were produced with two different oil phases with varying purity. The large size distribution of the nanocellulose along with the oil purity and nanoparticle concentration affected the emulsion stability. In general, a high aspect ratio, homogeneous oil, and high particle concentration are to strive for when producing stable Pickering emulsions. With this study, it has been proven that it is possible to use a side-stream product such as bark to produce an eco-friendly emulsifying agent that may replace petroleum-based surfactants and emulsifying agents via environmentally friendly routes and chemicals.

Lignin was recovered from the spruce inner bark via sequential extraction steps. Acetone and subcritical water extractions were applied to remove extractives and non-cellulosic polysaccharides before exposing the bark fibers to cyclic organosolv extractions with an acid catalyst. This process route to recover lignin differs from the more conventional processes to produce technical lignins. From the study, high-purity lignin was able to be recovered. Additional properties (e.g., antibacterial and antioxidative) were also detected because of the incorporation of traces of stilbene glucosides in the lignin. This makes spruce bark lignin extracted via organosolv more interesting and may be more attractive on the market as it makes it possible to be applied in more materials and products.

By using a side-stream product as a raw material, the environmental impact is decreased in comparison to if fossil-based resources were used. Moreover, by focusing on using more sustainable chemicals throughout the processes, the environmental impact is decreased further since fewer hazardous residues are generated. This may be beneficial for industries to reach carbon neutrality.

6. Outlook and future work

This thesis has demonstrated that the bark biorefinery concept is indeed feasible but there is yet much to explore and many challenges to meet.

Other components that are extracted during the sequential extractions such as extractives and pectic polysaccharides are interesting and may be studied further and used to produce various products or be used as valuable chemicals. The extractives from spruce bark contain a mixture of different compounds. Two of them are stilbene glucosides and tannins. These can for example be incorporated into the production of emulsions and serve as co-surfactants to aid emulsion stability. Pectin polysaccharides are naturally occurring surface-active agents that are suitable to produce Pickering emulsions. Another interesting continuation would be to use the recovered organosolv lignin to produce lignin nanoparticles which can be used to produce for example Pickering emulsions, coatings, and bio-composites.

Bark from Norway spruce was in focus of this thesis, but other tree species also contain unique compounds. One such species is birch. Birch bark contains interesting compounds such as tannins, suberin, and betulin. However, the research on birch bark is limited and the extraction processes to isolate these compounds are not particularly sustainable, especially during the purification step. It would therefore be of great interest to investigate greener production routes to obtain these highly interesting compounds. Moreover, combining the compounds from spruce and birch bark to produce all-bark composites is intriguing as great properties from both barks would elevate the properties of the end products.

There are still challenges remaining with using bark as a resource industrially for the production of materials. The scale-up of a bark biorefinery is energy demanding and the runnability is questionable. Therefore, it is necessary to further investigate the feasibility of a bark biorefinery pilot plant. Another challenge is the competition with energy production of incinerating the bark. Currently, mills and factories use the bark to produce energy for themselves and a shift to start using tree bark to produce new products requires an alternative source for energy production. This may not be economically beneficial, and it is therefore necessary to perform techno-economically and environmental assessments.

Acknowledgments

First of all, I would like to sincerely send my gratitude to my main supervisor Prof. Monica Ek for giving me this opportunity to perform this licentiate project and letting me join her research group. It really has been a journey that started back in the days with my master's thesis up until now with my licentiate degree. I really appreciate you for always believing in me and for your persistent guidance far beyond only research.

I would like to say a big thank you to my co-supervisor Prof. Åsa Emmer for being a part of my research journey. You have provided me with great scientific guidance regarding analytical chemistry.

I would like to acknowledge all the co-authors and collaborators: Barbara R-L, Maria K, Rauni S, Peter S, Tianxiao H, Kloce L, and Martin L for all the fruitful discussions. Thank you all for your contributions and nice collaborations.

I want to acknowledge Knut and Alice Wallenberg Foundation through the Wallenberg Wood Science Center for funding this licentiate thesis.

I would like to thank both former and present members of the Fiber and Polymer Technology department, and my colleagues in the Wood Chemistry and Pulp Technology division. A special thank you to Huisi L, Nicola G, Jowan R, Lukas C, Jonas G, and Björn B for providing a nice atmosphere and work environment. Thank you to former and present friends and colleagues at WWSC for the great time in the office and lab. A warm thank you to Maria K, Rebecca Ö, Farhiya S, Maria C, Luna X, Billy H, and Raffaele P for making it enjoyable to go to work.

I would like to send a special thank you to Dr. Barbara Rietzler-Lins. Your knowledge and guidance in the lab were very valuable to me, making it more manageable to start my doctoral studies. Thank you for taking the time to explain, sharing your ideas, and your support throughout this journey. I am forever grateful for your friendship!

Finally, I would like to thank my dear family for always supporting and believing in me. Your love and concern mean a lot. This work is dedicated to you.

Isabella Kwan
Stockholm, September 2023

References

(1) United Nations. *The Sustainable Development Agenda*. 2023. https://www.un.org/sustainabledevelopment/development-agenda/ (accessed 2023 June 21).

(2) United Nations. *Sustainable Development - The 17 Goals*. 2023. https://sdgs.un.org/goals (accessed 2023 June 9).

(3) Pásztory, Z.; Mohácsiné, I. R.; Gorbacheva, G.; Börcsök, Z. The Utilization of Tree Bark. *BioResources* **2016**, *11* (3), 7859-7888. DOI: 10.15376/biores.11.3.Pasztory.

(4) Fratzl, P.; Weinkamer, R. Nature's hierarchical materials. *Prog Mater Sci* **2007**, *52* (8), 1263-1334. DOI: 10.1016/j.pmatsci.2007.06.001.

(5) Speck, T.; Burgert, I. Plant Stems: Functional Design and Mechanics. *Annu Rev of Mater Res* **2011**, *41* (1), 169-193. DOI: 10.1146/annurev-matsci-062910-100425.

(6) Sjöström, E. The Structure of Wood. In *Wood Chemistry - Fundamentals and Applications*, Academic Press, Inc., 1981; pp 1-20.

(7) Saka, S. Structure and Chemical Composition of Wood as a Natural Composite Material. In *Recent Research on Wood and Wood-Based Materials*, Vol. 11; Elsevier Science Publishers Ltd, 1993; pp 1-20.

(8) Biermann, C. J. Pulping Fundamentals. In *Essentials of Pulping and Papermaking*, Academic Press Inc., 1993; pp 55-136.

(9) Sjöström, E. Wood Polysaccharides. In *Wood Chemistry - Fundamentals and Applications*, Academic Press, Inc., 1981; pp 49-67.

(10) Kolseth, P.; De Ruvo, A. The Cell Wall Components of Wood Pulp Fibers. In *Paper Structure and Properties*, Bristow, J. A., Kolseth, P. Eds.; Marcel Dekker, Inc., 1986; pp 3-25.

(11) Lavoine, N.; Desloges, I.; Dufresne, A.; Bras, J. Microfibrillated cellulose - its barrier properties and applications in cellulosic materials: a review. *Carbohydr Polym* **2012**, *90* (2), 735-764. DOI: 10.1016/j.carbpol.2012.05.026.

(12) Habibi, Y.; Lucia, L. A.; Rojas, O. J. Cellulose nanocrystals: chemistry, self-assembly, and applications. *Chem Rev* **2010**, *110* (6), 3479-3500. DOI: 10.1021/cr900339w.

(13) Siqueira, G.; Bras, J.; Dufresne, A. Cellulosic Bionanocomposites: A Review of Preparation, Properties and Applications. *Polymers* **2010**, *2* (4), 728-765. DOI: 10.3390/polym2040728.

(14) Rong, M. Z.; Zhang, M. Q.; Liu, Y.; Yang, G. C.; Zeng, H. M. The effect of fiber treatment on the mechanical properties of unidirectional sisal-reinforced epoxy composites. *Compos Sci Technol* **2001**, *61* (10), 1437-1447. DOI: 10.1016/S0266-3538(01)00046-X.

(15) Li, D.; Henschen, J.; Ek, M. Esterification and hydrolysis of cellulose using oxalic acid dihydrate in a solvent-free reaction suitable for preparation of surface-functionalised cellulose nanocrystals with high yield. *Green Chem* **2017**, *19* (23), 5564-5567. DOI: 10.1039/c7gc02489d.

(16) Spiliopoulos, P.; Solala, I.; Paakkonen, T.; Seitsonen, J.; van Bochove, B.; Seppala, J. V.; Kontturi, E. Native structure of the plant cell wall utilized for top-down assembly of aligned cellulose nanocrystals into micrometer-sized nanoporous particles. *Macromol Rapid Commun* **2020**, *41* (15), e2000201. DOI: 10.1002/marc.202000201.

(17) Bras, J.; Saini, S. Nanocellulose in functional packaging. In *Cellulose-Reinforced Nanofibre Composites*, 2017; pp 175-213.

(18) Foster, E. J.; Moon, R. J.; Agarwal, U. P.; Bortner, M. J.; Bras, J.; Camarero-Espinosa, S.; Chan, K. J.; Clift, M. J. D.; Cranston, E. D.; Eichhorn, S. J.; et al. Current characterization methods for cellulose nanomaterials. *Chem Soc Rev* **2018**, *47* (8), 2609-2679. DOI: 10.1039/c6cs00895j.

(19) Moon, R. J.; Martini, A.; Nairn, J.; Simonsen, J.; Youngblood, J. Cellulose nanomaterials review: structure, properties and nanocomposites. *Chem Soc Rev* **2011**, *40* (7), 3941-3994. DOI: 10.1039/c0cs00108b.

(20) Dufresne, A. *Nanocellulose - From Nature to High Performance Tailored Materials*; De Gruyter, 2018.

(21) Isogai, A.; Saito, T.; Fukuzumi, H. TEMPO-oxidized cellulose nanofibers. *Nanoscale* **2011**, *3* (1), 71-85. DOI: 10.1039/c0nr00583e.

(22) Wågberg, L.; Decher, G.; Norgren, M.; Lindström, T.; Ankerfors, M.; Axnäs, K. The build-up of polyelectrolyte multilayers of microfibrillated cellulose and cationic polyelectrolytes. *Langmuir* **2008**, *24*, 784-795. DOI: 10.1021/la702481v.

(23) Henriksson, M.; Henriksson, G.; Berglund, L. A.; Lindström, T. An environmentally friendly method for enzyme-assisted preparation of microfibrillated cellulose (MFC) nanofibers. *Eur Polym J* **2007**, *43* (8), 3434-3441. DOI: 10.1016/j.eurpolymj.2007.05.038.

(24) Françon, H.; Wang, Z.; Marais, A.; Mystek, K.; Piper, A.; Granberg, H.; Malti, A.; Gatenholm, P.; Larsson, P. A.; Wågberg, L. Ambient-Dried, 3D-Printable and Electrically Conducting Cellulose Nanofiber Aerogels by Inclusion of Functional Polymers. *Adv Funct Mater* **2020**, *30* (12), 1-11. DOI: 10.1002/adfm.201909383.

(25) Shiraishi, N.; Kajita, H.; Norimoto, M. *Recent Research on Wood and Wood-Based Materials - Current Japanese Materials Research*; Elsevier Science Publishers Ltd, 1993.

(26) Lin, S. Y.; Dence, C. W. *Methods in Lignin Chemistry*; Springer Berlin, Heidelberg, 1992. DOI: 10.1007/978-3-642-74065-7.

(27) Vaidya, A. A.; Murton, K. D.; Smith, D. A.; Dedual, G. A review on organosolv pretreatment of softwood with a focus on enzymatic hydrolysis of cellulose. *Biomass Convers Biorefin* **2022**, *12* (11), 5427-5442. DOI: 10.1007/s13399-022-02373-9.

(28) Pye, E. K.; Lora, J. H. The Alcell™ process - A proven alternative to kraft pulping. *TAPPI J* **1991**, 113-117.

(29) Diebold, V. B.; Cowan, W. F.; Walsh, J. K. Solvent Pulping Process. United States 1978.

(30) Lora, J. H.; Glasser, W. G. Recent Industrial Applications of Lignin: A Sustainable Alternative to Nonrenewable Materials. *J Polym Environ* **2002**, *10*, 39-48. DOI: 10.1023/A:1021070006895.

(31) Mikkonen, K. S. Recent Studies on Hemicellulose-Based Blends, Composites and Nanocomposites. In *Advances in Natural Polymers: Composites and Nanocomposites*, Vol. 18; Springer-Verlag Berlin Heidelberg, 2013; pp 313-336.

(32) Al-Dajani, W. W.; Tschirner, U. W. Pre-extraction of hemicelluloses and subsequent kraft pulping Part I: alkaline extraction. *TAPPI J* **2008**, *7* (6), 3-8. DOI: 10.32964/TJ7.6.3.

(33) Arola, S.; Malho, J. M.; Laaksonen, P.; Lille, M.; Linder, M. B. The role of hemicellulose in nanofibrillated cellulose networks. *Soft Matter* **2013**, *9* (4), 1319-1326. DOI: 10.1039/c2sm26932e.

(34) Hon, D. N. S.; Shiraishi, N. *Wood and Cellulosic Chemistry*; CRC Press, 2000.

(35) Imamura, H. Contribution of Extractives to Wood Characteristics. In *Natural Products of Woody Plants*, Springer-Verlag Berlin Heidelberg, 1989; pp 843-860.

(36) Lehr, M.; Miltner, M.; Friedl, A. Removal of wood extractives as pulp (pre-)treatment: a technological review. *SN Appl Sci* **2021**, *3* (12). DOI: 10.1007/s42452-021-04873-1.

(37) Ek, M.; Gellerstedt, G.; Henriksson, G. *Wood Chemistry and Wood Biotechnology*; De Gruyter, 2009. DOI: 10.1515/9783110213409.

(38) Eurostat. *Data Browser - Pulp, paper and paperboard*. Eurostat, 2022. https://ec.europa.eu/eurostat/databrowser/view/FOR_PP/default/bar?lang=en (accessed 2023 August 7).

(39) Feng, S.; Cheng, S.; Yuan, Z.; Leitch, M.; Xu, C. Valorization of bark for chemicals and materials: a review. *Renewable Sustainable Energy Rev* **2013**, *26*, 560-578. DOI: 10.1016/j.rser.2013.06.024.

(40) Henderson, P.; Szakálos, P.; Pettersson, R.; Andersson, C.; Högberg, J. Reducing superheater corrosion in wood-fired boilers. *Mater Corros* **2006**, *57* (2), 128-134. DOI: 10.1002/maco.200503899.

(41) Ashton, S.; Cassidy, P. Energy basics. *Sustainable Forestry for Bioenergy and Bio-based Products* **2007**.

(42) Corder, S. E. *Properties and Uses of Bark as an Energy Source*; Research Paper 31; 1976.

(43) Pásztory, Z.; Börcsök, Z.; Tsalagkas, D. Density optimization for the manufacturing of bark-based thermal insulation panels. *IOP Conference Series: Earth and Environmental Science* **2019**, *307*. DOI: doi:10.1088/1755-1315/307/1/012007.

(44) Kain, G.; Barbu, M.-C.; Hinterreiter, S.; Richter, K.; Petutschnigg, A. Using Bark as a Heat Insulation Material. *Bioresources* **2013**, *8* (3), 3718-3731.

(45) Gosswald, J.; Barbu, M. C.; Petutschnigg, A.; Tudor, E. M. Binderless Thermal Insulation Panels Made of Spruce Bark Fibres. *Polymers (Basel)* **2021**, *13* (11). DOI: 10.3390/polym13111799.

(46) Tsalagkas, D.; Börcsök, Z.; Pásztory, Z. Thermal, physical and mechanical properties of surface overlaid bark-based insulation panels. *Eur J Wood Wood Prod* **2019**, *77* (5), 721-730. DOI: 10.1007/s00107-019-01436-5.

(47) Bianchi, S.; Kroslakova, I.; Janzon, R.; Mayer, I.; Saake, B.; Pichelin, F. Characterization of condensed tannins and carbohydrates in hot water bark extracts of European softwood species. *Phytochemistry* **2015**, *120*, 53-61. DOI: 10.1016/j.phytochem.2015.10.006.

(48) Krogell, J.; Holmbom, B.; Pranovich, A.; Hemming, J.; Willfor, S. Extraction and chemical characterization of Norway spruce inner and outer bark. *Nord Pulp Pap Res J* **2012**, *27* (1), 6-17. DOI: 10.3183/npprj-2012-27-01-p006-017.

(49) Neiva, D. M.; Araújo, S.; Gominho, J.; Carneiro, A. d. C.; Pereira, H. Potential of Eucalyptus globulus industrial bark as a biorefinery feedstock: Chemical and fuel characterization. *Ind Crops Prod* **2018**, *123*, 262-270. DOI: 10.1016/j.indcrop.2018.06.070.

(50) Kemppainen, K.; Inkinen, J.; Uusitalo, J.; Nakari-Setala, T.; Siika-aho, M. Hot water extraction and steam explosion as pretreatments for ethanol production from spruce bark. *Bioresour Technol* **2012**, *117*, 131-139. DOI: 10.1016/j.biortech.2012.04.080.

(51) Neiva, D. M.; Gominho, J.; Fernandes, L.; Lourenço, A.; Chemetova, C.; Simões, R. M. S.; Pereira, H. The Potential of Hydrothermally Pretreated Industrial Barks From E. globulus as a Feedstock for Pulp Production. *J Wood Chem Technol* **2016**, *36* (6), 383-392. DOI: 10.1080/02773813.2016.1184280.

(52) Le Normand, M.; Moriana, R.; Ek, M. Isolation and characterization of cellulose nanocrystals from spruce bark in a biorefinery perspective. *Carbohydr Polym* **2014**, *111*, 979-987. DOI: 10.1016/j.carbpol.2014.04.092.

(53) Rietzler, B.; Ek, M. Adding value to spruce bark by the isolation of nanocellulose in a biorefinery concept. *ACS Sustainable Chem Eng* **2021**, *9* (3), 1398-1405. DOI: 10.1021/acssuschemeng.0c08429.

(54) SLU. *Skogsdata 2022*; Uppsala, 2022.

(55) Räisänen, T.; Athanassiadis, D. *Basic chemical composition of the biomass components of pine, spruce and birch*; 2013.

(56) Sain, S.; Matsakas, L.; Rova, U.; Christakopoulos, P.; Oman, T.; Skrifvars, M. Spruce Bark-Extracted Lignin and Tannin-Based Bioresin-Adhesives: Effect of Curing Temperatures on the Thermal Properties of the Resins. *Molecules* **2021**, *26* (12). DOI: 10.3390/molecules26123523.

(57) Rencoret, J.; Neiva, D.; Marques, G.; Gutierrez, A.; Kim, H.; Gominho, J.; Pereira, H.; Ralph, J.; Del Rio, J. C. Hydroxystilbene Glucosides Are Incorporated into Norway Spruce Bark Lignin. *Plant Physiol* **2019**, *180* (3), 1310-1321. DOI: 10.1104/pp.19.00344.

(58) Cherubini, F. The biorefinery concept: Using biomass instead of oil for producing energy and chemicals. *Energy Convers Manage* **2010**, *51* (7), 1412-1421. DOI: 10.1016/j.enconman.2010.01.015.

(59) Demirbas, A.; Demirbas, M. F. Biorefineries. In *Algae Energy - Algae as a New Source of Biodisel*, Springer-Verlag, 2010; pp 159-181.

(60) Maity, S. K. Opportunities, recent trends and challenges of integrated biorefinery: Part I. *Renewable Sustainable Energy Rev* **2015**, *43*, 1427-1445. DOI: 10.1016/j.rser.2014.11.092.

(61) Spliethoff, H.; Hein, K. R. G. Effect of co-combustion of biomass on emissions in pulverized fuel furnaces. *Fuel Process Technol* **1998**, *54* (1-3), 189-205. DOI: 10.1016/S0378-3820(97)00069-6.

(62) Faaij, A.; Meuleman, B.; Turkenburg, W.; Wijk, A. V.; Bauen, A.; Rosillo-Calle, F.; Hall, D. Externalities of biomass based electricity production compared with power generation from coal in the Netherlands. *Biomass Bioenergy* **1998**, *14* (2), 125-147. DOI: 10.1016/S0961-9534(97)10005-8.

(63) Nosek, R.; Holubcik, M.; Jandacka, J. The Impact of Bark Content of Wood Biomass on Biofuel Properties. *Bioresources* **2016**, *11* (1), 44-53. DOI: 10.15376/biores.11.1.44-53.

(64) Yuan, Z.; Ni, Y.; Van Heiningen, A. R. P. Kinetics of peracetic acid decomposition: Part I: Spontaneous decomposition at typical pulp bleaching conditions. *Can J Chem Eng* **1997**, *75* (1), 37-41. DOI: 10.1002/cjce.5450750108.

(65) Pals, M.; Lauberte, L.; Arshanitsa, A.; Vevere, L.; Jurkjane, V.; Telysheva, G. Organosolv delignification of residual plantation willow bark after extractive removal. In Research for Rural Development 2020 : annual 26th International scientific conference proceedings, 2020.

(66) Johansson, A.; Aaltonen, O.; Ylinen, P. Organosolv Pulping - Methods and Pulp Properties. *Biomass* **1987**, *13*, 45-65. DOI: 10.1016/0144-4565(87)90071-0.

(67) Bai, L.; Huan, S.; Zhao, B.; Zhu, Y.; Esquena, J.; Chen, F.; Gao, G.; Zussman, E.; Chu, G.; Rojas, O. J. All-Aqueous Liquid Crystal Nanocellulose Emulsions with Permeable Interfacial Assembly. *ACS Nano* **2020**, *14* (10), 13380-13390. DOI: 10.1021/acsnano.0c05251.

(68) Binks, B. P. *Modern Aspects of Emulsion Science*; The Royal Society of Chemistry, 1998. DOI: 10.1039/9781847551474.

(69) Barnes, G. T.; Gentle, I. R. *Interfacial science: an introduction*; Oxford University Press, 2011.

(70) Albert, C.; Beladjine, M.; Tsapis, N.; Fattal, E.; Agnely, F.; Huang, N. Pickering emulsions: preparation processes, key parameters governing their properties and potential for pharmaceutical applications. *J Control Release* **2019**, *309*, 302-332. DOI: 10.1016/j.jconrel.2019.07.003.

(71) Berton-Carabin, C. C.; Schroen, K. Pickering emulsions for food applications: background, trends, and challenges. *Annu Rev Food Sci Technol* **2015**, *6*, 263-297. DOI: 10.1146/annurev-food-081114-110822.

(72) Dickinson, E. Colloids in food: ingredients, structure, and stability. *Annu Rev Food Sci Technol* **2015**, *6*, 211-233. DOI: 10.1146/annurev-food-022814-015651.

(73) Tsabet, È.; Fradette, L. Effect of Processing Parameters on the Production of Pickering Emulsions. *Ind Eng Chem Res* **2015**, *54* (7), 2227-2236. DOI: 10.1021/ie504338d.

(74) Tsabet, È.; Fradette, L. Effect of the properties of oil, particles, and water on the production of Pickering emulsions. *Chem Eng Res Des* **2015**, *97*, 9-17. DOI: 10.1016/j.cherd.2015.02.016.

(75) Nollet, M.; Boulghobra, H.; Calligaro, E.; Rodier, J. D. An efficient method to determine the Hydrophile-Lipophile Balance of surfactants using the phase inversion temperature deviation of C(i) E(j) /n-octane/water emulsions. *Int J Cosmet Sci* **2019**, *41* (2), 99-108. DOI: 10.1111/ics.12516.

(76) Griffin, W. C. Classification of Surface-Active Agents by "HLB". *J Soc Cosmet Chem* **1949**, *1*, 311-326.

(77) Davies, J. T. A quantitative kinetic theory of emulsion type, I. Physical chemistry of the emulsifying agent. In *Proc Int Congr Surf Act*, 1957; pp 426-438.

(78) De, S.; Malik, S.; Ghosh, A.; Saha, R.; Saha, B. A review on natural surfactants. *RSC Adv* **2015**, *5* (81), 65757-65767. DOI: 10.1039/c5ra11101c.

(79) McClements, D. J.; Gumus, C. E. Natural emulsifiers - Biosurfactants, phospholipids, biopolymers, and colloidal particles: Molecular and physicochemical basis of functional performance. *Adv Colloid Interface Sci* **2016**, *234*, 3-26. DOI: 10.1016/j.cis.2016.03.002.

(80) Benvegnu, T.; Plusquellec, D.; Lemiègre, L. Surfactants from Renewable Sources: Synthesis and Applications. In *Monomers, Polymers and Composites from Renewable Resources*, Belgacem, M. N., Gandini, A. Eds.; Elsevier Ltd., 2008; pp 153-178.

(81) Williams, J. R.; Gong, H. Biological activities and syntheses of steroidal saponins: the shark-repelling pavoninins. *Lipids* **2007**, *42* (1), 77-86. DOI: 10.1007/s11745-006-1002-3.

(82) Guzmán, E.; Llamas, S.; Fernández-Peña, L.; Léonforte, F.; Baghdadli, N.; Cazeneuve, C.; Ortega, F.; Rubio, R. G.; Luengo, G. S. Effect of a natural amphoteric surfactant in the bulk and adsorption behavior of polyelectrolyte-surfactant mixtures. *Colloids Surf, A* **2020**, *585*. DOI: 10.1016/j.colsurfa.2019.124178.

(83) Kwan, I.; Huang, T.; Ek, M.; Seppänen, R.; Skagerlind, P. Bark from Nordic tree species – a sustainable source for amphiphilic polymers and surfactants. *Nord Pulp Pap Res J* **2022**, *37* (4), 566-575. DOI: 10.1515/npprj-2022-0003.

(84) Ramsden, W. Separation of solids in the surface-layers of solutions and suspensions (observations on surface-membranes, bubbles, emulsions, and mechanical coagulation) - preliminary account. *Proc R Soc London* **1903**, *72*, 156-164. DOI: 10.1098/rspl.1903.0034.

(85) Pickering, S. U. CXCVI. - Emulsions. *J Chem Soc, Trans 91* **1907**, 2001-2021.

(86) Yuan, T.; Zeng, J.; Wang, B.; Cheng, Z.; Chen, K. Pickering emulsion stabilized by cellulosic fibers: morphological properties-interfacial stabilization-rheological behavior relationships. *Carbohydr Polym* **2021**, *269* (118339), 1-10. DOI: 10.1016/j.carbpol.2021.118339.

(87) Zhu, F. Starch based Pickering emulsions: Fabrication, properties, and applications. *Trends Food Sci Technol* **2019**, *85*, 129-137. DOI: 10.1016/j.tifs.2019.01.012.

(88) Oza, K. P.; Frank, S. G. Microcrystalline cellulose stabilized emulsions. *J Dispersion Sci Technol* **1986**, *7* (5), 543-561. DOI: 10.1080/01932698608943478.

(89) Kalashnikova, I.; Bizot, H.; Cathala, B.; Capron, I. New Pickering emulsions stabilized by bacterial cellulose nanocrystals. *Langmuir* **2011**, *27* (12), 7471-7479. DOI: 10.1021/la200971f.

(90) Kedzior, S. A.; Gabriel, V. A.; Dube, M. A.; Cranston, E. D. Nanocellulose in Emulsions and Heterogeneous Water-Based Polymer Systems: A Review. *Adv Mater* **2020**, e2002404. DOI: 10.1002/adma.202002404.

(91) Jimenez Saelices, C.; Capron, I. Design of Pickering Micro- and Nanoemulsions Based on the Structural Characteristics of Nanocelluloses. *Biomacromolecules* **2018**, *19* (2), 460-469. DOI: 10.1021/acs.biomac.7b01564.

(92) Gestranius, M.; Stenius, P.; Kontturi, E.; Sjöblom, J.; Tammelin, T. Phase behaviour and droplet size of oil-in-water Pickering emulsions stabilised with plant-derived nanocellulosic materials. *Colloids Surf, A* **2017**, *519*, 60-70. DOI: 10.1016/j.colsurfa.2016.04.025.

(93) Bai, L.; Huan, S.; Xiang, W.; Rojas, O. J. Pickering emulsions by combining cellulose nanofibrils and nanocrystals: phase behavior and depletion stabilization. *Green Chem* **2018**, *20* (7), 1571-1582. DOI: 10.1039/c8gc00134k.

(94) Capron, I.; Rojas, O. J.; Bordes, R. Behavior of nanocelluloses at interfaces. *Curr Opin Colloid Interface Sci* **2017**, *29*, 83-95. DOI: 10.1016/j.cocis.2017.04.001.

(95) Capron, I.; Cathala, B. Surfactant-free high internal phase emulsions stabilized by cellulose nanocrystals. *Biomacromolecules* **2013**, *14* (2), 291-296. DOI: 10.1021/bm301871k.

(96) Kalashnikova, I.; Bizot, H.; Cathala, B.; Capron, I. Modulation of cellulose nanocrystals amphiphilic properties to stabilize oil/water interface. *Biomacromolecules* **2012**, *13* (1), 267-275. DOI: 10.1021/bm201599j.

(97) Karlsson, M.; Giummarella, N.; Linden, P. A.; Lawoko, M. Toward a Consolidated Lignin Biorefinery: Preserving the Lignin Structure through Additive-Free Protection Strategies. *ChemSusChem* **2020**, *13* (17), 4666-4677. DOI: 10.1002/cssc.202000974.

(98) Björkman, A. Isolation of Lignin from Finely Divided Wood with Neutral Solvents. *Nature (London)* **1954**, *174* (4440), 1057-1058. DOI: 10.1038/1741057a0.

(99) TAPPI. *Acid-insoluble lignin in wood and pulp*; Atalanta, GA, USA, 2006.

(100) Scandinavian Pulp Paper and Board. *Total acidic group content*; 2002.

(101) Gellerstedt, G. Gel Permeation Chromatography. In *Methods in Lignin Chemistry*, Lin, S. Y., Dence, C. W. Eds.; Springer, 1992; pp 487-497.

(102) Sette, M.; Wechselberger, R.; Crestini, C. Elucidation of lignin structure by quantitative 2D NMR. *Chemistry* **2011**, *17* (34), 9529-9535. DOI: 10.1002/chem.201003045.

(103) Argyropoulos, D. S. Quantitative Phosphorus-31 NMR Analysis of Lignins, a New Tool for the Lignin Chemist. *J Wood Chem Technol* **1994**, *14* (1), 45-63. DOI: 10.1080/02773819408003085.

(104) Henschen, J.; Li, D.; Ek, M. Preparation of cellulose nanomaterials via cellulose oxalates. *Carbohydr Polym* **2019**, *213*, 208-216. DOI: 10.1016/j.carbpol.2019.02.056.

(105) Benselfelt, T.; Kummer, N.; Nordenstrom, M.; Fall, A. B.; Nystrom, G.; Wagberg, L. The colloidal properties of nanocellulose. *ChemSusChem* **2023**, *16* (8), e202201955. DOI: 10.1002/cssc.202201955.

(106) Kwan, I.; Rietzler, B.; Ek, M. Emulsions of cellulose oxalate from Norway spruce (Picea abies) bark and dissolving pulp. *Holzforschung* **2023**, *77* (7), 554-565. DOI: 10.1515/hf-2022-0191.

(107) Asakura, S.; Oosawa, F. Interaction between particles suspended in solutions of macromolecules. *J Polym Sci* **1958**, *33* (126), 183-192. DOI: 10.1002/pol.1958.1203312618.

(108) Tingaut, P.; Zimmermann, T.; Sèbe, G. Cellulose nanocrystals and microfibrillated cellulose as building blocks for the design of hierarchical functional materials. *J Mater Chem* **2012**, *22* (38), 20105-20111. DOI: 10.1039/c2jm32956e.

(109) Binks, B. P.; Lumsdon, S. O. Effects of oil type and aqueous phase composition on oil–water mixtures containing particles of intermediate hydrophobicity. *Phys Chem Chem Phys* **2000**, *2* (13), 2959-2967. DOI: 10.1039/b002582h.

(110) Souza, A. G.; Ferreira, R. R.; Paula, L. C.; Setz, L. F. G.; Rosa, D. S. The effect of essential oil chemical structures on Pickering emulsion stabilized with cellulose nanofibrils. *J Mol Liq* **2020**, *320* (114458), 1-10. DOI: 10.1016/j.molliq.2020.114458.

(111) McClements, D. J. Critical review of techniques and methodologies for characterization of emulsion stability. *Crit Rev Food Sci Nutr* **2007**, *47* (7), 611-649. DOI: 10.1080/10408390701289292.

(112) Arditty, S.; Whitby, C. P.; Binks, B. P.; Schmitt, V.; Leal-Calderon, F. Some general features of limited coalescence in solid-stabilized emulsions. *Eur Phys J E Soft Matter* **2003**, *11* (3), 273-281. DOI: 10.1140/epje/i2003-10018-6.

(113) Rietzler, B.; Karlsson, M.; Kwan, I.; Lawoko, M.; Ek, M. Fundamental Insights on the Physical and Chemical Properties of Organosolv Lignin from Norway Spruce Bark. *Biomacromolecules* **2022**, *23* (8), 3349-3358. DOI: 10.1021/acs.biomac.2c00457.

(114) Nakagame, S.; Chandra, R. P.; Kadla, J. F.; Saddler, J. N. The isolation, characterization and effect of lignin isolated from steam pretreated Douglas-fir on the enzymatic hydrolysis of cellulose. *Bioresour Technol* **2011**, *102* (6), 4507-4517. DOI: 10.1016/j.biortech.2010.12.082.

(115) Santos, R. B.; Hart, P. W.; Jameel, H.; Chang, H. M. Wood Based Lignin Reactions Important to the Biorefinery and Pulp and Paper Industries. *Bioresources* **2013**, *8* (1), 1456-1477.

(116) Sipponen, M. H.; Rahikainen, J.; Leskinen, T.; Pihlajaniemi, V.; Mattinen, M.-L.; Lange, H.; Crestini, C.; Österberg, M. Structural changes of lignin in biorefinery pretreatments and consequences to enzyme-lignin interactions. *Nord Pulp Pap Res J* **2017**, *32* (4), 550-571. DOI: 10.3183/npprj-2017-32-04_p550-571_sipponen.

(117) Bauer, S.; Sorek, H.; Mitchell, V. D.; Ibanez, A. B.; Wemmer, D. E. Characterization of Miscanthus giganteus lignin isolated by ethanol organosolv process under reflux condition. *J Agric Food Chem* **2012**, *60* (33), 8203-8212. DOI: 10.1021/jf302409d.

(118) Karlsson, M.; Romson, J.; Elder, T.; Emmer, A.; Lawoko, M. Lignin Structure and Reactivity in the Organosolv Process Studied by NMR Spectroscopy, Mass Spectrometry, and Density Functional Theory. *Biomacromolecules* **2023**, *24* (5), 2314-2326. DOI: 10.1021/acs.biomac.3c00186.