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ABSTRACT

Strategies for a green bioeconomy transition are of worldwide importance and closely related to society's well-being. There is a growing demand for sustainable solutions that increase the efficient use and recycling of materials by utilizing side streams and reducing the dependence on non-renewable raw materials. The aim of this doctoral thesis was to address the value creation from underutilized plant biomass fractions, focusing on biorefinery and food systems through environmentally friendly and industrially feasible processes. Different underutilized biomass resources were investigated, and conifer-derived green needles were concluded to be the most promising in terms of chemical composition and bioactivities, which were further explored in bread models.

Study **I** investigated common reed, reed canary grass, oil hemp, and fiber hemp as feedstock for a proposed biorefining process step, focusing on the fractionation and recovery of extractives and hemicelluloses as target components. The chemical characterization assessed the content of polyphenols, hemicelluloses, fatty acids, sugars, lipids, and proteins in the biomasses. Employing two-stage pressurized water extraction (90 °C/60 min + 160 °C/60 min) indeed improved the isolation of extractives and hemicelluloses, indicating overall higher yields of hemicelluloses, phenolics, and bioactivity compared to the single stage at 90 °C/60 min. In addition to introducing a biorefinery route approach, this study is the first detailed investigation into the composition of extractives from these biomass fractions. Consequently, it can serve as a foundation for future research.

Study **II** explored a biorefinery-inspired possibility for a value-added utilization of industrially sorted Norway spruce green needle-rich logging residues. Pressurized liquid extraction optimization using response surface methodology identified optimal processing conditions – 120 °C for 10 min with water, and 125 °C for 68 min with ethanol/water – to maximize desired chemical composition (total phenolics and condensed tannins) and bioactivities (antioxidant and antibacterial properties). Under the optimized conditions, aqueous ethanol extraction resulted in a higher overall yield with increased antioxidant activities and bacterial inhibition compared to water.

Study **III** examined a technological application of green needles and fine twigs (NT) from Norway spruce, silver fir and Japanese red pine by replacing water in bread at 0, 35, and 70% levels to assess effects on secondary metabolites, bioactivity, nutrition, and quality. NT-fortified bread showed good stability of compounds analyzed via high-performance liquid chromatography (HPLC-DAD) after 24 and 72 h, with a significant increase in several polyphenols after 72 h, correlating with over an 80% enhancement in antioxidant activity. A total of 115

compounds were identified, including flavonoids, phenolic acids, alkaloids, stilbenes, lignans, resin acids, and gibberellins. Overall, a 35% fortification was sufficient to improve functionality, extend shelf-life, and maintain nutritional and textural properties, while also enhancing overall acceptability and purchase intent. Among all formulations, bread fortified with pine NT at 35% levels exhibited the best overall balance of these factors, making it a promising option for further development as a bioactive, consumer-oriented product. The findings show the valorization potential of underutilized conifer NT as natural antioxidants and will help provide the industry with phytochemical compositional information. Also, this study emphasizes the broader applicability of these side streams by employing a green extraction technique (hydrodynamic cavitation) that yields valuable compounds, promotes sustainability, and supports the circular economy through a cost-effective process.

Overall, this research offers essential insights into the effects of external factors and processing parameters on the properties of the studied biomass resources. It also highlights the potential of the underutilized fractions of plant species in Finland for commercial use, providing a promising outlook for future biorefinery approaches.

SUOMENKIELINEN ABSTRAKTI

Keinot, jotka mahdollistavat siirtymisen vihreään biotalouteen, ovat globaalisti merkittäviä ja kytkeytyvät suoraan yhteiskunnan hyvinvointiin. Kestäviä ratkaisuja tarvitaan edistämään materiaalien tehokasta käyttöä ja kierrätystä hyödyntämällä sivuvirtoja sekä vähentämällä riippuvuutta uusiutumattomista raaka-aineista. Väitöskirjan tavoitteena oli tutkia alihyödynnettyjä kasvibiomassoja biojalostamoiden ja elintarvikejärjestelmien näkökulmasta, keskittyen ympäristöystävällisten ja teollisesti toteuttamiskelpoisiin prosesseihin. Tutkimus osoitti havupuiden vihreiden neulasten olevan kemiallisen koostumuksensa ja bioaktiivisuutensa suhteen lupaavimpia analysoiduista biomassoista, joten niitä tarkasteltiin myös leipämallissa.

Väitöskirjan ensimmäisessä osassa (Study **I**) käytettiin ruokohelpeä, järviruokoa ja kuitu- sekä öljyhamppua raaka-aineina biojalostusprosessissa, joka tähtäsi erityisesti uuteaineiden ja hemiselluloosajakeiden talteenottoon. Kemiallisen karakterisoinnin avulla määritettiin biomassajakeiden polyfenoli-, hemiselluloosa-, rasvahappo-, sokeri-, lipidi- ja proteiinipitoisuudet. Kaksivaiheinen paineistettu vesiuutto (90 °C/60 min + 160 °C/60 min) johti liuenneiden kiinteiden aineiden, hemiselluloosien, fenolien ja bioaktiivisten aineiden suurempiin kokonaispitoisuuksiin verrattuna yksivaiheiseen uuttoon (90 °C/60 min). Tutkimuksessa esiteltiin ensimmäistä kertaa yksityiskohtainen analyysi valittujen biomassauutteiden koostumuksesta, mikä loi perustan jatkotutkimuksille.

Toisessa osassa (Study **II**) keskityttiin mahdollisuuteen hyödyntää teollisesti lajiteltuja kuusen tuoreita ja neulaspitoisia hakkuutähteitä biojalostuksen raaka-aineena. Tutkimuksessa pyrittiin maksimoimaan uutteiden kondensoituneiden tanniinien ja kokonaisfenolien pitoisuudet sekä antioksidatiivinen ja antibakteerinen aktiivisuus. Optimaaliset paineistetun nesteuuton olosuhteet määritettiin käyttäen vastepintamenetelmää ja ne olivat vesiuutolle 120 °C ja 10 min ja 125 °C ja 68 min etanoli/vesiuutolle. Optimaalisissa olosuhteissa etanolivesiuutto johti suurempaan kokonaissaantoon, lisääntyneeseen antioksidanttiaktiivisuuteen sekä tehokkaampaan bakteerien estoon verrattuna pelkkään vesiuuttoon.

Viimeisessä osassa (Study **III**) tutkittiin metsäkuusen, saksanpihdan ja japaninpunamännyn vihreiden neulasten ja oksankärkien (NT) käyttöä elintarvikesovelluksessa. Uutteiden vaikutusta leivän laatuun, ravintoainepitoisuuteen ja koostumukseen selvitettiin korvaamalla leipätaikinassa käytettävää vettä NT-uutteilla pitoisuuksissa 0, 35 ja 70 %. Mittaukset korkean erotuskyvyn nestegromatografi-diodirividetektoinnilla (HPLC-DAD) osoittivat, että sekundaarimetaboliitit säilyvät stabiileina 24 ja 72

tuntia leipien paistamisen jälkeen. Uuterikastetuissa leivissä antioksidanttiaktiivisuus kasvoi 72 tunnin kuluessa paistamisesta yli 80 %, mikä korreloi positiivisesti ($p<0.05$) polyfenolien kokonaispitoisuuden kanssa. Tunnistetut 115 yhdistettä olivat muun muassa flavonoideja, fenolihappoja, alkaloideja, stilbeenejä, lignaaneja, hartsihappoja ja gibberelliinejä. Uutelisäys 35 % paransi myös leivän toiminnallisia ominaisuuksia ja säilyvyyttä heikentämättä sen ravitsemuksellisia tai rakenneominaisuuksia. Leivät arviointiin miellyttävimmiksi ja mikäli niitä olisi kaupallisesti saatavilla, ostohalukkuutta lisääviksi. Japaninpunamäntyuutteen 35 % lisäys osoittautui lupaavimmaksi vaihtoehdoksi jatkokehitykselle. Tutkimus osoitti alihyödynnettyjen havupuiden neulasten ja oksankärkien potentiaalin luonnollisina antioksidantteina ja lisäksi tarjosi fytokemiallista koostumustietoa. Tutkimuksessa käytetty hydrodynaaminen kavitaatio on ympäristöystävällinen uuttotekniikka, jonka avulla voidaan tuottaa arvokkaita yhdisteitä kestävästi ja tukea kiertotaloutta kustannustehokkaasti.

Tutkimuksen johtopäätökset lisäävät tietoa ulkoisten tekijöiden ja prosessoinnin vaikutuksesta biomassan ominaisuuksiin. Lisäksi väitöskirja nostaa esiin vajaakäyttöisten kasvilajien ja -jakeiden kaupallisen hyödyntämisen mahdollisuuksia tarjoten innovatiivisia ratkaisuja tulevaisuuden biojalostamoihin.

LIST OF ABBREVIATIONS

AOX	Antioxidant activity
MS	Mass spectrometry
RSM	Response surface methodology
DPPH	2,2-diphenyl-1-picrylhydrazyl
4-O-Me-GlcA	4-O-methyl glucuronic acid
ASE	Accelerated solvent extractor
ANOVA	Analysis of variance
Ara	Arabinose
-A	Autumn
BPI	Base peak ion
FNT70	Bread fortified with 70% fir needle and twig extract
PNT70	Bread fortified with 70% pine needle and twig extract
SNT70	Bread fortified with 70% spruce needle and twig extract
CBC	Cannabichromene
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBG	Cannabigerol
CCD	Central composite design
CR	Common reed
CR	Common reed
CT	Condensed tannins
Cu(II)-Nc	Copper(II)-neocuproine
CUPRAC	Cupric reducing antioxidant capacity
THC	Delta-9-tetrahydrocannabinol
DNA	Deoxyribonucleic acid
DAD	Diode-array detection
DW	Dry weight
E. coli	*Escherichia coli*
EtOH/H_2O	Ethanol/water
DOE	Experimental design
FRAP	Ferric reducing activity power
-FF	Fiber fraction
FH	Fiber hemp
FH	Fiber hemp
FLD	Fluorescence detection
FW	Fresh weight
GalA	Galacturonic acid
GAE	Gallic acid equivalent
GC-MS	Gas chromatography combined with mass spectrometry

GC-FID	Gas chromatography-flame ionization detection
Glc	Glucose
GlcA	Glucuronic acid
AUNPs	Gold nanoparticles
NT	Green needles and fine twigs
HPAEC	High-performance anion-exchange chromatography
HPLC	High-performance liquid chromatography
HC	Hydrodynamic cavitation
H_3O^+	Hydronium ion (protonated water molecule)
IEA	International energy agency
ISO	International Organization for Standardization
LC	Liquid chromatography
Man	Mannose
Luke	Natural resources institute finland
OH	Oil hemp
ORAC	Oxygen radical absorbance capacity
PHWE	Pressurized hot-water extraction
PLE	Pressurized liquid extraction
QTOF	Quadrupole-time-of-flight
RCG	Reed canary grass
RCG	Reed canary grass
Rha	Rhamnose
-SF	Screening fines
SFN	Silver fir needle
S. aureus	*Staphylococcus aureus*
-S	Summer
THCA	Tetrahydrocannabinolic acid
TDS	Total dissolved solids
TPC	Total phenolic content
UHPLC	Ultra high performance liquid chromatography
UV	Ultraviolet
Xyl	Xylose

LIST OF ORIGINAL PUBLICATIONS

I Fidelis, M.; Tienaho, J.; Brännström, H.; Korpinen, R.; Pihlava, J.-M.; Hellström, J.; Jylhä, P.; Liimatainen, J.; Möttönen, V.; Maunuksela, J.; Kilpeläinen, P. Chemical composition and bioactivity of hemp, reed canary grass and common reed grown on boreal marginal lands. *RSC Sustainability*, **2023**, *1*, 2202-2223.

II Tienaho, J.; Fidelis, M. ; Brännström, H. ; Hellström, J. ; Rudolfsson, M.; Kumar Das, A.; Liimatainen, J.; Kumar, A.; Kurkilahti, M.; Kilpeläinen, P. Valorizing assorted logging residues: response surface methodology in the extraction optimization of a green Norway spruce needle-rich fraction to obtain valuable bioactive compounds. *ACS Sustainable Resource Management*, **2024**, *1*, 237–249.

III Fidelis, M.; Tienaho, J.; Meneguzzo, F.; Pihlava, J.-M.; Rudolfsson, M.; Järvenpää, E.; Imao, H.; Hellström, J.; Liimatainen, P.; Kilpeläinen, P.; Yang, B.; Jyske, T. Spruce, pine and fir needles as sustainable ingredients for whole wheat bread fortification: Enhancing nutritional and functional properties. *LWT – Food Science and Technology*, **2024**, *213*, 117055.

1 INTRODUCTION

In recent years, the global use of renewable natural resources for new applications has seen a consistent rise (European Commission, 2018; Lehtonen et al., 2024). Finland's current industrial strategy aims to develop value-added products and new technologies for biomass and side stream valorization through sustainable and efficient use of raw materials, following the bioeconomy strategy targets (Finnish Government, 2022). According to IEA Bioenergy Task 42, "bioeconomy refers to the production of renewable biological resources and their conversion into food, feed, fibers, materials, chemicals, fuels, energy and minerals through efficient and innovative technologies" (IEA Bioenergy, 2016).

Agricultural residues such as leaves, stalks, straw, husk, pulp, and peel obtained after harvesting and processing food crops are used for biofuels and chemical production worldwide. However, numerous dedicated non-food crops can also be used for biorefinery applications and value-added products (Kumar and Verma, 2021). Common reed and reed canary grass, traditionally grown for paper production and soil remediation, have yet to be fully exploited for biorefinery purposes (Antonkiewicz et al., 2019; Brix et al., 2014; Kołodziej et al., 2023). Fiber hemp is gaining attention due to its rapid growth, adaptability, and broad commercial use in textiles, paper, food and feed, biofuels, biodegradable plastics, and construction materials (Rehman et al., 2021). Although oil hemp cultivars are primarily cultivated for seed commercialization, other plant parts also have potential for food and feed due to their health-promoting properties (Saastamoinen et al., 2016). However, there is still limited information on alternative biorefining methods for utilizing the leaves and stalks of reed and hemp biomass beyond their conventional uses.

Large quantities of logging residues are generated annually, with Finland alone producing approximately 4.4 million dry tons of Norway spruce residues (Natural Resources Institute Finland, 2023). Norway spruce needles constitute about 30% of the total crown biomass (Hakkila, 1992). These residues are rich in extractable compounds that hold significant potential value. In addition to large biomacromolecules, namely cellulose, hemicelluloses, and lignin, lignocellulosic biomass contains numerous low-molecular-mass constituents known as extractives, such as phenolic compounds, terpenes, terpenoids, alkaloids, lipids, and resins (Verkasalo et al., 2021). These secondary metabolite-related extractives play a crucial role in protecting standing trees from a variety of environmental and biological stressors, including drought, high humidity, temperature, fungi, bacteria, parasites, and other phytopathogens (Bennett and Wallsgrove, 1994). Extractives from woody biomass, due to their antioxidant and antimicrobial effects, are highly valuable as food and cosmetic preservatives and hold potential for medicinal applications (Santos et al., 2022). With rising

demand for bioproducts, there is increasing interest in utilizing these resources (Verkasalo et al., 2021). Forest biomass-based extractives are promising raw materials for the production of a wide range of high-value products, including pharmaceuticals (Routa et al., 2017), biochemicals, and dietary supplements (Holmbom, 2011; Kemppainen, 2015). Despite this potential, there is no substantial industrial utilization of valuable compounds derived from logging residues or needles in Finland or Sweden. Moreover, the processing and refining methods for logging residues and green needles require substantial development. Previous research has primarily focused on the cellulose, hemicelluloses, and lignin fractions of commercially available biomass, often overlooking other plant parts, such as leaves, inflorescences, and stems, in favor of primary components like cellulose. Although conifer needles have been extensively studied for their physiological characteristics, research specifically targeting their potential applications in the food industry remains limited.

One potential market for such extractives is cereal-based foods, particularly bread, which accounts for more than 50% of the energy intake in developed societies. Known for its high fiber content, whole wheat bread is linked to enhanced gut health and a lower risk of heart disease. Even though bread is a promising vehicle for functional supplements, its functional potential remains underexplored (Cappelli and Cini, 2021; Dziki et al., 2014; James et al., 1997).

In selecting target biomass plant feedstocks, this study focused on species with distinct compositions and high availability in Finland, particularly forest residues and those grown on marginal lands (e.g., underutilized agricultural areas and former peat production sites). Common reed, reed canary grass, fiber hemp, oil hemp, and conifer needles were chosen based on their potential for biorefinery applications targeting high-value chemicals and food ingredients. Thus, the research of this thesis focuses on three valorization approaches and the role of processing parameters on the studied side streams. Studies **I** and **II** focused on exploring different underutilized side streams, and the most promising was chosen for the bread model (Study **III**). The findings of this work advance the understanding of value creation from previously underutilized biomass resources using different green processing techniques while also showcasing its potential to enhance the nutritional and functional properties of whole-wheat bread. This work provides essential information for exploiting Finnish cultivated species for the commercial use of underutilized plant fractions.

The literature review section of the thesis provides a detailed overview of the aboveground biomasses of herbaceous and coniferous species under investigation. The review emphasizes the valorization strategies implemented to establish a sustainable framework for biomass utilization, including biorefining processes, extraction optimization to efficiently recover target value-added compounds, and technological applications.

2 REVIEW OF THE LITERATURE

2.1 Aboveground biomass

According to Reichle (2023), the biosphere's overall biomass composition shows the distribution of approximately 550 gigatons of carbon (Gt C) across all life kingdoms. Overall, plants are the dominant source of biomass among all life forms. About 320 Gt C is found in aboveground biomass, representing ~60% of global biomass (**Figure 1**). This includes carbon stored in living plant tissues located above the Earth's surface, such as stems, bark, branches, and twigs. Belowground biomass, mainly consisting of approximately 130 Gt C in plant roots and 100 Gt C in soil and subsurface microbes, significantly contributes to this total. Terrestrial and marine bacteria together contribute around 9 Gt C and fungi account for 12 Gt C (Bar-On et al., 2018; Reichle, 2023).

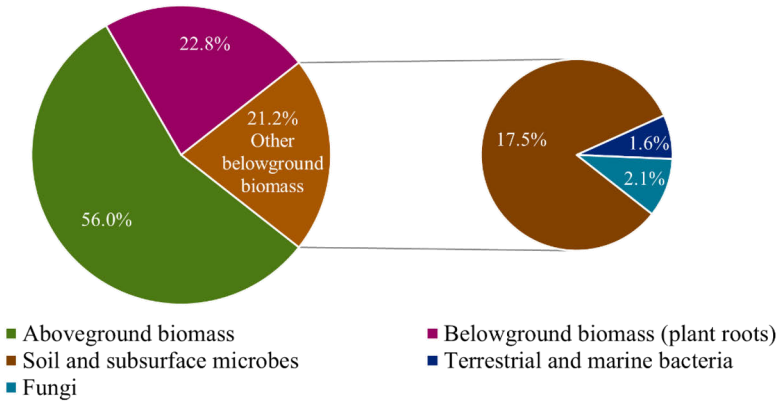

Figure 1. Distribution of global biomass among all of the kingdoms of life Adapted from Bar-On et al. (2018) and Reichle (2023).

Plant biomass is the biodegradable fraction (e.g., leaves, stems, roots, seeds, fruits, wood, and straw) resulting from agriculture, forestry, and industrial and municipal waste valued for renewable energy and sustainable bioproducts (María Diaz-Montaño, 2022). **Figure 2** and **Figure 3** illustrate the aboveground biomasses with an indication of the plant part(s) used in the present thesis. Lignocellulosic biomass is the most abundant biomass and it represents a major carbon source for chemical compounds, biofuels, and bioenergy. Lignocellulosic biomass materials consist of a closely associated network of high-molecular-mass polysaccharides, i.e., cellulose (40 – 60%) and hemicelluloses (15 – 40%), along with lignin (10 – 35%) and various low-molecular-mass extractives, including lipids, resins, steroids, terpenes, terpenoids, and phenolic compounds (McKendry, 2002; Mujtaba et al., 2023).

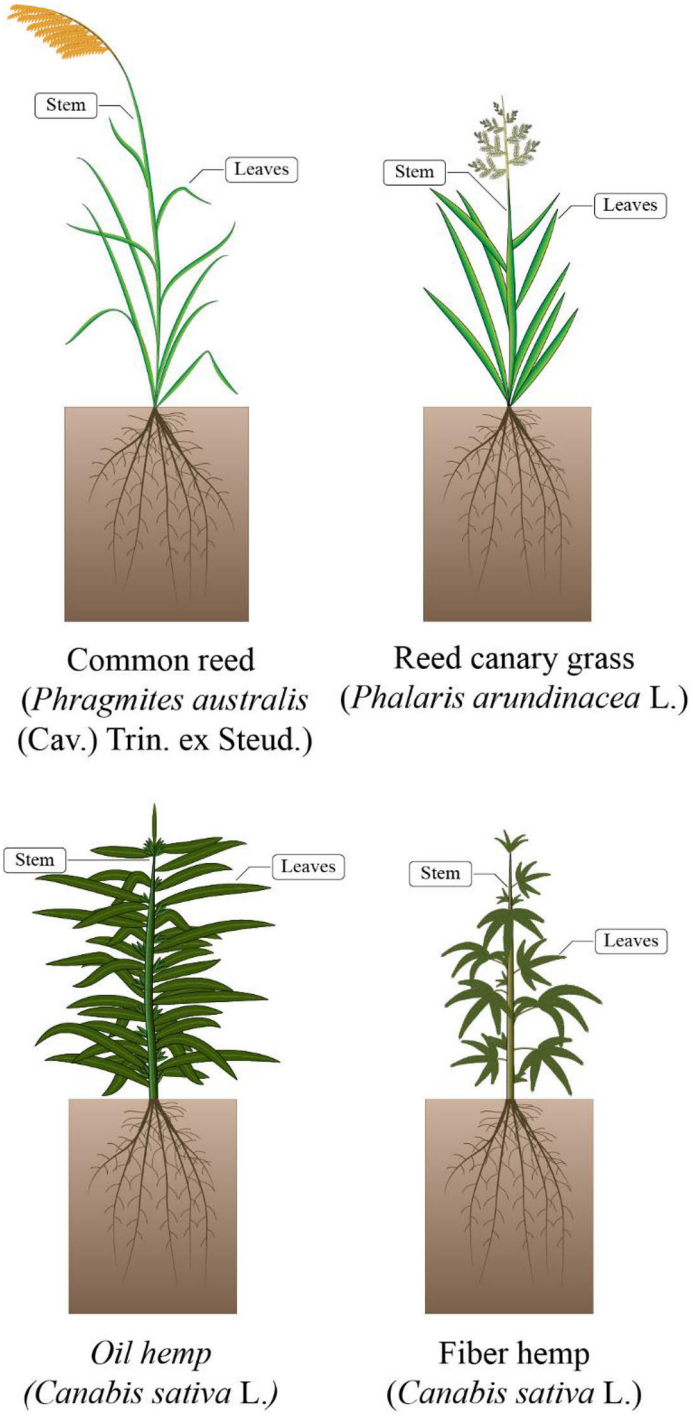

Figure 2. Stem and leaves of common reed, reed canary grass, and hemp.

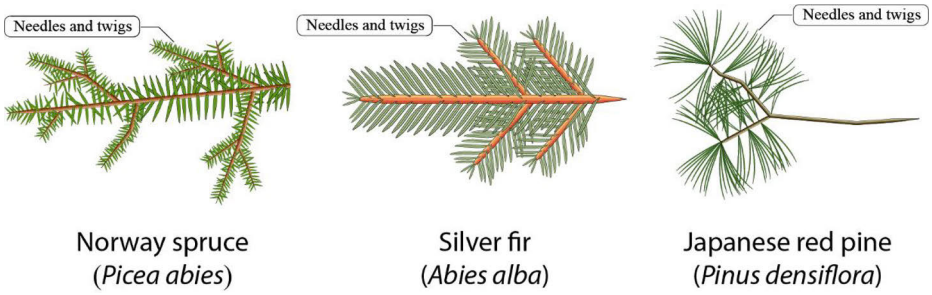

Figure 3. Needles and twigs from conifer tree branches.

Industrial crops are classified into oil (e.g., camelina, safflower, and rapeseed), lignocellulosic (e.g., perennial crops/grasses, fiber crops, and woody species), carbohydrate (e.g., corn, cassava, and potato), and specialty types (e.g., calendula, lavender, and peppermint) (Monti and Lizarazu, 2022). Beyond biofuels and chemicals, there has been considerable exploitation of biomass for the development of bio-based food products and other applications (e.g., biogels, antibacterial film, antioxidants) due to their abundance, renewability, biodegradability, and economic advantage (Jyske et al., 2020; Okonkwo et al., 2023; Parenti et al., 2022). Researchers have studied the potential of all-cellulose nanocomposite film made from bagasse cellulose nanofibers for food packaging applications. Promising properties indicated a favorable multi-execution material with potential for application in cellulose-based food bundling (Ghaderi et al., 2014). Additionally, lignin is a green and under-utilized raw material that has been investigated for its potential as a natural preservative, emulsifier agent, and additive to improve mechanical properties and gas barriers in food packaging materials (Okonkwo et al., 2023).

Marginal lands, including abandoned or underutilized agricultural land, indicate formerly cultivated land areas, which are unusable for agriculture due to soil degradation, improper management, or climate changes (Fahd et al., 2011). The distinction between agricultural lands as productive, marginal, or unproductive depends on their use and management objectives (Csikós and Tóth, 2023; Dauber et al., 2012). Key EU policies and initiatives prioritize sustainable bioenergy, rural development, and ecosystem restoration in these areas (Muscat et al., 2022). In fact, cultivating industrial crops on marginal lands for non-food applications presents an opportunity to enhance the bio-based industry by generating high-value products and bioenergy. Despite crop impact variations, their use can reduce competition for land, enhance the value of underutilized land, and contribute to a more resource-efficient economy with minimal indirect land use changes, finally leading to diversified incomes for farmers (Csikós and Tóth, 2023; Elbersen et al., 2017; MAGIC, 2017; Zhu et al., 2018). In order to support

the selection of optimal crops for specific types of marginal land, an EU-funded project has developed a database to assess 37 industrial crops for yield efficiency across different soil conditions (MAGIC, 2017).

Existing research on biomasses from marginal lands has primarily concentrated on bioenergy and fiber production from grasses. However, the high costs and logistical difficulties associated with non-wood fibers have constrained their application in pulp and cellulose-based products (Finell et al., 2011; Hellqvist et al., 2003; Saijonkari-Pahkala, 2001; Sixta, 2006). However, fresh or dried biomass contains valuable compounds, such as cellulose, hemicelluloses, carbohydrates, polyphenols, proteins, and lipids, which have diverse applications across industries (Chen et al., 2022; Valoppi et al., 2019; Voogt et al., 2023; Walsh and de Jong, 2012).

Peat, the surface organic soil layer, is formed from partially decomposed organic matter from plant material under waterlogging, oxygen deficiency, high acidity, and nutrient deficiency (Joosten and Clarke, 2002). Peatland is defined as wetland ecosystems with or without vegetation containing peat soil, emerging from high to low latitudes and from high mountains to the sea, including mires drained or degraded for forestry, agriculture, horticulture and energy production (Joosten and Clarke, 2002; Xu et al., 2018). The diversity in peatland plant communities can be divided into vascular plants (e.g., sedges, grasses, herbaceous plants, dwarf-shrubs, shrubs, and trees) and mosses (Similä et al., 2014). Moreover, peat serves various purposes, including horticulture (as a soil enhancer and growing medium component), energy production, ex-situ applications in chemistry (e.g., wax, dye, and activated carbon), animal bedding, filtration and absorption (e.g., oil spill cleanup and heavy metal removal), and peat textiles production (e.g., cotton grass fibers and paper) (Joosten and Clarke, 2002). Finland holds the second-largest peatland area in Europe, with 8.3 Mha (Patronen, 2020), of which 120 kha have been converted for peat production (Salo, 2019). However, peat production is quickly declining due to the goal of cutting fuel peat usage in energy generation by at least 50% by 2030 (Finnish Government, 2019).

2.1.1 Hemp

Hemp (*Cannabis sativa* L., Cannabaceae) is an herbaceous, wind-pollinated annual plant that originated from Central Asia and has been used in folk medicine and as a source of textile fiber since ancient times (Small and Cronquist, 1976; Vonapartis et al., 2015). *Cannabis* has been considered to have only one species, *C. sativa* L., that can be divided into subspecies *indica*, known for its higher levels of the psychoactive compound delta-9-tetra hydrocannabinol (THC), and subsp. *sativa*, which has lower THC levels (Small and Cronquist, 1976).

Although various hybrids exist between subspecies, industrial hemp (i.e., oil and fiber hemp) varieties (non-drug types) can be classified under subsp. *sativa*, whereas most medical *Cannabis* varieties, commonly referred to as "marijuana," fall under subsp. *indica*. According to Statista, over 54,000 ha are currently being dedicated to hemp cultivation in Europe. France has the most significant area, with almost 20,000 ha dedicated to it, followed by Germany with over 5,000 ha. Finland is in the 11th position, with over 880 ha of farmland devoted to growing hemp in 2020 (Trenda, 2023).

Industrial hemp has been cultivated as a sustainable multi-purpose crop valued for fiber, oil, fuel, food, and medicinal purposes. Its cultivation is considered environmentally friendly due to its minimal requirement for pesticides and herbicides, fast growth rate, and carbon sequestration ability, making it a renewable resource (Rupasinghe et al., 2020). Fiber hemp (e.g., var. Uso 31), a fiber crop, has a wide range of commercial applications, including paper, food and feed, biofuel, biodegradable plastics, and construction materials (Rehman et al., 2021). It is a adaptable and phytosanitary plant, allowing for the integration into various crop rotation systems (Alaru et al., 2011). Oilseed hemp (e.g., var. FINOLA) is primarily cultivated for its seeds and the oil they produce, which are valued for food, cosmetics, and feed applications. While the grain is the main fraction, the green biomass of the plant can be also utilized (Saastamoinen et al., 2016).

Hurds, leaves, and inflorescences, major components of hemp biomass, often considered low-value residues, are rich in micronutrients and phytochemical antioxidants, offering potential for conversion into high-value resources (Kitrytė et al., 2018; Moscariello et al., 2021). In general, hemp contains phenolic compounds, particularly flavonoids and terpenes, such as mono- and sesquiterpenes, which are primary components of hemp essential oils that contribute to its characteristic flavor and aroma (André et al., 2020). Phytocannabinoids, unique terpenophenolic compounds in hemp, have attracted interest for their medicinal and therapeutic potential, exhibiting a range of biological activities (Kitrytė et al., 2018). Key phytocannabinoids present in hemp are cannabidiol (CBD), cannabidiolic acid (CBDA), cannabichromene (CBC), THC, and cannabigerol (CBG) (Kitrytė et al., 2018). Hemp hurds, or shives, the woody inner part resulting from the separation of the bast fibers during fiber production, are characterized by their substantial carbohydrate composition, predominantly comprising glucose and xylose. This composition offers a promising substrate for producing bio-based chemicals, including furfural, lactic acid, and ethanol (Brazdausks et al., 2017).

Research indicates that hemp fractions offer natural antioxidants and anti-inflammatory effects. Studies have demonstrated that bioactive compounds in hemp can slow the oxidation of vegetable oils (Cantele et al., 2020) and enhance

the beneficial omega-6/omega-3 ratio in seed triglycerides (Werz et al., 2014). Previous research indicates that cannabinoids, including phyto-, endo-, and synthetic types, may have therapeutic properties against various cancers (i.e., brain, prostate, breast, skin, pancreas, and colon) by regulating cellular mechanisms. Both *in vitro* and *in vivo* models indicated anti-proliferative, anti-metastatic, anti-angiogenic, and pro-apoptotic responses (Alexander et al., 2009; Sarfaraz et al., 2005). Furthermore, hemp waste biomass has shown effectiveness in eliminating malaria vectors, suggesting its potential as a sustainable and eco-friendly insecticide (Rossi et al., 2020).

2.1.2 Common reed

Common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) is a perennial, emergent aquatic plant with annual cane-like stems developed from an extensive rhizome system. The stems can reach up to 6 m in height and 4 - 10 mm in diameter. This species is prevalent across temperate and tropical regions, thriving in low-lying wetlands, including fresh and salt-water marshes, drainage ditches, shallow lake edges, sandy banks, roadsides, woodlands, and rocky places. It exhibits optimal growth at temperatures between 20 °C and 30 °C (Brix et al., 2014; Mal and Narine, 2004; Shaltout et al., 2006).

Common reed, also known as lake reed, has been mainly used for paper, cardboard, hardboard, synthetic textiles, roofing, fencing, and insulation (Brix et al., 2014; Mal and Narine, 2004). Common reed has also been used for phytoremediation in constructed wetlands systems aiming to treat wastewater from papermaking (Brix et al., 2014). Recently, a study explored the potential use of common reed in the biotechnological production of lactic acid, traditionally used in the food industry as a preserver and mild acidifier. The findings highlighted the successful production of lactic acid with both high yield and purity through chemical treatment and enzymatic hydrolysis of the biomass (Schroedter et al., 2020). Recent projects encourage the sustainable use of reed as a new sustainable biomaterial and energy resource in Finland. For instance, the project "Reed Occurrence and Biomasses by the Archipelago Sea" (Regional Council of Southwest Finland, 2021 – 2023) aimed to promote a profitable reed business, enabling regular and large-scale harvesting of reeds and the removal of biomass from the water for further utilization. An ongoing project (IIIA Interreg EU, 2023 – 2026) has been promoting the harvesting and use of reed as a sustainable biomaterial or bioenergy source in Finland and Estonia (BalticReed, 2023).

The literature lacks comprehensive research on the extractive composition of *P. australis* stalks and leaves. A study has reported the presence of glycosides, tannins, phenolic compounds, flavonoids, and terpenoids in the aqueous extracts

of common reed rhizome (Derouiche et al., 2017). The entire plant material has been shown to be a source of protein, calcium, and potassium, with a good quality amino acid composition (Beyzi et al., 2022). In addition, antibacterial and antioxidant properties of *P. australis* were previously found (Al-Akeel et al., 2010; Derouiche et al., 2017). El-Borady et al. (2021) developed an eco-friendly method to produce gold nanoparticles (AuNPs) from common reed leaf extract, demonstrating their potential in antioxidant, anticancer, and photocatalytic applications, and suggesting a sustainable approach to utilizing common reed biomass in green nanotechnology.

2.1.3 Reed canary grass

Reed canary grass (*Phalaris arundinacea* L., Gramineae) is a tall (1 – 3 m high) native perennial grass of the temperate regions of Europe, Asia, and North America that typically grows best under cool and moist conditions. Reed canary grass thrives in wet areas, such as floodplains and lake shores, and requires a minimum growing season of 111 days. Also, it is notably drought-resistant and has a higher tolerance for flooding than other cool-season grasses, exhibiting high yield even with low fertilization (Kitczak et al., 2023; Reinhardt et al., 2022; Von Cossel et al., 2019; Wrobel et al., 2009). As a result of climate change, biomass plants, such as reed canary grass are more suited to the northern regions of Europe, indicating potential for cultivation in Finland's former peat production areas (Ferdini et al., 2023; Järveoja et al., 2016). Among multiple purposes, reed canary grass has been used as livestock feed, in water phytoremediation, biomass generation, forage, and soil erosion control (Lavergne and Molofsky, 2004). Moreover, given the strong demand for wood-based raw materials, *P. arundinacea*, one of the highest-yielding cool-season lignocellulosic grasses, is a promising sustainable alternative feedstock for biofuel production (Finell et al., 2011; Kitczak et al., 2023; Waliszewska et al., 2021; Wrobel et al., 2009).

Reed canary grass mainly consists of lignocellulosic biomass, which includes cellulose, hemicelluloses, and lignin as its primary components, with xylose and glucose as the most abundant hemicellulosic sugars (Finell et al., 2011). For example, a study reported a similar performance to conventional synthetic plastics when using *P. arundinacea* fibers to reinforce plant fiber composites at reduced cost and improved thermal stability (Zhao et al., 2021). Additionally, the plant contains alkaloids (tryptamine, carboline, gramine, and hordenine), which have been investigated due to their impact on forage quality, palatability, and potential medicinal properties (Coulman et al., 1977; Østrem, 2008). So far, no research has reported comprehensive chemical fingerprints and health benefits of reed canary grass.

2.1.4 Conifer branches and needles

Softwood species, such as Norway spruce (*Picea abies*), Japanese red pine (*Pinus densiflora*), and silver fir (*Abies alba*), grow in different parts of the globe. Norway spruce is native to Northern, Central, and Eastern Europe. Red pine is found in Japan, Korea, and Northeastern China. Silver fir is native to European mountains and widespread in southern and central-eastern Europe (Dobrowolska et al., 2017).

Logging residues refer to the aboveground biomass remaining at the felling sites after the stem wood has been harvested, which includes the tops and branches of felled trees as well as small diameter trees from thinnings (Moskalik and Gendek, 2019). These residues constitute a substantial part of the total nutrient pool initially present in the growing stand (Palviainen et al., 2004), e.g., nearly 80% of the total nitrogen and as much as 90% of the total phosphorus of the standing tree biomass pools of these nutrients. Forest litter plays an essential role in the formation of soil humus, which is important for soil fertility and nutrient cycling (Wei et al., 2020). As a result, logging residues are typically left on-site to allow the nutrients to return to the forest soil (Kumar et al., 2021; Törmänen and Smolander, 2022).

Extractives from woody biomass can be categorized into three main groups: aliphatic compounds (such as terpenes, terpenoids, fatty acids, and resin acids), phenolics (e.g., flavonoids, tannins, stilbenes, and lignans), and other compounds, e.g., amino acids, sugars, quinones, and alkaloids (Verkasalo et al., 2021). Logging residues, particularly needles, are rich in valuable extractable compounds, including vitamins, extractives (comprising up to 43% of the dry matter), and protein (approximately 10%) (Jyske et al., 2020; Voipio and Laakso, 1992). Green needles, in particular, are an underutilized source of polyphenols, including flavonoids, hydroxycinnamic acids, and stilbenes, which are well-known for their biological and nutraceutical properties (Mofikoya et al., 2023, 2022; Slimestad et al., 1992). Thus far, more than 200 compounds have been identified in conifer sprouts and needles, with their chemical composition differing from that of sap (the younger wood layers) and heartwood (the older, central part) (Mofikoya, 2022; Mofikoya et al., 2023, 2020).

Recently, the utilization of logging residues as a feedstock for producing forest-based bioenergy has grown significantly (Amiandamhen et al., 2020; Ranius et al., 2018; Spinelli et al., 2019) driven by the need to meet renewable energy targets, such as those outlined in the EU Directive 2009/28/EC. However, the significant transportation costs and dry mass losses pose challenges to the economic viability of bioenergy production from these sources (Eliasson et al., 2022). In addition to nutrient recycling and bioenergy production, logging residues hold potential for various high-value applications, including their use in pharmaceuticals and cosmetic ingredients (Routa et al., 2017), reinforcement

biomass for biocomposites (Xu et al., 2022), biopolymers (Jiang et al., 2018), bioplastics (Hemmilä et al., 2017), platform and specialty chemicals, dietary supplements (Holmbom, 2011; Kemppainen, 2015), foams/emulsions, coatings (Kumar et al., 2022), and growing media (Çetinkaya and Bilir, 2020).

The extraction of valuable compounds from abundant logging residues prior to their use in bioproducts, biochemicals, or bioenergy applications offers a promising and attractive strategy to enhancing resource efficiency and promoting sustainable development (Xu et al., 2022). Secondary metabolites from woody biomass, crucial for defending trees against environmental and biological stressors, often exhibit antioxidant and antimicrobial properties, making them valuable as preservatives in the food and cosmetic sectors, with potential for medical application (Bennett and Wallsgrove, 1994; Santos et al., 2022). Studies have identified volatile compounds such as terpenoids, polyphenolic compounds, and piperidine alkaloids as key contributors to the broad-spectrum antimicrobial properties of coniferous species (Eberhardt and Young, 1994; Fyhrquist et al., 2017; Garzoli et al., 2021; Metsämuuronen and Siren, 2014; Muilu-Mäkelä et al., 2022; Visan et al., 2021).

The limited industrial use of needle biomass for biochemical production highlights the need for refining methods suited to its complex composition. Proper sorting of these materials before extracting high-value chemicals could enhance the quality of the residual fraction for further applications. However, current research often relies on small-scale handpicking, with little information available on up-scaled sorting processes (Xu et al., 2022).

2.1.5 Conifer-based products: utilization as ingredients and their challenges

Conifer-based products are widely available in many parts of the world for different purposes. For instance, the inner bark of Scots pine [*pettu* in Finnish, *bark* in Swedish] has been traditionally used in food preparation in northern Fennoscandia. Additionally, sprouts of Norway spruce have been traditionally used as herbal tea and in folk medicine (Jyske et al., 2020). Bronchosan dry, tickly cough syrup® is an example of commercially available medicinal product that contains Norway spruce shoots. Pine trees have a global distribution, and in Asia, pine (*Pinud densiflora*) needles, cones, bark, and pollen are commonly consumed as either food or dietary supplements, e.g., needle powder**,** tonic, wine, and tea (Kim and Chung, 2000). Pine seeds, known for their high nutritional value and appealing flavor, are traditionally consumed both raw and in culinary dishes (Ferreira-Santos et al., 2020). Red pine needles have been traditionally used in herbal medicine for many years and are still commonly employed in Japan for nourishing and tonic preparations (Lee et al., 2021). Flavangenol® and

Pycnogenol®, produced from pine bark extract (e.g., *P. maritima*, and *P. pinaster*), have been commercially used as nutritional supplements and phytochemical treatments for multiple diseases, leveraging their well-known antioxidant properties (Mármol et al., 2019). Additionally, various parts of the conifer, such as cones, needles, bark, and oil, are recognized and approved as food ingredients within the EU Novel Food Catalogue. In Estonia, pine needles are used in traditional herbal teas, and in Korea, pine needle-derived products like powders, wines, and teas are increasingly popular (Kim and Chung, 2000; Sak et al., 2014). Recently, interest has increased in incorporating pine needles into herbal teas and various culinary recipes (Koutsaviti et al., 2021).

A recent study found that adding silver fir needle (SFN) extracts to whole wheat bread raises its antioxidant capacity by 87% and improves dough and bread volume, highlighting SFNs extracted via hydrodynamic cavitation as a beneficial ingredient for enhancing bread's functional and antioxidant properties (Parenti et al., 2022). Moreover, the supplementation of beer with a Scots pine needle extract revealed a decrease in oxidative stress in the brain under acute pathological conditions, indicating its potential to lessen alcohol's adverse effects due to its antioxidant properties (Penkina et al., 2017). A study examined the nutritional and microbial qualities of spruce sprouts and older needles. Findings revealed superior antioxidant potential, dry matter, energy, and calcium levels in older needles than sprouts, which were richer in vitamin C, potassium, magnesium, and phosphorus. Sensory evaluations had positive consumer reception when incorporating sprout powder in ice cream and sorbet products, suggesting its potential as a conifer-based ingredient (Jyske et al., 2020).

Kothari et al. (2021) found that fermented pine (*P. densiflora*) needle extract in hen diets improved egg production, enhancing egg yolk antioxidants, color, and shell strength. This evidence supports the usefulness of these plant extracts for improving the quality of food products. Enriching staple foods with functional components is a common strategy to boost the delivery of their health benefits, showcasing the remarkable role of these unconventional plants in health-oriented diets. Kim et al. (2021) reported significant reductions in physiological markers when *P. densiflora* needle powder extract was used as a dietary supplement for mice with obesity induced by a high-fat diet, particularly in terms of body weight, body fat mass, and plasma leptin concentrations, along with improvements in glucose metabolism. These findings indicate that the extract may help modulate energy homeostasis via the hypothalamus, which is a regulator of body energy balance. Another study unveiled the antibacterial and antioxidant properties of 7-year-old self-fermented red pine needle extracts. These beneficial effects were attributed to the synergistic interactions among compounds during self-fermentation (Park et al., 2008). Other studies reported antioxidant, antimicrobial, anti-diabetic, antimutagenic, antitumor,

cytoprotective, and antiapoptotic effects of *P. densiflora* needle extracts (Kwak et al., 2006; Park et al., 2011).

Under Regulation (EC) No 178/2002, 'novel food' refers to foods that had not been consumed to a significant degree within the EU before 15 May 1997. These foods must be evaluated for safety and receive authorization from the European Food Safety Authority before being sold in the EU (Regulation EC 178/2002, 2002). Specifically, pine, silver fir, and Norway spruce parts are not considered 'novel' in food or food supplements according to the provisions of the Novel Food Regulation (EU) 2015/2283, and their access to the market is not subject to pre-market authorization (Regulation EC 2015/2283, 2015). *Abies alba* Mill. parts, including bark, branch, needle, seed, and resin, were already used in food supplements in the EU before 15 May 1997. The same is valid for *Picea abies* (L.) H.Karst. (i.e., young shoots (sprouts), leaves (needles), flowers, cones, and resin), and *Pinus sylvestris* L. (i.e., cone syrup, cone, needles, bud, bark, and young shoots). To date, patent databases list various inventions related to the use of conifer needles for food or pharmaceutical purposes (**Table 1**).

Table 1. Different application found for needles in patent databases.

Needle-based ingredient	***Purpose***	***Patent reference***
Spruce conifer needles	Carbonated beverage	WO2020225480A1
Pine needles	Green tea beverage	CN111345376
Pine needles	Tea beverage	CN112931723A
Pine needles	Laying hen feed additive	CN112931723A
Pine needles	Medical purposes (hair growth)	KR100668878B1
Pine needle extract	Encapsulation for pharmaceutical and the food industry formulations	PL217762B1
Conifer green needles	Trichomoniasis infection treatment	EP2305279A1
Pine needle enzyme	Immunity boosting	CN105495613A
Fresh pine needles	Composite material	CN112154860

Conifer resins, complex mixtures of monoterpenes, diterpenes, and smaller amounts of sesquiterpenes, serve as crucial protective agents by sealing wounds, trapping insects, and inhibiting pathogenic microorganisms (Mofikoya et al., 2023). In addition, essential oils extracted from conifer needles or bark have been utilized in ointments, bath oils, and inhalants for the treatment of infectious diseases (Grassmann et al., 2003; Koutsaviti et al., 2021). Other commercial resin applications include adhesives, food additives, varnishes, and cosmetics products (Mofikoya, 2022).

In food safety and preservation, terpenes have emerged as effective antimicrobial agents. The combined potential of linalool, pinenes, citral, and

mild heating has shown efficacy in preserving orange-based soft drinks (Belletti et al., 2010). Also, other conifer-derived terpenes (e.g., hexanal, citral, thymol, and carvacrol) effectively reduced the growth of foodborne pathogens, such as *Listeria monocytogenes* and *Salmonella enterica* by damaging their lipid membranes (Kamdem et al., 2011; Lu and Wu, 2010; Ravishankar et al., 2010; Trombetta et al., 2005). Despite the generally low toxicity of terpenes, their safety, particularly concerning mutagenicity and genotoxicity, requires individual evaluation due to varying effects based on the type and concentration of terpene (Bakkali et al., 2008; Lewis et al., 1994).

Besides phenols and terpenoids, conifers possess additional minor secondary metabolites, including volatile piperidine alkaloids. These alkaloids are extensively found within Pinaceae, with a higher prevalence in *Picea* and *Pinus* than in the *Abies* (Stermitz et al., 2000, 1994; Virjamo et al., 2013). Piperidine alkaloids are considered insect antifeedants (Shtykova et al., 2008), but they have also been reported to be toxic and teratogenic in a frog embryo test (Eisner et al., 1986; Schneider et al., 1991; Stermitz et al., 1994). Although traditionally considered more important for plant-herbivore defense, recent research highlights the role of piperidine alkaloids in microbial defense. Epidihydropinidine, in particular, has shown promising antibacterial and anti-*Candida* activity (Fyhrquist et al., 2017), while 1,6-dehydropinidine has been found to exhibit antibacterial properties against *Streptococcus equi* Subsp. *equi* (Virjamo et al., 2020).

Nevertheless, incorporating extracts rich in bioactive compounds, such as those from conifer needles, into food and beverages requires a thorough evaluation of multiple factors to ensure product safety. This includes considering the plant stage of maturity, quality and stability of raw materials, processing and extraction methods, nutritional properties, and detailed safety assessment (Jyske et al., 2020; Klavins et al., 2023; Padam et al., 2014). Moreover, high concentrations of phenolic compounds in extracts might introduce bitterness, aftertaste, and color changes to the final product. Hence, optimizing the use of plant extracts to leverage their health-related properties without compromising sensory qualities is crucial (Awad et al., 2021).

2.2 Valorization strategies to improve utilization of plant biomass

The valorization of biomass refers to converting waste or residual biomass from agricultural, forestry, or industrial activities into valuable products, such as biofuels, chemicals (e.g., sugar alcohols, glycerine, furfurals, and resins), materials (e.g., cellulose fiber, and bioplastics), and energy. This approach aims to enhance the sustainability and efficiency of biomass utilization by minimizing

waste and exploiting the full potential of biomass resources. Valorization supports the principles of the circular economy and bioeconomy by promoting the use of renewable resources (Ning et al., 2021). The IEA Bioenergy Task 42 defines circular economy as an "economy that is restorative and regenerative by design, and which aims to keep products, components and materials at their highest utility and value at all times, distinguishing between technical and biological-cycles" (IEA Bioenergy, 2016).

According to IEA Bioenergy, "biorefining is the sustainable biomass processing into commercial biobased products and bioenergy" (IEA Bioenergy, 2016). Recently, there has been an increasing interest in the lignocellulose-based biorefinery concept from researchers and industries, as it offers a renewable feedstock for a wide range of application areas, including energy, food, nutrition, and the chemical industry (Mujtaba et al., 2023). The biorefinery concept has been proposed to optimize overall technical, economic, and energetic efficiencies, reduce environmental impact, develop new value-added products, and generate new employment opportunities (Samer, 2022). However, the efficiency of biomass conversion technologies in producing value-added products is significantly influenced by the biomass type and its composition. Hence, either in chemical, biochemical, or hydrothermal conversion technologies, biomass must be broken down into smaller molecules (e.g., oligosaccharides and monosaccharides) to enable efficient conversion into target products. This highlights the critical role of pretreatment and hydrolysis in product yield (Irmak, 2017). For example, lignocellulosic biomass can be utilized in biorefineries to produce sugars, which are then transformed into biofuels or chemicals. However, this fractionation process necessitates an initial pretreatment method that enhances the availability of enzymes or microorganisms to the plant matrix, aiming to facilitate the hydrolysis of cellulose and hemicelluloses into fermentable sugars (Moreno-González and Ottens, 2021).

Considerable pretreatment methods have been established, including physical, chemical, and biological methods (Moreno-González and Ottens, 2021). However, scaling up biomass utilization from laboratory to industrial levels emphasizes critical challenges in pretreatment methods. Physical pretreatment is favored for its low cost and simplicity, while chemical pretreatment faces barriers, such as high costs, equipment corrosion, and complex solvent recovery processes. Extraction with green solvents, in turn, offer an environmentally friendly alternative but require further development for efficient use (e.g., process optimization, and life cycle analysis). Biological pretreatment, environmentally benign due to mild processing conditions, is limited by high costs, feedstock compatibility, and process duration (Millati et al., 2020; Ning et al., 2021). Indeed, plant material pretreatment and preservation are crucial

factors that significantly impact extraction yields and require careful consideration. When plant material is extracted, it typically yields a wide variety of components (Pfennig et al., 2011). As a result, the extract often needs further processing and refining to achieve the desired final form for specific application (Tienaho et al., 2024).

In addition to identification, collection, pretreatments, and conversion process, the valorization may also involve other steps, such as reducing volume, altering phases, removing solids, purification, formulation of recipe, as well as environmental and economic impacts (**Figure 4**) (Moreno-González and Ottens, 2021; Mujtaba et al., 2023).

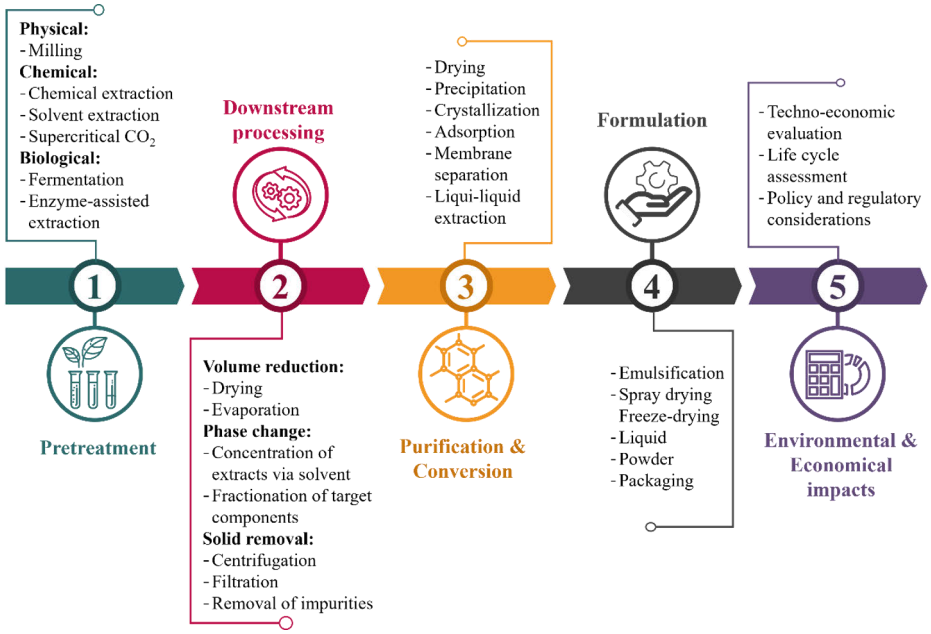

Figure 4. Generalized process flow diagram for the processing of biomass and side streams. Adapted from Moreno-González and Ottens (2021) and Mujtaba et al. (2023).

2.2.1 Extraction of multiple value-added compounds

Fractionation, extraction, and chemical processes utilizing solvents are frequently employed due to their effectiveness in isolating specific compounds, ease of implementation, and wide applicability (Reyes et al., 2022). The properties of solvents, such as polarity, significantly influence the composition of the biomass extract. Besides physical solubility, the effectiveness of extraction is closely tied to the similarity in functional groups between the solvent and the solute. It is well established that less polar solvents generally yield lower

amounts of polyphenols. Typically, highly hydroxylated aglycone forms of polyphenols are soluble in water, alcohols (e.g., methanol and ethanol), or their mixtures. Conversely, less polar and highly methoxylated aglycone forms are extracted using less polar solvents, such as acetone or ethyl acetate (Dorta et al., 2012; Kaczorová et al., 2021).

Furthermore, it is preferable to use solvents that are considered environmentally friendly, taking into account their impact on the environment, safety, and health (Prat et al., 2016). Water, for example, is an eco-friendly solvent capable of extracting polar compounds, and it allows for chemical modifications to adjust pH (e.g., using Na_2CO_3) (Kilpeläinen et al., 2023) and react with condensed tannins (e.g., $NaHSO_3$) to improve extraction yields (Ma et al., 2018). Ethanol is one of the most commonly used solvents due to its ability to mix with both water and organic solvents, its effectiveness in dissolving both polar and non-polar compounds, and its low toxicity (Kerton and Marriot, 2013).

The conventional extraction of bioactive compounds predominantly employs solvent extraction methods, utilizing a range of organic solvents. However, the growing demand for higher yields of targeted bioactive compounds from plant matrices, coupled with the critical demand for environmentally sustainable production, has underscored the need to develop innovative green extraction techniques (Fidelis et al., 2019). In this context, a promising strategy for efficient phytochemical recovery involves multi-step fractionation processes that leverage green technologies (Arshadi et al., 2016; Huang et al., 2008). Additionally, significant attention has been directed toward the separation of biomass into its three primary components, namely hemicelluloses, cellulose, and lignin, further enhancing the potential for sustainable extraction and utilization (Kilpeläinen et al., 2014). Although plant-derived hemicelluloses have not yet fully achieved their potential in industrial applications, research has highlighted their value in various areas, such as serving as a delivery system for essential fatty acids in food emulsions (Valoppi et al., 2019) and medical and pharmaceutical applications (Liu et al., 2019). Techniques like pressurized hot-water extraction (PHWE) present several benefits over traditional extraction methods such as Soxhlet and sonication (Leppänen et al., 2011; Sluiter et al., 2008), as PHWE is generally quicker and a more environmentally friendly approach, requiring less solvent. PHWE has already proven successful and scalable in the recovery of hemicelluloses and polyphenols (Kilpeläinen et al., 2014; Ravber et al., 2015). From a technical perspective, PHWE is a method carried out at temperatures between 100 °C and water's critical temperature of 374 °C. This process can be performed in either static (batch) or dynamic (flow-through) modes.

Pressure and temperature greatly influence the solvent's dielectric constant/polarity, consequently impacting the extractability of a wide range of compounds (Gil-Martín et al., 2022). In the PHWE process, at high pressure and

temperatures above its boiling point (around 200 – 275 °C), water remains liquid but considerably less polar due to the breakdown of intermolecular hydrogen bonds (Matshediso et al., 2015; Smith, 2002). This results in a lower dielectric constant, allowing water to behave similarly to organic solvents and become capable of dissolving less polar compounds. Pressurized water can also break internal matrix bonds, increasing the diffusivity and extractability of analytes (Gil-Martín et al., 2022). Higher-temperature extractions generally enhance extraction efficiency by increasing solute release at matrix sites, leading to faster mass transfer rates, and higher yields. The polarity of the solvent is a key factor in determining the solubility of polyphenols; therefore, reducing polarity and weakening hydrogen bonds can enhance the dissolution of semi-polar components (Co et al., 2012; Fidelis et al., 2018). At elevated temperatures, water acts as a source of hydronium ions (H_3O^+), which promotes the hydrolysis of polysaccharides and proteins into smaller molecules, e.g., monosaccharides, oligosaccharides, peptides, and amino acids (Plaza and Turner, 2015).

Hydrodynamic cavitation extraction (HC) is an emerging technique for recovering bioactive compounds and lipids from algal sources and the delignification of lignocellulosic biomass. HC is a process in which hydrostatic pressure decreases below the local saturated vapor pressure when liquid passes through constricted spaces, such as Venturi tubes and orifice plates (Meneguzzo et al., 2019; Wu et al., 2019). Cavitation generates vapor-filled microbubbles that grow and collapse below the liquid's boiling point (Kumar and Moholkar, 2007). Subsequently, highly reactive microelements are formed when bubbles implode, characterized by extreme temperatures and pressure waves (Albanese et al., 2019; Wu et al., 2019). Cavitation effects enhance and accelerate chemical and physical processes, promoting the breakdown of materials and the release of compounds. This phenomenon also improves mass transfer rates and effectively ruptures cell walls, leading to the disintegration of the matrix. HC presents a promising, rapid, energy-efficient, and scalable method for recovering valuable compounds, producing bioenergy and chemical derivatives, such as antioxidants, cellulose- and lignin-based products (Albanese et al., 2019; Wu et al., 2019).

2.2.2 Process optimization

The efficient recovery of target bioactive compounds is influenced by numerous factors, especially plant fraction, assortment, chemical properties (e.g., molecular structure and polarity), particle size, solvent type, extraction temperature and time, and the application of enzymes (Lucci et al., 2017). The properties of the extracted compounds must be considered to prevent adverse chemical modifications during extraction, such as hydrolysis, oxidation, or isomerization reactions (Tura and Robards, 2002). Typically, extractions can be

improved and higher yields achieved by raising the temperature and increasing the solvent-to-solid ratio (Seidel, 2012; Zhang et al., 2018). However, excessively high temperatures can cause thermal degradation, resulting in solvent losses and extracts that contain impurities or undesired compounds. Moreover, the efficiency of extraction increases only up to a certain threshold, beyond which prolonged extraction may lead to the degradation of the desired compounds (Esclapez et al., 2011). Consequently, it is essential to optimize key factors to maximize efficiency, reduce resource consumption, and ensure the effectiveness and feasibility of the extraction process (Dai and Mumper, 2010).

In order to account for interactive effects among all system variables, statistical optimization can be conducted through response surface methodology (RSM) to improve system performance by maximizing benefits and achieving optimal outcomes (Dean et al., 2017). RSM has become one of the most popular optimization tools over recent years, and its significance is linked to its primary functions: to develop, improve, and optimize a process. It encompasses crucial applications in designing, developing, and formulating new products. These applications include mapping a response surface for a specific purpose, optimizing the response, and selecting operating conditions (Myers et al., 2016; Şahin et al., 2017). RSM enables the analysis of interactions or the effect of factors, i.e., independent variables (e.g., pH, solvent system, and temperature), alone or combined, where the variable of interest is influenced by others, to optimize the response. Moreover, it allows for the generation of a mathematical model that describes the chemical and biochemical processes. This is achieved using statistical and mathematical techniques for fitting empirical models based on experimental data obtained from an experimental design (Baş and Boyacı, 2007; Bezerra et al., 2008).

Among the experimental design (DOE) techniques using RSM, central composite, mixture design, Box Behnken, and Doehlert matrix are widely employed for optimization (Ferreira et al., 2018). Central composite design (CCD) is an RSM approach used for process modeling from two or more factors (e.g., extraction time and temperature), generally involving five levels. The levels indicate the distinct settings (values) at which each factor (e.g., extraction time and temperature) is tested. These levels typically include factorial points (low and high levels, a.k.a main effects and interactions between factors), axial/star points (extreme levels beyond the factorial levels to explore curvature in the response surface), and central points (the midpoint level of all factors for error and curvature assessment). CCD allows the determination of linear and quadratic models and facilitates the modeling and analysis of the effects of multiple factors on a response variable (Bezerra et al., 2008; Ferreira et al., 2018, 2007).

3 AIMS OF THE STUDY

The aim of this study was to investigate the potential utilization of underutilized plant fractions of reed canary grass, common reed, hemp, and conifer needles in biorefinery and food application. Whole wheat bread was chosen as carrier system for bioactive compounds. All the proposed valorization approaches concentrated on environmentally friendly and industrially feasible processes. Thus, the role of different processing parameters on the studied biomasses was also explored.

The objectives of the individual studies were as follows:

i To screen the lipophilic and hydrophilic fractions of reed and hemp biomasses in terms of chemical composition and bioactivity, as well as explore an extraction-based biorefining process using a green technique for the fractionation of hemicelluloses and extractives. (Study **I**)

ii To optimize water and aqueous ethanol extractions of needle-rich biomass from logging residue, evaluating the effects of time and temperature to recover extracts rich in condensed tannins and total phenolics exhibiting antioxidant and antibacterial properties. (Study **II**)

iii To explore the impact of incorporating green needle and twig extracts on the quality (texture and color), chemical and nutritional composition, bioactivity, and sensory properties of whole-wheat bread. Additionally, the effect of storage time on the stability of phenolic compounds was assessed. (Study **III**)

4 MATERIALS AND METHODS

4.1 Outline of studies

The outline of Studies **I–III** is presented in **Figure 5**. For detailed information, see the original publications. Studies **I** and **II** focused on investigating reed and hemp biomass grown on marginal and peatlands, and conifer needles, respectively, whereas Study **III** explored the effect of NT extracts on whole wheat bread's quality, chemical and nutritional composition, and sensory properties.

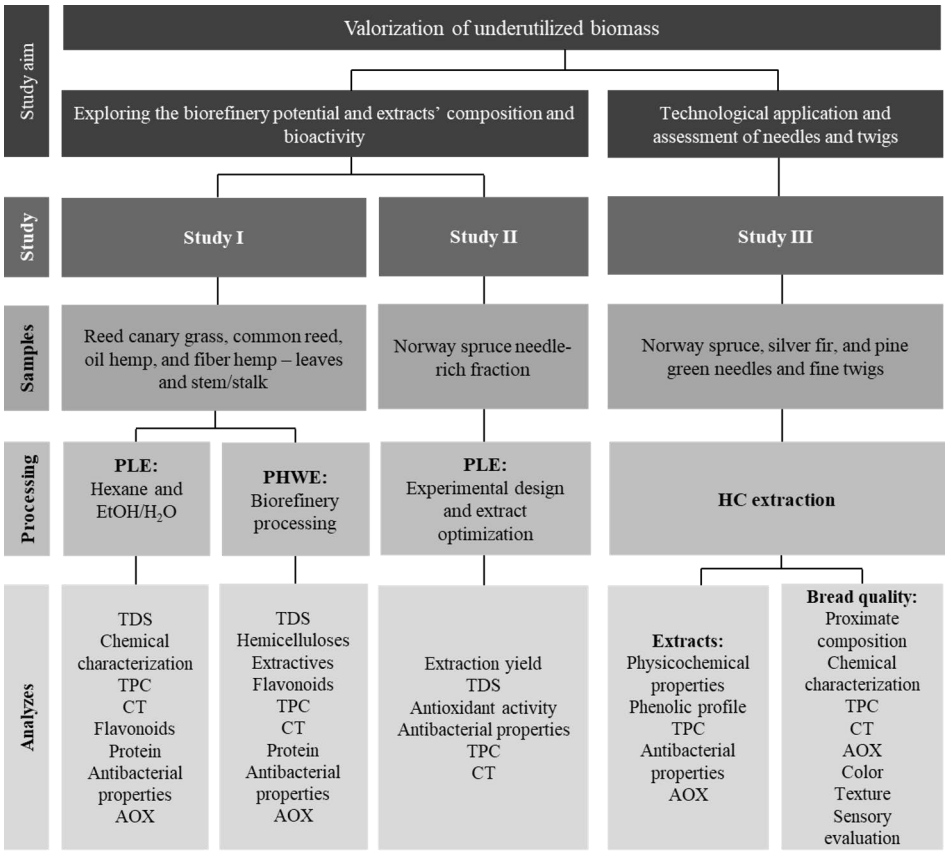

Figure 5. Outline of Studies **I-III**. Abbreviations: PLE: pressurized liquid extraction, HC: hydrodynamic cavitation extraction, TDS: total dissolved solids, TPC: total phenolic content, CT: condensed tannins, and AOX: antioxidant activity.

4.2 Plant materials and sample preparation

The raw materials used in Study **I** were four herbaceous plants grown in Finland: reed canary grass (*Phalaris arundinacea* var. Pedja), common reed (*Phragmites australis*), fiber hemp (*Cannabis sativa* var. Uso 31), and oil hemp (*Cannabis sativa* var. FINOLA). In Study **II**, green needles were obtained from logging residues (branches) of Norway spruce (*Picea abies* [L.] Karst). In Study **III**, green needles and fine twigs (NT) of Japanese red pine (*Pinus densiflora* Siebold & Zuccarini), silver fir (*Abies alba* Mill.), and Norway spruce (*Picea abies* (L.) H. Karst) were used. Detailed information on plant materials is given in **Table 2**.

The aboveground plant biomass samples were collected in summer and autumn to assess extractive differences (Study **I**). The screening fines were chosen for further biorefining process since this fraction represents the less fibrous part of the material that is unsuitable for pulping and papermaking but usable for other value-added purposes. Fiber hemp samples collected in summer were not included in this study due to the eventual dryness and poor quality of available plants, which did not represent average annual growth. In Study **II**, the separation of green needles from the rest of the branch material was performed in a upscaled process (Mäkelä et al., 2016). Logging residues were carefully harvested and preserved in their freshest and greenest state to prevent the degradation of valuable bioactive compounds. In Study **III**, green NT samples were treated equally in terms of harvesting, drying, and milling.

Table 2. Collection of experimental samples.

Study	*Plant material*	*Location*	*Sampling date*	*Part of the plant*	*Sampling*
Study I	Common reed (*Phragmites australis*)	Siikajoki, Finland (64.8° N, 24.8° E)	Week 30/2021 Week 42/2021	Screening fines (leaves), fiber fraction (stem)	Sandy sea shore
	Reed canary grass (*Phalaris arundinacea* var. Pedja)	Siikajoki, Finland (64.6° N, 25.1° E)	Week 30/2021 Week 42/2021	Screening fines (leaves), fiber fraction (stem)	Agricultural peatland (Sphagnum peat)
	Fiber hemp (*Cannabis sativa* var. Uso 31)	Siikajoki, Finland (64.6° N, 25.4° E)	Week 42/2021	Screening fines (leaves), fiber fraction (stalk)	Fine-sandy moraine (organic content 6 – 11.9%)
	Oil hemp (*Cannabis sativa* var. FINOLA)	Hausjärvi, Finland (60.7° N, 25.0° E)	Week 26/2021 Week 38/2021	Screening fines (leaves), fiber fraction (stem)	Fine silt (organic content 3 – 5.9%)
Study II	Norway spruce (*Picea abies* (L.) H. Karst)	Håknäs, Sweden (63°54’0”N and 19°74’1”E)	May 2021	Green needles from logging residues	70-year-old tree stand
Study III	Norway spruce (*Picea abies* (L.) H. Karst)	Umeå, Västerbotten, Sweden (63°38’04.1”N; 19°59’41.2”E)	November 2022	Needles and twigs were harvested from the lower branches of older trees, and the entire tree in younger specimens	4 large trees (around 70-year-old) and 6 small trees (around 20 years old)
	Japanese red pine (*Pinus densiflora* Siebold & Zuccarini)	Minamiminowa Village, forest near Ina city Nagano prefecture, about 930 m a.s.l., Japan (35°53’57”N; 137°54’58”E)	October 2022	Green needles and twigs were harvested from the crown parts of the trees	3 trees (between 4 to 5 m)
	Silver fir (*Abies alba* Mill.)	“Teso” Forest in Tuscany Apennines, about 1050 m a.s.l. (44°03’54”N; 10°48’34”E)	October 2022	Needles and twigs from older trees were collected from lateral branches up to 2 meters above the ground, while those from younger trees were taken from crown branches	8 trees, around 4 high-rise trees (around 60 years old) and 4 low-rise trees (5 to 20 years old).

4.3 Extraction

Detailed description of the extraction methods can be found in Studies **I-III**. Due to the heterogeneous composition of plant biomass resources and the varying recalcitrance of biological tissues, the experimental conditions for the proposed extraction methods and their respective analytical techniques were specifically optimized for each case. In particular, reed and hemp biomass (screening fines, fiber fraction and unscreened material) were extracted using an accelerated solvent extractor (ASE-350, Dionex, USA) with hexane at 90 °C for 15 min (3 cycles×5 min each). A second extraction was carried out with an ethanol/water (95/5, v/v) at 100 °C for 15 min (3 cycles×5 min each) (Study **I**, **Table 3**). Hexane and ethanol/water solvents were selected to assess the diverse composition of lipophilic and hydrophilic fractions. For the proposed biorefinery processing, the single- (90 °C for 60 min or 160 °C for 60 min) and two-stage (90 °C for 60 min followed by 160 °C for 60 min) PHWE of screening fines were performed using the ASE system. In the two-stage extraction method, the same raw material was used in both stages, with only the water replaced for each new extraction. The solid-to-liquid ratio was set at 1:10 (w/v) for hexane and ethanol/water extractions, while a ratio of 1:22 was selected for PHWE, based on prior studies using wood biomass (Kilpeläinen et al., 2023, 2014).

Spruce needles were extracted using an ASE system (Study **II**, **Table 3**). Water and ethanol/water (90/10, vol/vol) were chosen to have water as a polar solvent for hydrophilic compounds and ethanol/water as a general solvent for both lipophilic and hydrophilic compounds to cover whole polarity ranges. The amount of fresh sample was adjusted according to its moisture content to ensure that each extraction contained 10 g of oven-dried sample in a 100 mL extraction vessel. The temperatures ranged from 40 to 135 °C and the extraction times from 10 to 70 min, according to the experimental design.

Given the complex nature of plant biomass, the ASE system was selected for its versatility, repeatability, and suitability for biorefinery processes, requiring less solvent and time than traditional methods. Its adjustable solvent polarity and temperature make ASE highly adaptable for extracting a wide range of compounds through PLE or PHWE. Additionally, the use of pressure enables extraction at temperatures below the solvent's boiling point, preserving sensitive compounds and improving extraction efficiency (Kilpeläinen et al., 2014; Leppänen et al., 2011; Ravber et al., 2015).

Spruce, pine and fir green NT were extracted in water using semi-industrial-scale (200 L) HC processes (Study **III**, **Table 3**). This extraction process, optimized for food applications, has proven to be a viable and effective method for the integral valorization of industrial by-products (Meneguzzo et al., 2019). Samples of NT were collected at 10, 20, and 30 minutes, as well as at the point when the temperature reached 47 °C, which varied slightly (52 to 58 minutes)

with the raw material and the solid-to-liquid ratio. The fresh biomass to water ratio was 16 kg/160 L for spruce, 9.1 kg/170 L for silver fir, and 7.9 kg/170 L for pine, corresponding to 100 g/L, 53.5 g/L, and 46.5 g/L, respectively. During the HC treatment, the temperature rose from the original room temperature (18 – 19 °C) to 47 °C. The rationale for the different quantities of raw materials was to work to the full potential of the HC pilot device (200 L), using all the available quantity of each biomass.

Table 3. Extraction methods used.

Extraction	***Biomass***	***Study***
Pressurized liquid extraction using hexane and ethanol/water (95/5, v/v)	Leaves and stalk/stem of reed canary grass, common reed, oil, and fiber hemp	**I**
Two-stage pressurized hot-water extraction (biorefinery approach)	Leaves of reed canary grass, common reed, oil and fiber hemp	**I**
Pressurized hot-water extraction	Spruce needles	**II**
Hydrodynamic cavitation	Spruce, fir, and pine needles and twigs	**III**

4.4 Composition analyses

4.4.1 Total dissolved solids (Studies I–III)

TDS were determined in 3 mL aliquots of extracts duplicates after overnight oven-drying at 105 °C and examined gravimetrically (Studies **I-III**). TDS includes all solid and suspended material.

4.4.2 Protein content and total phenolic content (Studies I–III)

In Study **I**, protein content was analyzed by Bradford protein assay. The method relies on the absorbance change of the Coomassie Brilliant Blue G-250 dye, which shifts from red under acidic conditions to deep blue upon binding with proteins (Bradford, 1976).

TPC by the Prussian Blue method (Studies **I**–**II**) was quantified based on the methodology described by Price and Butler (1977) and adapted by Margraf et al. (2015). In Study **III**, extracts were analyzed for their TPC by the Folin-Ciocalteu method (Ainsworth and Gillespie, 2007; Singleton et al., 1999; Singleton and Rossi, 1965). The standard curve was prepared using gallic acid (0, 25, 100, 250, and 500 mg/L; stock solution 5 g/L (29.4 mM).

4.4.3 Condensed tannins (Studies I–III)

CT (proanthocyanins) was analyzed using a thiolytic degradation method combined with ultra-high performance liquid chromatography equipped with diode-array detection and fluorescence detection (UHPLC-DAD/FLD) (Korkalo et al., 2020). Quantification was performed using external standards of catechin, epicatechin, gallocatechin, epigallocatechin, and thiolyzed procyanidin B2.

4.4.4 Phenolic profile (Study III)

In Study **III**, control bread, bread fortified with 70% spruce NT (SNT70), bread fortified with 70% silver fir NT (FNT70) and bread fortified with 70% pine NT (PNT70) were analyzed by Agilent 1100-series high-performance liquid chromatography with diode-array detection (HPLC-DAD) and the analytical column was Phenomenex Kinetex® C18 (150 × 3.0 mm; 5 µm; 100 Å). The quantification was performed to explore the presence and stability of identified compounds during storage (24 and 72 h after baking). The HPLC pumps, autosampler, column oven, and diode array system were operated by the ChemStation computer program. The chromatograms were obtained at 245, 280, and 350 nm wavelengths. For identification purposes, ultraviolet (UV) spectra were recorded at 190 − 600 nm. Based on the in-house UV spectral libraries and combined with the retention times, some compounds were identified, while the others were just tentatively identified. Quantitation of the compounds was done by corresponding reference compounds, as in the case of ferulic acid, or by the compound with the closest resemblance by UV spectrum. In the case of SNT extract at 350 nm, the compound with UV spectrum resemblance of umbelliferone was expressed as peak area unit/g; otherwise, the results were given as mg/100 g dry weight (DW).

Further characterization of the compounds was conducted by high-resolution ultra-high performance liquid chromatography quadrupole-time-of-flight tandem mass spectrometry spectrometry (UHPLC-QTOF-MS/MS, Waters Acquity UPLC - Xevo G2 QTOF) with a Waters Acquity HSS T3 (2.1 mm x 100 mm; 1.8 µm) column. The analytical conditions used were described by Karonen and Pihlava (2022) and Pihlava et al. (2018). Putative identification of the main peaks in the negative and positive base peak ion (BPI) chromatograms was performed. The liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) data was also screened using the targeted compound approach method (Pihlava et al., 2018). Only the control bread and NT-fortified breads stored for 24 h were analyzed by LC-MS/MS.

4.4.5 Extractives (Study I)

Extractive composition was determined using gas chromatography combined with mass spectrometry (GC-MS) or flame ionization detection (GC-FID). Comprehensive description of the methods is provided in Study **I**. The silylated samples were analyzed using GC-MS (HP6890-5973 GC-MSD instrument, USA), with a HP-5 GC column (Agilent Technologies Inc., USA; 30 m x 0.25 mm i.d., film thickness 0.25 µm). Heneicosanoic acid (C21:0, 0.02 mg/mL), betulinol (0.02 mg/mL), cholesteryl heptadecanoate (Ch17, 0.02 mg/mL), and 1,3-dipalmitoyl-2-oleyl-glycerol (TGstd, 0.02 mg/mL) were used as internal standards.

The group composition (including fatty acids, sitosterol, steryl esters, triglycerides, and sterols) was analyzed by obtaining mass spectra in electron ionization mode (70 eV). The fragmentation patterns were then compared with reference standards from commercial libraries (NIST14 and Wiley11) and the MS libraries available at Luke's laboratory. Furthermore, the silylated samples were analyzed using the GC-FID (Shimadzu GC-2010, Kyoto, Japan) equipped with a HP-1 column (Agilent Technologies Inc., Santa Clara, CA, USA; 15 m x 0.53 mm i.d., film thickness 0.15 µm).

4.4.6 Monomer composition (Study I)

Non-cellulosic polymeric carbohydrates, including hemicelluloses (polysaccharides) and pectins (chains of D-galacturonic acid units linked by α-(1→4)-glycosidic bonds), were identified in biomass extracts through acid methanolysis and gas chromatography (GC-FID with an HP-1 Column (25 m x 0.2 mm i.d., film thickness 0.11 µm)), as reported previously (Sundheq et al., 1996). Detailed description of the method can be found in Study **I**. After acid methanolysis, the remaining non-cellulosic polysaccharides were decomposed to monomeric sugar units. Arabinose (Ara), glucose (Glc), glucuronic acid (GlcA), galactose (Gal), galacturonic acid (GalA), mannose (Man), rhamnose (Rha), xylose (Xyl), and 4-O-methyl glucuronic acid (4-O-Me-GlcA) were used as standards for identification and quantification.

4.5 Antioxidant activity (Studies I–III)

Three antioxidant mechanisms were measured through microplate methodologies: single electron transfer (2,2-diphenyl-1-picrylhydrazyl (DPPH), cupric ion-reducing antioxidant capacity (CUPRAC), and ferric ion-reducing antioxidant power (FRAP)), transition metal ion chelation (Fe(II)), and hydrogen atom transfer (oxygen radical absorbance capacity, ORAC).

The DPPH free-radical scavenging assay was performed according to the method described by Brand-Williams et al. (1995) (Studies **I - III)** to monitors signal intensity loss over time as the antioxidant scavenges the DPPH radical. The CUPRAC was estimated using copper(II)-neocuproine (Cu(II)-Nc) reagent as the chromogenic oxidant (Apak et al., 2008) (Studies **I** and **II**). The absorbance of the Cu(I)-chelate, produced through the redox reaction of reducing polyphenols and vitamins, was recorded at 450 nm against a control sample after 30 minutes of incubation. The Fe(II) chelating ability assay was assessed by the reaction between a phenolic compound and iron (Fe(II)) (Study **I**). In a slightly acidic medium (pH 6), the remaining Fe(II) reacted with ferrozine, forming a blue-colored complex that can be monitored spectrophotometrically (Santos et al., 2017). The color reduction, which represents an estimation of the binding ability of the extract absorbance of the Fe(II)-ferrozine complex, was measured at 562 nm. The FRAP method was conducted to measure the ability of antioxidants to reduce ferric Fe(III) to ferrous Fe(II) ions (Benzie and Strain, 1996) (Studies **II** and **III**). The ORAC test measured a potential antioxidant's ability to prevent peroxyl radicals from harming the fluorescent fluorescein molecule (Studies **I - III**). The method was modified from those described by Huang et al. (2002) and Prior et al. (2003).

4.6 Antibacterial activity (Studies I–III)

The antibacterial activity was determined using three recombinant whole-cell bacterial biosensor strains. Two were constitutively luminescent light-emitting strains, as reported by Vesterlund et al. (2004). *Escherichia coli* K12 + pcGLS11 and *Staphylococcus aureus* RN4220 + pAT19 (Studies **I** and **III**). The third biosensor strain was a genotoxicity or deoxyribonucleic acid (DNA)-damage-induced stress-responsive strain, *E. coli* DPD2794 (recA::lux) (Vollmer et al., 1997) (Study **III**). The bacterial strains were cultivated according to the method reported previously (Tienaho et al., 2021, 2015). The detailed description of the methods can be found in Studies **I – III.**

4.7 Nutritional and quality assessments of the bread models (Study III)

4.7.1 Bread making

The most promising extracts of NT from pine, fir, and spruce in terms of antioxidant activity were selected for further supplementation in bread. Dough batches (500 g) were prepared using a standard bread formulation (Parenti et al., 2022), with slight modifications. The ingredients consisted of whole wheat flour,

fresh yeast, salt, water, and liquid extract (**Table 4**). Kneading was carried out with a mixer equipped with a dough hook (Hobart N50-110, Canada) at room conditions (22 ± 2 °C and 50% relative humidity). The leavening phase was performed for 90 min at room conditions and then duplicate bread samples were baked for 50 min at 150 °C in an oven (Electrolux Professional Skyline Premium, Italy). Whole wheat bread samples were supplemented with NT from spruce (SNT), fir (FNT), and pine (PNT) liquid extracts by substituting water required in the recipe at 0% (control, 100% water), 35% (35:65, w/w), and 70% (70:30, w/w) levels. These additions corresponds to 15% and 31% of the total bread weight in a 500 g dough formulation with 35% and 70% extract additions, respectively. Incorporation was constant for all the extracts. The TDS for PNT, FNT, and SNT extracts were 5.74 g/L, 6.96 g/L, and 19.38 g/L corresponding to 46.5 g/L, 53.5 g/L, and 100 g/L of solid-to-liquid ratios used for the extractions, respectively. The extract additions were selected considering the minimum impact on the absolute threshold of needle extract taste perception reported in a previous study (Parenti et al., 2022). After baking, bread prototypes were allowed to cool to ambient conditions for subsequent analyses. Fresh samples were stored in plastic bags under the same room conditions for 24 and 72 h to evaluate technological properties, antioxidant activity, secondary metabolite profiling, and chemical composition.

Table 4. Ingredient composition of the bread dough samples.

Ingredients (g)	***Control (water)***	***35% NT extract addition (w/w)***	***70% NT extract addition (w/w)***
Flour	310	310	310
Fresh yeast	13	13	13
Salt	4.5	4.5	4.5
Water	221.6	144.0	66.6
SNT, PNT, FNT liquid extract	-	77.6	155.4

4.7.2 Proximate composition, sugar profile, and mineral elements

The proximate composition, sugar profile and mineral elements were determined using standardized methods at certified laboratories (Eurofins Scientific Finland Oy). The moisture, ash, fat, protein, and total carbohydrate results were expressed as g/100 g fresh weight (FW). Sugar profile (fructose, galactose, glucose, lactose, maltose, and sucrose) consisted of an aqueous ethanol extraction of the sugars in the bread sample, followed by clarification with Carrez reagents. After Carrez treatment and filtration, the samples were diluted and analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Mineral elements, including calcium

(Ca), copper (Cu), iron (Fe), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES).

4.7.3 Instrumental evaluation of color

The ground inner part of fresh bread with and without the outer surface were analyzed for their color parameters in the visible light wavelength range 400–700 nm by a Minolta CM-508s spectrophotometer equipped with SpectraMagic software (Konica Minolta, Tokyo, Japan), which uses a CieLab method (International Commission on Illumination L*a*b* scale). The software calculates values for sample color lightness (L*, scale 100 … 0) and actual color in red-green (a*, scale +100 … −100) or yellowblue (b*, scale +100 … −100) color spaces.

4.7.4 Texture profile analysis

Bread crumb hardness, fracturability, adhesiveness, springiness, cohesiveness, and chewiness were determined using a texture analyzer (TA.TX plus 100 Stable Microsystems®, London, England), using a radiused cylinder probe with a 12.7 mm diameter (hemispherical). Two control breads and SNT70 breads were sliced, and cubes were cut using a two-blade cutter set to 1-1.5 cm width. Each sample taken from a different slice was subjected to a double compress test with a 2 s waiting time between the two cycles, performing a 40% compression at 1 mm/s with a trigger force of 0.196 N.

4.7.5 Sensory evaluation

The appearance, smell/odor, taste, and texture of the coded bread samples were evaluated by a panel at a sensory evaluation laboratory (Luke, Jokioinen, Finland), consisting of 8 people involved in food product development activities weekly and consume bread on a daily basis. The laboratory complies with the ISO 8589:2007 standard (Sensory analysis – General guidance for the design of test rooms). The samples were presented in cardboard plates with three-digit characters to each evaluator on cardboard plates labeled with three-digit characters to each evaluator in a randomized sequence. A seven-point hedonic scale was used, ranging from 0 (dislike extremely) to 6 (like extremely). The panelists were instructed to rinse their mouths out with water between evaluating each sample. The study also assessed the participants' willingness to purchase the product, measured on a scale from 1 (very low willingness to purchase) to 8 (very high willingness to purchase), represented by the average scores obtained.

4.8 Statistical analyses (Studies I–III)

The experimental data were expressed as means and standard deviation. The Shapiro–Wilk test was used to assess the data's normality and the Brown-Forsythe test to assess the homogeneity of the data variance. One-way analysis of variances (ANOVA) and the Tukey post hoc test were used to compare the mean values. Differences that reached a confidence level of 95% ($p < 0.05$) were considered statistically significant. An unpaired student t-test was used to compares the means of two independent variables. Correlation analyses were applied to investigate the contribution of compounds to bioactivities. The statistical analysis was performed using the TIBCO Statistica v.13.3 software and IBM SPSS Statistics 26 (SPSS Inc., NY, USA).

The experimental design was created using Design Expert DX13 V. 13.0.8.0 (StatEase, Minneapolis, USA) program. The Response Surface Methodology (RSM) was used to investigate the effects of time and temperature (factors) on the TDS, TPC, CT, antioxidant capacities FRAP and ORAC, and antibacterial properties (*E. coli* and *S. aureus*). A central composite response surface design was used for each solvent with 18 runs (time and temperature combinations) in randomized order. Mathematical modeling, information on optimized factors, and the two-factor central composite design used for optimization can be found in Study **II** (supplementary material). Analysis results were utilized to identify optimized conditions for forest residue extractions with each solvent. Optimization was performed using the Design Expert desirability function (Myers et al., 2016).

5 RESULTS AND DISCUSSION

This thesis focused on different valorization approaches useful for biorefinery and food applications and the role of processing parameters in the studied aboveground biomass properties. The results were distributed separately according to the outcomes of each publication (Studies **I–III**).

5.1 Biomass properties (Study I)

In the first part of Study **I**, the extractives and bioactivities of reed canary grass (RCG), common reed (CR), and oil and fiber hemp (OH and FH, respectively) biomass fractions (i.e., screening fines and fiber) were explored as a screening step and a benchmark to evaluate the proposed biorefinery processing with pressurized hot water. Hexane extraction was initially performed first to obtain lipophilic (non-polar) fractions, followed by ethanol/water ($EtOH/H_2O$; 95/5, v/v) extractions to obtain hydrophilic fractions.

5.1.1 Total dissolved solids, total phenolic content, and protein content of the extracts

Figure 6 illustrates the results for each biomass fraction, collected during summer and autumn, and extracted first with hexane followed by $EtOH/H_2O$. Data showed considerable variation in analyses used for the screening assessment. The results revealed clear seasonal distinctions between extractable hydrophilic and lipophilic fractions in the same biomass. Generally, most biomass fractions extracted with $EtOH/H_2O$ showed a higher extraction yield, as indicated by total TDS, compared to those extracted with hexane, regardless of the collection period. The only exception was autumn OH. Regarding harvesting time alone, reed samples yielded higher TDS when collected in summer, whereas oil hemp showed an exceptionally higher yield in the autumn. It is worth noting that autumn OH samples were collected around the typical harvest time for other parts of the plant besides seeds, which might explain the increased amount of solids in the autumn.

Both reed fractions showed overall higher total phenolic content (TPC) and protein content when extracted with $EtOH/H_2O$. In contrast, hemp fractions had higher yields in hexane extracts, indicating a higher prevalence of lipophilic components in hemp fractions. Moreover, the protein content was shown to be dependent on the harvesting period. The results indicated that OH samples had higher protein amounts in the summer. Conversely, CR and RCG collected in the autumn, when CR is typically harvested, resulted in greater protein content.

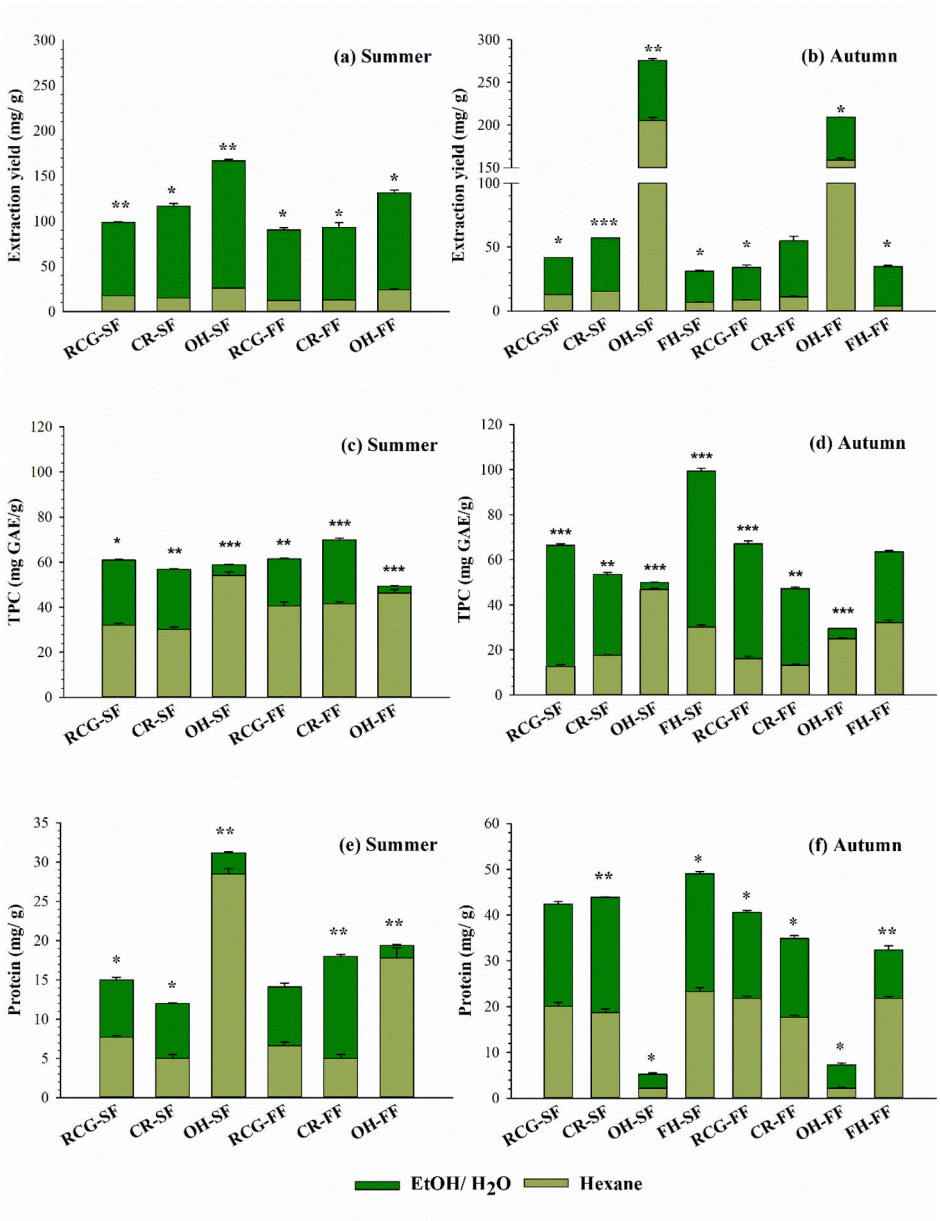

Figure 6. Comparison between hexane and aqueous ethanol extractions regarding extraction yield, total phenolic content, and protein content of reed and hemp biomass fractions during (a) summer and (b) autumn. Unscreened samples are excluded. *, **, and *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$ respectively. RCG: reed canary grass; CR: common reed; OH: oil hemp; FH: fiber hemp; -SF: screening fines; -FF: fiber fraction.

5.1.2 Extractives composition

Within the compound groups determined by GC-FID, fatty acids, sitosterol, steryl esters, triglycerides, and "sterols etc" (i.e.g, sterols and other compounds) were quantified (Study **I**, Table 3). Lipophilic summer OH was the only raw material with detectable amounts of tannin (0.3 ± 0.0 mg/g dw) and a degree of polymerization (DP, 4.7 ± 0.7). Hemp tannins were essentially (epi)catechin polymers, i.e., procyanidins, agreeing with a previous study (Mattila et al., 2018).

Nine fatty acids were identified in hexane-rich fractions, including acid 16:0 (palmitic acid), acid 18:1 (oleic acid), acid 18:2 (linoleic acid), acid 18:0 (stearic acid), acid 20:0 (arachidic acid), acid 22:0 (behenic acid), acid 24:0 (lignoceric acid), acid 26:0 (hexacosanoic acid), and acid 28:0 (octacosanoic acid), the most abundant being oleic and palmitic acids (Study **I**, Table 4). However, only two fatty acids were found in EtOH/H_2O: acid 16:0 and acid 18:0.

Sucrose, fructose, α-glucose, and β-glucose were the dominant sugars found in most EtOH/H_2O samples, while sucrose was exclusively identified in OH hexane (Study **I**, Table 4). The primary sugar alcohols detected included pinitol, myo-inositol, xylitol, and sorbitol, all of which are extensively studied for their biological properties. For instance, pinitol has garnered attention for its role in insulin regulation (Gao et al., 2015). Sorbitol, a natural sugar alcohol, is widely used across various industries, including food, pharmaceuticals (Maniganda et al., 2014), and cosmetics (Chanasattru et al., 2009). Research has shown that administering myo-inositol led to a reduction in both the number and dimensions of surface tumors and the extent of adenocarcinomas, highlighting its potential use in the chemoprevention of early pulmonary lesions (Chhetri, 2019). Additionally, lignocellulosic biomasses are renewable and cost-effective sources of polysaccharides that can be used for xylitol production, which has applications in food and pharmaceutical sectors.

Furthermore, the phytocannabinoids were found in both lipophilic and hydrophilic hemp extracts (Study **I**, Table 4). The most abundant one, CBDA, was identified in lipophilic OH and FH hemp extracts. CBD was found in both OH and FH autumn but mainly in OH lipophilic extract. In contrast, tetrahydrocannabinolic acid (THCA) was identified only in OH summer lipophilic fraction. These results corroborate earlier research, which found similar contents in hemp inflorescence (cv. FINOLA vs Futura 75) also extracted with an accelerated solvent extractor (Pavlovic et al., 2019).

Typically, RCG is produced for energy generation, and it is harvested in early spring to reduce harmful constituents (e.g., alkali and chlorine) and moisture content (Burvall, 1997). In Finland, FH is generally harvested during the spring when the soil is frozen. With this timing, unwanted plant components can be reduced, fiber processing or combustion properties can be improved (Prade et al.,

2012) and soil compacting and rutting can be avoided. In the case of OH, generally, only seeds are harvested during spring, while other parts are usually harvested during early autumn (Norokytö, 2013). The present study explored the potential to increase recovery yields of specific extractives by bringing the harvest time forward. However, increasing biomass recovery would increase the need for fertilization (Prade et al., 2012). From a biorefining perspective, the harvest time can affect the extractable substances. Therefore, given that compounds (e.g., sugars, sterols, and fatty acids) with higher concentrations in summer can be of significant value for potential commercial applications, it may be worth exploring the option of harvesting immature plants to capitalise on their higher concentrations. This is the first time that the detailed extractives content and composition of the extracts obtained from different biomass resources from marginal and peatlands have been investigated, and thus it can form the basis for future research.

5.1.3 Antioxidant activity

Consistent with TPC results, reed biomass extracts exhibited higher antioxidant activity (AOX) as determined by DPPH, CUPRAC, and ORAC assays compared to hemp when extracted with EtOH/H_2O (Study **I**, Table 6). Hemp extracts appears to benefit more from lipophilic extraction, resulting in a higher concentration of antioxidant compounds in hexane. Reed extracts, unlike other outcomes, exhibited greater Fe(II) chelating ability than hemp in both hydrophilic and lipophilic fractions. Differences in AOX could be attributed to the distinct availability of extractable components due to the varied chemical composition of plants (Palmieri et al., 2020).

Differences in TPC, protein, and AOX measurements can be attributed to several factors, including processing methods, chemical properties, environmental conditions, cultivar types, growing practices, and soil composition. Moreover, the choice of extraction solvent plays a crucial role in determining reaction outcomes, as its polarity influences the reaction mechanism. In addition, selecting the appropriate solvent and extraction conditions can lower solvent viscosity, improve cell membrane permeability, and enhance the diffusion of polyphenols (Hendricks et al., 2011; Kleinhenz et al., 2020; Setford et al., 2017).

5.1.4 Antibacterial properties

In terms of bacterial inhibition, low activity was observed against the non-specific toxicity-responsive *E. coli* K12+pcGLS11 strain (Study **I**, Table 7). The maximum inhibition was detected in lipophilic fractions of FH, specifically its

fiber fraction (19.1% inhibition) and screening fines (11.8% inhibition). For the *S. aureus* strain, three hexane extracts were able to completely inhibit bacterial luminescent light production: autumn RCG fiber fraction and both the screening fines and fiber fraction of FH, indicating high toxicity to the strain.

Overall, the lipophilic extracts showed slightly higher antibacterial activity in the constitutively luminescent light-producing strains of *E. coli* strain DPD2794 and *S. aureus* compared to ethanol-water extracts, with reed samples showing more activity than hemp. This suggested that the initial extraction with hexane may recover a higher concentration of antibacterial compounds from reed and hemp biomasses compared to the subsequent ethanol-water extraction, which yielded fewer effective compounds against the *E. coli* strain. Indeed, hexane extracts showed superior antioxidant potential overall.

5.2 Extraction-based biorefinery approach for reed and hemp biomass resources (Study I)

The second part of Study **I** explored the potential of reed and hemp biomass as renewable resources for biorefinery processes, proposing a green, simple, and adaptable PHWE-based treatment method. The aim was to identify optimal temperature and pH to effectively hydrolyze hemicelluloses and pectin from biomass sources (Väisänen et al., 2019). Experimental conditions were based on PHWE studies using fiber hemp and woody biomass (Kilpeläinen et al., 2014; Rasi et al., 2019; Väisänen et al., 2019). The extraction process was conducted in two stages: the first stage was designed to recover extractives and secondary metabolites at low temperatures, while the second stage targeted hemicellulose-rich fraction at high temperatures. A standardized extraction time of 60 min was established for PHWE at 90 °C and 160 °C. This approach was compared with single-stage extractions.

The screening fines fraction was selected for investigation, as it is less fibrous and more promising in terms of bioactivity. The samples were evaluated for their TDS (i.e., extraction yield), carbohydrates, TPC, AOX, and antibacterial activity.

5.2.1 The effect of extraction stages on extraction yield

The samples were extracted under three conditions: 90 °C for 60 min (single stage), 160 °C for 60 min (single stage), and with two-stage extraction (90 °C for 60 minutes followed by 160 °C for 60 minutes on the same raw material). The TDS yield increased with higher extraction temperatures (**Figure 7**). It was also evident that summer-collected samples resulted in higher TDS than their autumn counterparts. In the sequential extraction process, the second stage contributed over 50% of the total TDS yield for most samples, except for OH, which reached

49%. A single-stage extraction at 160 °C may suffice, depending on the target application. Breaking down the extraction process allows each stage to focus on specific fractionation, potentially improving overall efficiency.

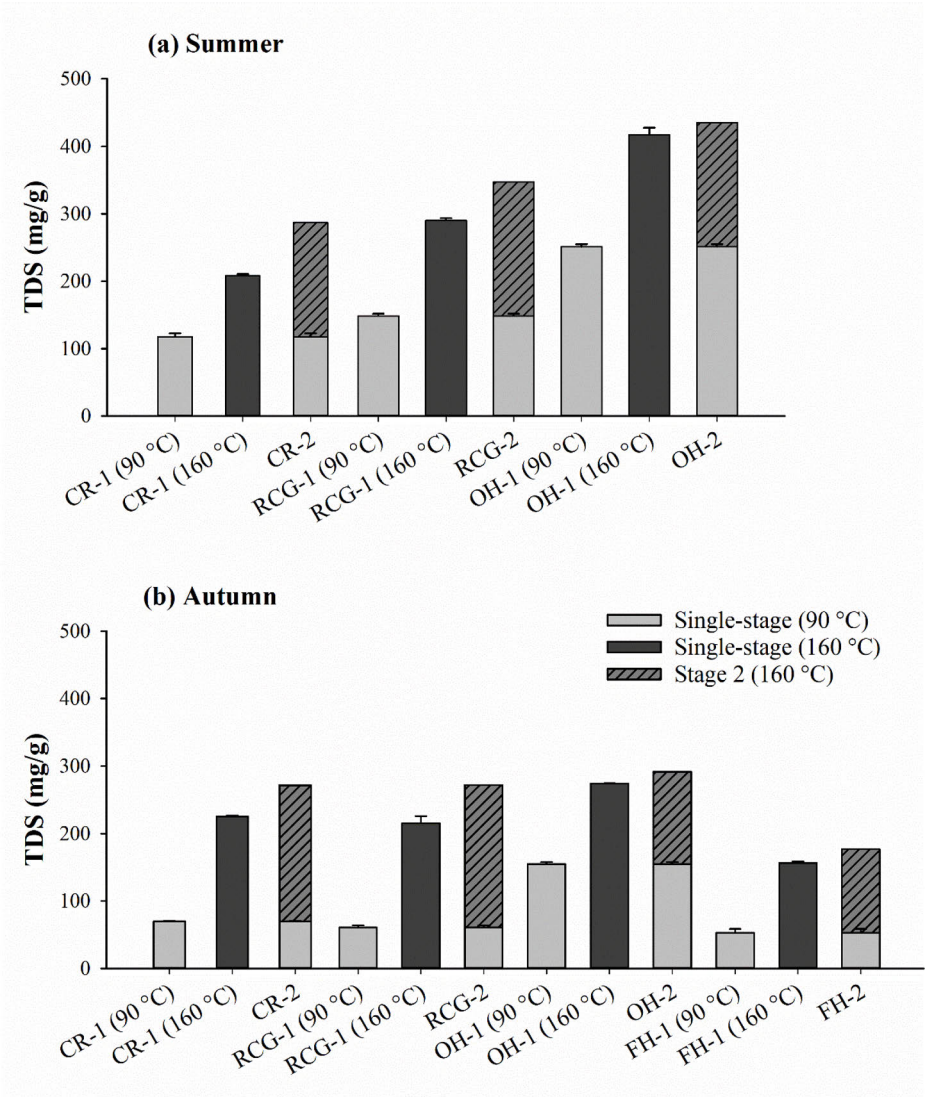

Figure 7. TDS determined in the PHWE extracts at single stages (90 °C and 160 °C) and after the first and second sequential extractions of samples collected in summer (a) and autumn (b). CR: common reed, RCG: reed canary grass, OH: oil hemp, and FH: fiber hemp (Study **I**).

5.2.2 The effect of extraction stages on total phenolics and protein content

Overall, two-stage extraction revealed higher TPC values compared to single-stage processes in most samples, except for CR-A (which showed increased TPC at 90 °C) and RCG-A (with higher values at 160 °C), as shown in **Figure 8a**. In addition to the advantages of using two different temperatures in the two-stage approach, the increased yield of soluble phenolics can be attributed to the prolonged extraction time of 120 min compared to the 60 min used in single-stage process. Furthermore, as a result of higher temperatures and pressures employed by the PHWE technique, water enhances the solubility of a broader spectrum of compounds, including less polar phenolic compounds (Matshediso et al., 2015; Smith, 2002). At elevated temperatures, water acts as a H_3O^+ source, facilitating also the hydrolysis of polysaccharides and proteins into smaller molecules, such as oligosaccharides, monosaccharides, peptides, and amino acids (Plaza and Turner, 2015).

Concerning protein content, only OH-A and RCG-S benefitted from two-stage extraction (**Figure 8b**). In contrast, a single-stage extraction at 90 °C was more effective in increasing the protein content of CR-A, RCG-A, and FH-A. Comparatively, a previous study reported increased protein content in hemp leaves (247 mg/g) than in stalk (35 mg/g) or in decorticated hemp with hurd (42 mg/g) (Väisänen et al., 2019). These findings corroborate the present results as OH screening fines were composed partially of leaves that indeed contain higher protein levels. Higher overall protein and TPC levels in CR were anticipated in autumn-collected samples, as this is the typical harvest period for most applications.

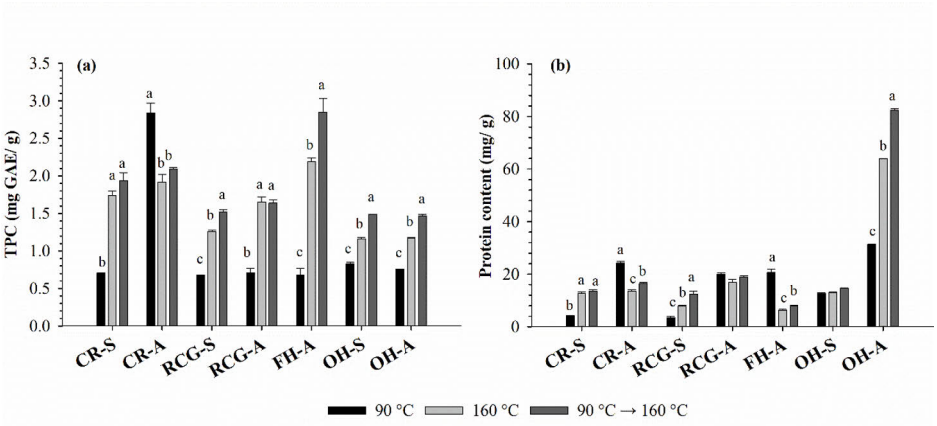

Figure 8. TPC (a) and protein content per extract DW (b). Different lowercase letters in each sample represent statistically different results between extraction temperatures ($p < 0.05$). GAE: gallic acid equivalent, CR: common reed, RCG: reed canary grass, OH: oil hemp, FH: fiber hemp, -A: autumn, and -S: summer.

5.2.3 The effect of extraction stages on bioactivities

The data showed a significant increase in AOX with elevated extraction temperatures, likely due to the presence of polyphenols, as supported by TPC results (**Figure 9a**). However, the presence of polyphenols alone does not fully explain the antioxidant capacity observed in extracts obtained at higher temperatures (Plaza et al., 2013). Evidence has shown that the increased antioxidant capacity in extracts from natural matrices at elevated temperatures using PHWE may also be attributed to the formation of new compounds either from Maillard or caramelization reactions (Plaza and Marina, 2023). Also, hemicelluloses and lignin begin to undergo hydrolysis and can be subsequently extracted at elevated temperatures (Che Sulaiman et al., 2017).

It has been shown that prolonged extraction time can decrease the yield of polyphenolic and antioxidant compounds (Che Sulaiman et al., 2017). Indeed, several biomass extracts demonstrated lower AOX with a two-stage extraction process (120 min) compared to a single-stage extraction at 160 °C for 60 min. Comparatively, RCG and CR autumn reached a higher AOX by DPPH and CUPRAC (**Figure 9**) within the investigated samples when using increased temperatures, while OH and CR extracts stood out in terms of ORAC. Furthermore, unlike other tested methods, the first extraction temperature (90 °C) indicated higher AOX in all the extracts, especially OH autumn, when tested by the Fe(II) chelating ability. The overall low outcome might suggest that the hemicellulose-rich extracts have less capacity to chelate ferrous ions. In fact, a previous study evaluated the AOX of hemicelluloses from Norway spruce galactoglucomannan and found no activity using a similar metal chelating assay, Cu^{2+} chelating ability (Granato et al., 2022). Such findings supported the outcomes.

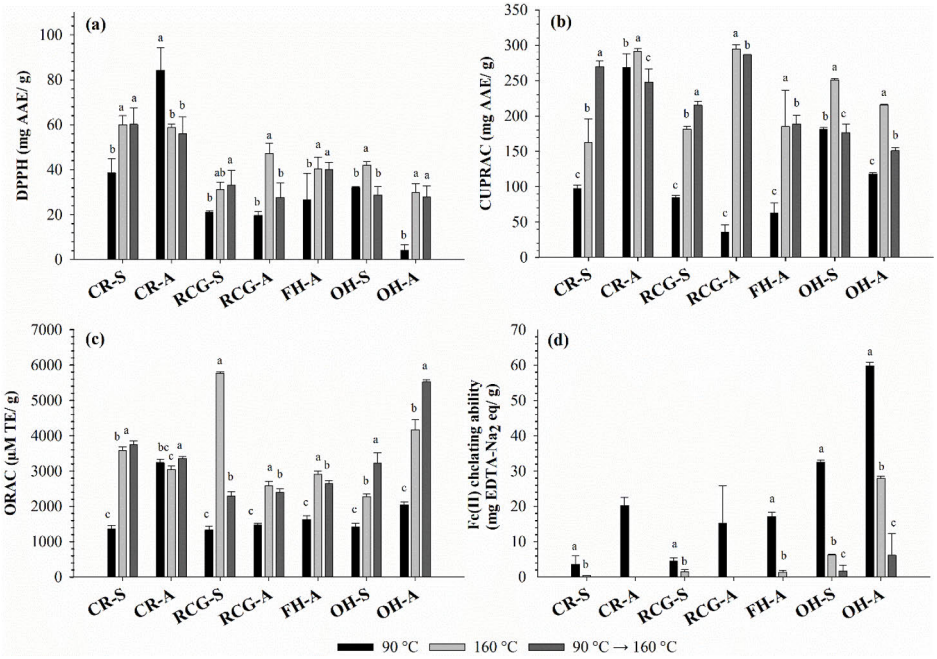

Figure 9. Effects of extraction stages on the antioxidant activity of biomass samples: (a) DPPH, (b) CUPRAC, (c) ORAC, and (e) metal chelating ability. CR-S and CR-A: common reed summer and autumn, respectively, RCG-S and RCG-A: reed canary grass summer and autumn, OH-S and OH-A: oil hemp summer and autumn, respectively, and FH-A: fiber hemp autumn, respectively.

The temperature also influenced the antibacterial activity (**Figure 10**). At elevated temperatures, the plant extracts (e.g., RCG at 160 °C) showed over 50% inhibition against *E. coli*, while inhibition of *S. aureus* reached over 70% in some the extracts (e.g., RCG at 160 °C and FH two-stage extraction). In general, PHW extracts indicated considerable antibacterial effects, particularly in autumn fractions, which achieved greater inhibition against *E. coli*. Based on these findings, the extracts may hold potential for various antibacterial applications across different fields.

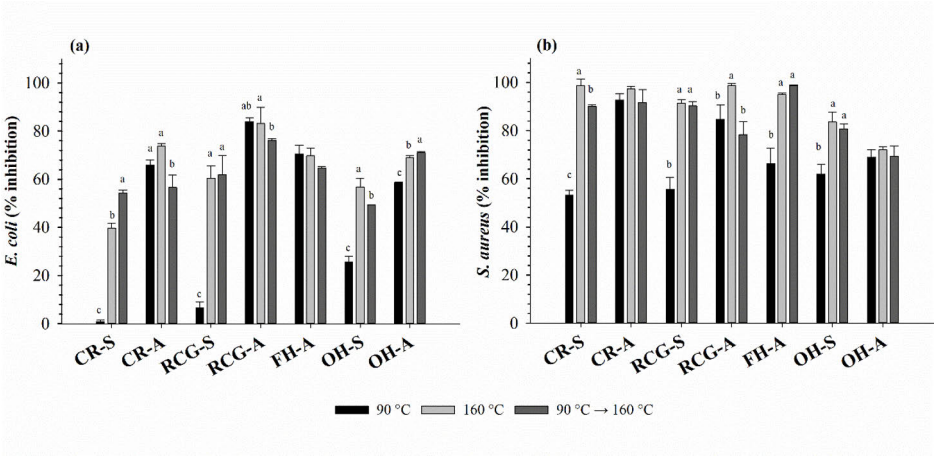

Figure 10. Effects of extraction treatment on the inhibition of (a) *E. coli* and (b) *S. aureus*. CR-S and CR-A: common reed summer and autumn, respectively, RCG-S and RCG-A: reed canary grass summer and autumn, respectively, OH-S and OH-A: oil hemp summer and autumn, respectively, and FH-A: fiber hemp autumn, respectively.

5.2.4 The effect of extraction stages on non-cellulosic polysaccharide composition

Increased extraction temperatures typically increased the total amount of extracted hemicelluloses and pectins analyzed by GC-FID (**Figure 11**). In general, over 60% of the total carbohydrate content in the two-stage extraction was obtained from its second step (160 °C/ 60 min). The CR and RCG benefited from the two-stage extraction approach, showing higher sugar levels in the second stage compared to the first stage (80% in CR summer, 60% in CR autumn, 75% in RCG summer, and 94% in RCG autumn). In contrast, hemp extracts (OH and FH) showed similar efficiency between the single-stage process at 160 °C and the two-stage process, which aligns with the trends observed in TDS yield. Previous studies confirmed the critical role of extraction temperature, time and flow rate on the hemicellulose yield (Kilpeläinen et al., 2014). For instance, a study on steam treatment of industrial hemp found that higher temperatures (100 °C, 120 °C, and 160 °C) led to increased extraction of hemicelluloses, while lower temperatures favored the extraction of glucose and pectin (Väisänen et al., 2019). Another research found that higher temperatures can enhance the yield of high-molar-mass hemicelluloses without significantly degrading the extracted polysaccharides (Leppänen et al., 2011).

Since hemicelluloses consist of various sugar units, the composition and arrangement of these units can vary depending on the plant species and tissue type. Regarding the breakdown of hemicellulose, in both harvest seasons, the CR and RCG extracts mainly contained xylose, glucose, arabinose, and galactose,

whereas glucose, arabinose, and galactose were dominant hemicellulose monomers in OH. The higher amount of glucose in OH can be attributed to the sample material containing leaves. Indeed, a study found that hemp leaves have a higher glucose content than xylose, attributed to the composition of their cell walls (Väisänen et al., 2019).

The data also demonstrated that the harvesting time can influence the composition. Specifically, when collected in late autumn (typical harvesting period), CR exhibited higher overall yields than those samples collected in summer, when the plants were still immature. On the other hand, RCG and OH had higher recovery in summer.

Overall, two-stage extraction was suitable for obtaining increased amount of hemicelluloses in the second stage while allowing the co-extraction of other bioactive compounds from screening fines fractions of reed and hemp. Research has demonstrated the successful conversion of hemicelluloses into higher-value end-use applications. For example, studies have highlighted that wood hemicelluloses can effectively stabilize emulsions against lipid oxidation in yogurt, serve as delivery systems for fatty acids, and improve the bioavailability of bioactive compounds (Lehtonen et al., 2016; Mikkonen et al., 2016; Valoppi et al., 2019; Zhao et al., 2020). Moreover, this study shows that biomass resources from underutilized or abandoned lands can provide green feedstocks for biorefineries, which can be further valorized in diverse fields (e.g., chemicals, food, pharmaceutical, and cosmetics). Still, further upscaling of the extraction to the pilot scale should be considered as it can provide comprehensive information on the process performance and channeling of water, which could not be detected in laboratory-scale processes (Kilpeläinen et al., 2014).

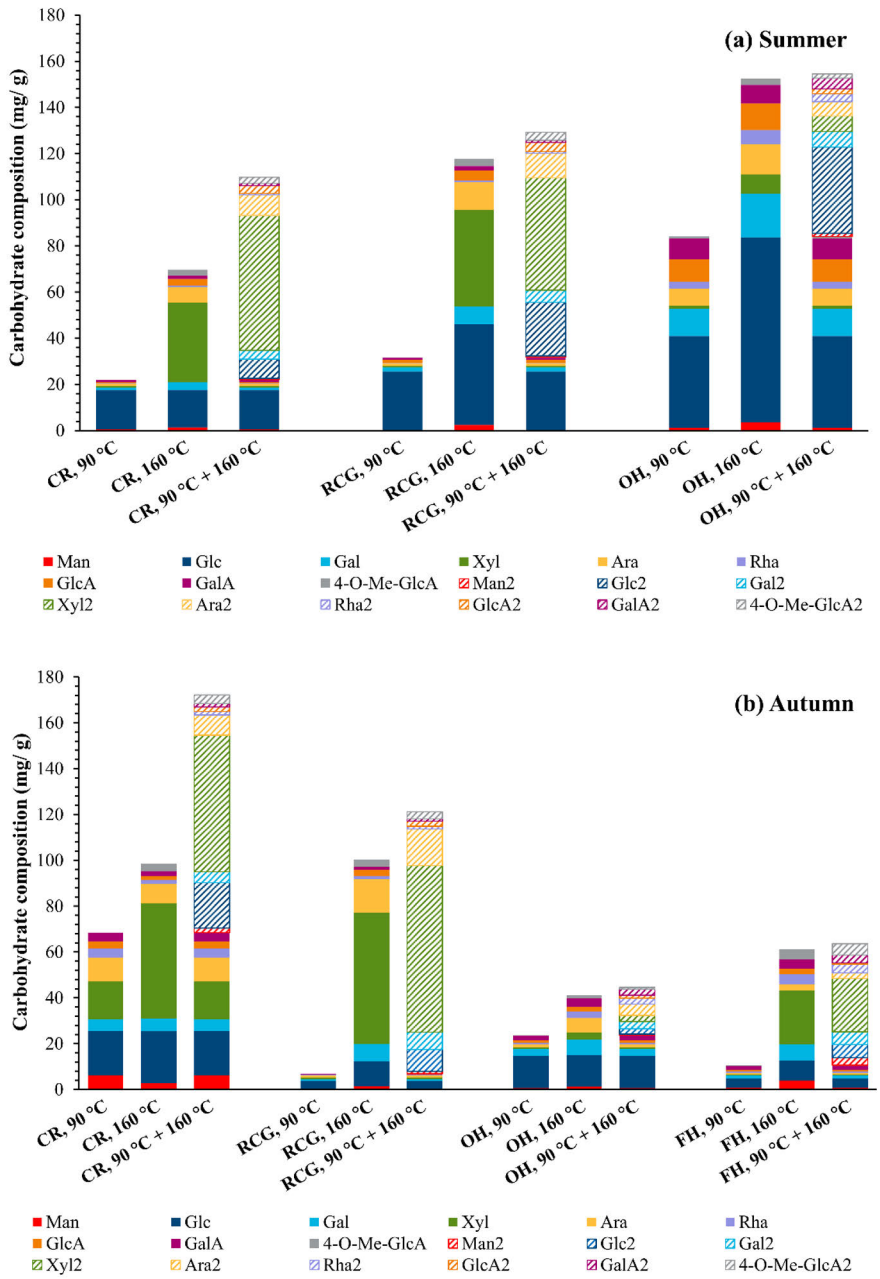

Figure 11. Effect of extraction stages on carbohydrate composition and content (mg/g DW of the original biomass) of PHWE biomasses collected in summer (a) and autumn (b). Different patterns distinguish hemicelluloses extracted in the first stage (filled shapes) from those in the second stage (line patterns), indicating the same carbohydrate but from different stages. CR*:* common reed, RCG*:* reed canary grass, OH*:* oil hemp, and FH*:* fiber hemp (Study **I**).

5.2.5 Structure-activity relationship

The Pearson correlation analysis showed that, for the summer samples, TPC significantly correlated ($p \leq 0.05$) with DPPH, CUPRAC, ORAC, *E. coli*, and *S. aureus* (**Table 5**). Protein was associated only with ORAC. Regarding autumn-collected samples, the TPC showed positive correlation with DPPH, CUPRAC, and *S. aureus*. No association between protein and bioactivities was found in autumn samples.

In the context of carbohydrates, xylose (from summer samples) exhibited a significant positive correlation with TPC, DPPH, CUPRAC, *E. coli*, and *S. aureus*, but showed a slightly negative correlation with the Fe(II) chelating ability. Similar behavior was also found in arabinose (summer), significantly correlated with TPC, CUPRAC, *E. coli*, and *S. aureus*. The sugar acid 4-O-Me-GlcA (summer) positively correlated with TPC, CUPRAC, *E. coli*, and *S. aureus*. Finally, GalA (summer) showed a positive correlation with the Fe(II) chelating ability. In fact, in most *in vitro* antioxidant systems, polysaccharides can effectively act as free radical scavengers, reducing agents, and ferrous chelators. Here, synergistic effects might occur when other antioxidants are possibly conjugated or mixed with polysaccharides, such as proteins, peptides, and polyphenols. However, different chemical characteristics influence the antioxidant potential of polysaccharides, including the molecular weight, glycosidic branching, compositions of monosaccharides, and intermolecular associations of polysaccharides (Lo et al., 2011; Wang et al., 2016).

Finally, this study revealed important insights into reed and hemp biomass fractionation for separately obtaining hemicellulose-rich and extractive-rich fractions, essential for assessing the technical feasibility of biorefinery applications.

Table 5. Correlation analysis and preliminary structure-activity relationship.

Parameter	*Season*	*TPC*	*DPPH*	*CUPRAC*	*Fe(II) chelating ability*	*ORAC*	*E. coli*	*S. aureus*
DPPH	S	**0.740***						
	A	**0.724****						
CUPRAC	S	**0.872****	**0.755***					
	A	**0.651***	**0.626***					
Fe(II) chelating ability	S	-0.484	-0.396	-0.152				
	A	-0.386	-0.406	-0.370				
ORAC	S	**0.734***	0.413	0.658	-0.015			
	A	0.221	0.200	0.238	-0.334			
E. coli	S	**0.831****	0.481	**0.912****	-0.136	**0.748***		
	A	-0.171	-0.393	-0.285	0.018	-0.282		
S. aureus	S	**0.948*****	0.662	**0.913****	-0.466	0.605	**0.905****	
	A	**0.696***	**0.652***	0.433	-0.517	0.184	-0.372	
TDS	S	0.292	-0.263	0.105	-0.011	0.334	0.193	0.293
	A	-0.02	-0.153	0.141	**0.568****	**-0.522***	0.249	-0.13
Man	S	0.287	-0.232	0.048	-0.029	0.454	0.163	0.233
	A	0.33	0.382	0.054	-0.127	0.003	-0.342	**0.458***
Glc	S	-0.136	**-0.564***	-0.222	0.182	0.318	-0.034	-0.065
	A	0.231	0.515*	0.306	0.153	-0.205	-0.363	0.404
Gal	S	0.068	-0.326	0.018	0.27	0.401	0.176	0.107
	A	0.128	-0.018	0.145	0.24	-0.534*	0.207	0.112
Xyl	S	**0.889****	**0.662****	**0.846****	**-0.529***	0.271	**0.794****	**0.908****
	A	0.331	0.276	0.432	-0.271	-0.256	0.352	0.133
Ara	S	**0.658****	**0.061**	**0.620****	-0.131	0.499	**0.726****	**0.756****
	A	-0.065	0.054	0.185	-0.042	-0.266	0.184	0.037

(**Table 5** Continued)

Parameter	*Season*	*TPC*	*DPPH*	*CUPRAC*	*Fe(II) chelating ability*	*ORAC*	*E. coli*	*S. aureus*
Rha	S	-0.016	-0.372	-0.042	0.401	0.542*	0.098	-0.007
	A	0.281	0.112	-0.096	0.124	-0.077	-0.235	0.242
GlcA	S	0.067	-0.189	0.066	0.251	0.205	0.153	0.133
	A	-0.146	0.107	0.071	0.128	-0.401	-0.019	0.131
GalA	S	-0.228	-0.359	-0.132	**0.671****	0.36	-0.049	-0.208
	A	0.241	0.114	-0.109	0.302	-0.164	-0.299	0.229
4-O-Me-GlcA	S	**0.871****	0.316	**0.807****	-0.374	0.627*	**0.872****	**0.922****
	A	**0.583****	0.159	0.214	-0.087	-0.342	0.374	0.191
Protein content	S	0.087	-0.168	0.278	**0.558****	**0.731****	0.460*	0.093
	A	-0.191	-0.239	-0.236	0.213	0.324	0.25	**-0.584****

***,**,* Correlation is significant at the 0.001 level (2-tailed), 0.01 level (2-tailed), and 0.05 level (2-tailed). Bold values represent statistically significant results. S: summer and A: autumn.

5.3 Extraction optimization of green Norway spruce needles (Study II)

In Study **II**, focus was given to response surface methodology and process optimization as a step further to obtain valuable extracts with a high concentration of condensed tannins and total phenolics exhibiting antioxidant and antibacterial properties. Water was selected as a polar solvent for hydrophilic compounds, aqueous ethanol as a general solvent for both lipophilic and hydrophilic compounds, a non-polar solvent, limonene, for lipophilic compounds to cover the whole polarity range, whereas water with Na_2CO_3 + $NaHSO_3$ addition for extracting tannins. This thesis presents two experimental designs – using water and aqueous ethanol as solvents – that best align with the research objectives of employing methods and solvents that are both industrially feasible and as green as possible.

5.3.1 Response surface methodology

Eighteen experimental points, including eight center point replicates, were used to optimize the extraction temperature (40 – 135 °C) and time (10 – 70 min). Using RSM, regression models were obtained to model the effects of time and temperature on extraction yield, extract's chemical composition (phenolic content and condensed tannins) and bioactivities (antioxidant activity and antibacterial properties). In the present study, experimental data were adjusted using the quadratic model, which took into account singular (isolated) and binary effects.

Regression analysis aims to estimate the coefficients of the model equation to minimize the sum of squared differences between the observed and the predicted responses. The statistical quality of the models was evaluated using the coefficient of determination (R^2), adjusted R^2, the normality of residuals (p-value), and the lack of fit (**Table 6,** Study **II** - ESI Tables 1, 2, 3, and 4). A ± 95% confidence interval was determined for each regression coefficient. Responses were selected for optimization based on the following criteria: an adjusted R^2 value above 0.7 (explaining at least 70% of the variance), statistical significance ($p \leq 0.05$), adequate precision (signal-to-noise ratio greater than 4), and a statistically non-significant lack of fit ($p > 0.1$). Responses failing to meet these criteria were excluded from the optimization process. Additionally, TDS lacking bioactivity were not considered, and the composition of CT (procyanidins or prodelphinidins) was deemed non-critical for the optimization.

The results demonstrated that all proposed regression models were statistically significant, indicating their effectiveness in describing the effects of time and temperature on the responses and their suitability for optimization. In addition, the R^2 values explained over 70% of the variance in both extraction

processes, except for the *S. aureus* response. The close alignment between R^2 and adjusted R^2 values further confirmed the high statistical quality of the RSM models, suggesting they effectively describe the phenolic content, condensed tannins, and bioactivities in needle samples.

The 2D contour plots were generated using mathematical equations to predict the interactions between variables and their effects on response variables (**Figures 12 – 14**). For aqueous extracts, the interaction between time and temperature significantly increased ($p \leq 0.05$) the responses of FRAP, ORAC, TPC, *S. aureus*, condensed tannins, and CT (DP). Similarly, in hydroalcoholic extracts, time and temperature combined increased FRAP, ORAC, TPC, *E. coli*, and condensed tannins (**Table 6**).

Table 6. Selected models, p-value, lack of fit (*F*-value), R^2 and adjusted R^2 for each solvent are marked in bold.

	Aqueous ethanol					*Water*				
Responses	***Model***	***p-value****	***Lack of fit****	***R^2***	***Adjusted R^2***	***Model***	***p-value****	***Lack of fit****	***R^2***	***Adjusted R^2***
TDS (extracts)	Quadratic	< 0.0001	0.5018	0.9831	0.9754	Quadratic	0.0002	0.6273	0.9845	0.9774
TDS (original dry sample)	Quadratic	none	none	none	0.981	Quadratic	none	none	none	0.9564
pH	none	none	none	none	none	Quadratic	none	none	none	0.9776
Brix	none	none	none	none	none	Quadratic	none	none	none	0.9789
FRAP	**Quadratic**	**< 0.0001**	**0.339**	**0.9785**	**0.9687**	**Linear**	**< 0.0001**	**0.0734**	**0.933**	**0.9234**
ORAC	**Linear**	**< 0.0001**	**0.1541**	**0.7791**	**0.7475**	**Linear**	**< 0.0001**	**0.1524**	**0.751**	**0.7154**
TPC	**Linear**	**< 0.0001**	**0.2844**	**0.8575**	**0.8371**	**Linear**	**< 0.0001**	**0.533**	**0.7604**	**0.7262**
E. coli	**Cubic**	**0.0253**	**0.3479**	**0.9017**	**0.7753**	Cubic	0.0444	0.0546	0.9317	0.8438
S. aureus	Mean	< 0.0001	none	none	none	**Linear**	**< 0.0001**	**0.8295**	**0.8492**	**0.8276**
Condensed tannins	**Quadratic**	**0.0003**	**0.1055**	**0.8466**	**0.7769**	**Cubic**	**0.013**	**0.4692**	**0.9698**	**0.931**
CT (DP)	Quadratic	0.0468	0.1871	0.6086	0.4308	**Cubic**	**0.0069**	**0.8209**	0.9492	**0.8839**

*For the best model, p-value of the model should be statistically significant ($p<0.05$), whereas the p-value associated with the lack of fit should remain non-significant ($p>0.05$). Bold values represent models fitting the data.

5.3.2 Extraction yield, total phenolic content, and condensed tannin content

Extraction yield, TPC, and CT were used to model the effects of factors time and temperature on the chemical composition of needle-rich fractions. In general, results from extraction run reponses revealed an increase in the extraction yields and TPC with the rise in extraction temperature and time for both water and aqueous ethanol extractions (**Figures 12 – 15,** Study **II**, Table 1, Supplementary Table 5). Comparatively, previous studies reported similar results for water extraction when compared with spruce bark PHWE yields or TPC (Kemppainen et al., 2014; Kilpeläinen et al., 2023; Pap et al., 2021; Raitanen et al., 2020; Rasi et al., 2019).

The yield of condensed tannins was influenced by both extraction time and temperature (**Figure 12** and **Figure 14**). Results indicated high-yield ridges at the 90 – 100 °C temperature range in water and aqueous ethanol extractions. Within this range, extending the extraction time slightly increased the yield for aqueous ethanol extractions. Evidence has shown that shorter extraction times may be preferable when using PHWE to extract tannins and other polyphenols, as this approach minimizes the risk of thermal degradation of these compounds (Mäkinen et al., 2020).

5.3.3 Bioactivities

For aqueous ethanol extraction, antioxidant activity, measured by FRAP, was highest at 120 °C with a 60-minute aqueous ethanol extraction, whereas it declined at temperatures exceeding 135 °C. In contrast, the ORAC values for aqueous ethanol extracts, as well as both FRAP and ORAC values for water extracts, exhibited a linear increase with rising extraction temperatures and extended extraction times. Lower FRAP antioxidant activity compared to previous studies can result from the industrially feasible assortment not producing a completely pure needle fraction for the extraction (Jyske et al., 2020). However, the ORAC values observed in this study were comparable to those reported in earlier research (Jyske et al., 2020), suggesting that ORAC-active compounds remain relatively stable and are not significantly influenced by differences in sampling, assortment, or handling methods (Study **II**, Supplementary Table 5). From the antioxidant response surface models of aqueous ethanol and water extracts (**Figures 12 – 15**), the variation in the center point was low in all but ORAC for water extracts.

Overall, the obtained antibacterial results are typical for unpurified extracts in the case of the aqueous ethanol extracts with *E. coli* and *S. aureus* (Study **II**, Supplementary Table 5). The lower inhibition against *S. aureus* observed in this

study suggested that the concentration of the samples (1 mg/mL) used herein may be insufficient for unpurified extraction products. Furthermore, unpurified extraction products also likely contain carbohydrates, which could serve as a nutrient source for the bacteria, potentially affecting the results.

In the bacterial tests using aqueous ethanol and water extracts, it is apparent that the variation at the center point is minimal, except for *S. aureus* in aqueous ethanol extracts, which was excluded from the optimization process (**Figures 12 – 15**). The variation indicates that the *S. aureus* strain is more sensitive to the effects of the solvents compared to *E. coli*. Additionally, the response surface model for water extracts with *E. coli* shows a dip near the center of the surface. Despite the model's desirable coefficient of determination ($R^2 = 0.8438$), it was not used for optimization due to its atypical behavior, likely resulting from overcompensation by the cubic model.

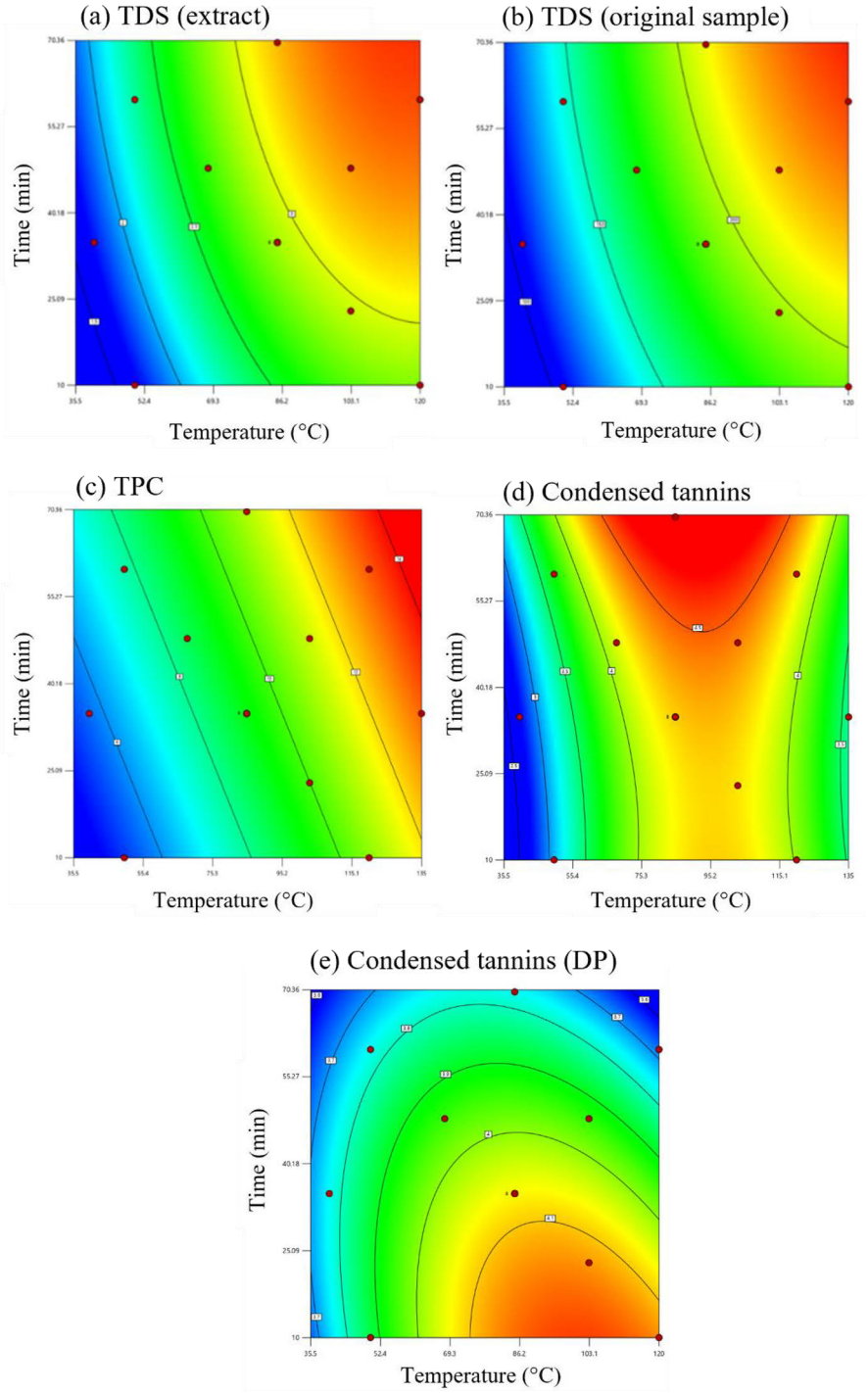

Figure 12. Contour plots representing the effects of extraction time and temperature on: (a) TDS of the extract, (b) TDS of the original sample, (c) TPC, (d) condensed tannins, and (e) condensed tannins (degree of polymerization, DP) in aqueous ethanol-extracted needle-rich fractions.

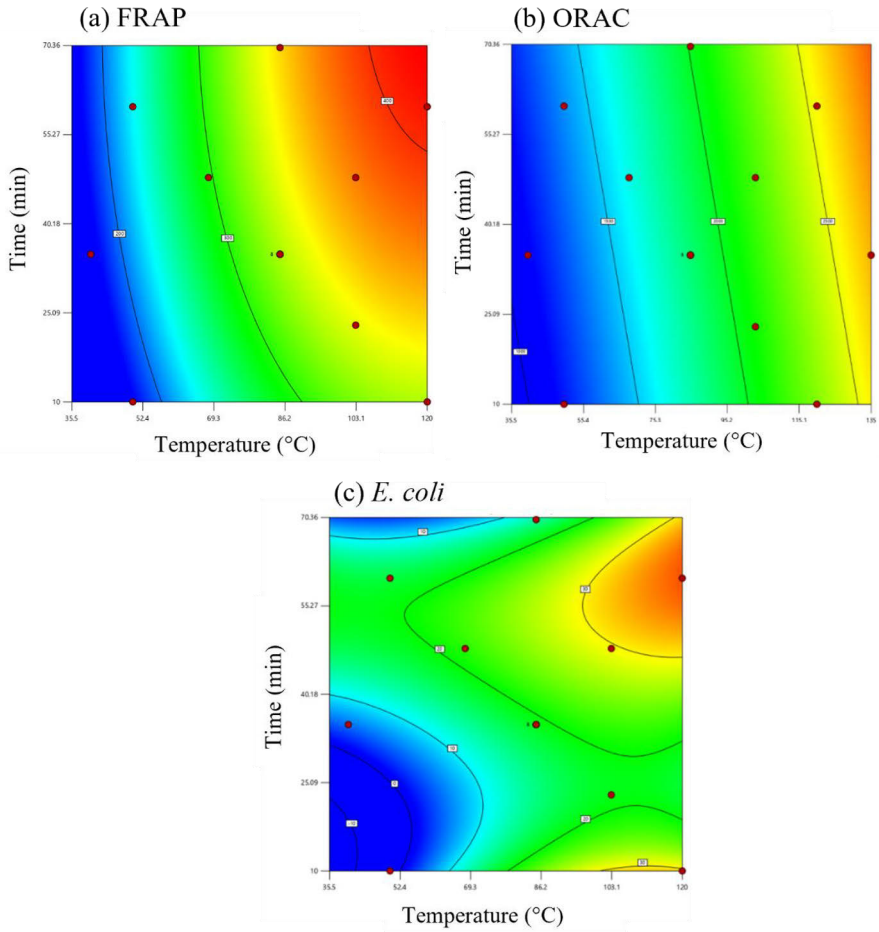

Figure 13. Contour plots representing the effects of extraction time and temperature on the bioactivities of water-extracted needle-rich fractions: (a) FRAP, (b) ORAC, and (c) antibacterial activity against *E. coli*.

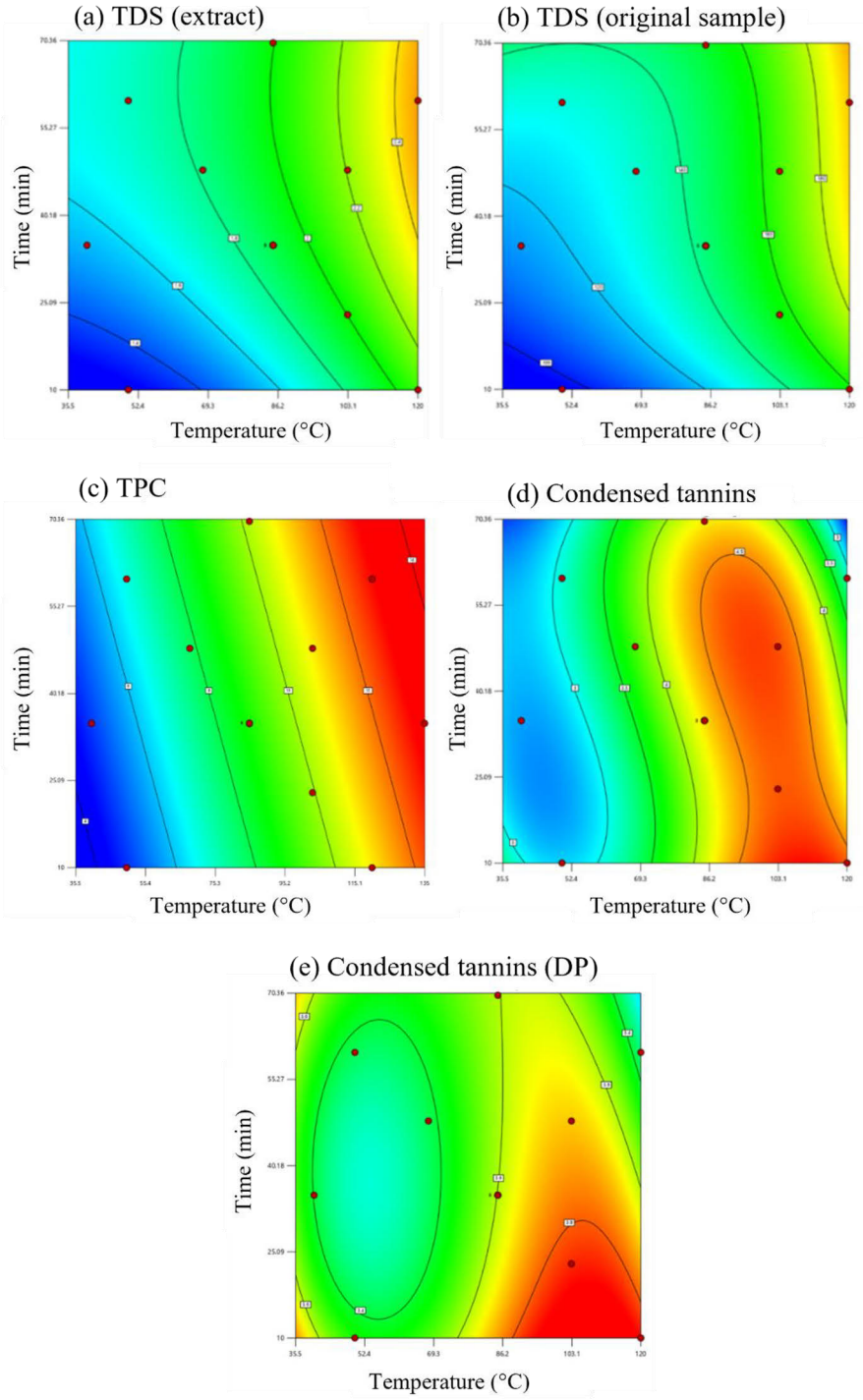

Figure 14. Contour plots representing the effects of extraction time and temperature on: (a) TDS of the extract, (b) TDS of the original sample, (c) TPC, (d) condensed tannins, and (e) condensed tannins (degree of polymerization, DP) in water-extracted needle-rich fractions.

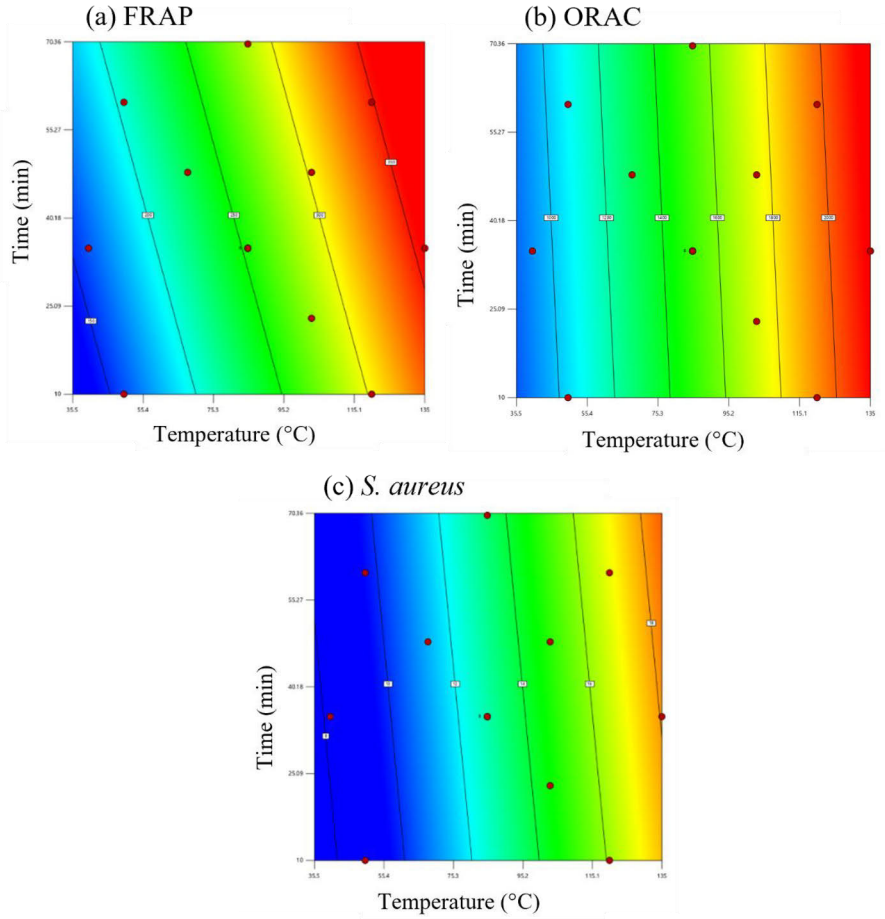

Figure 15. Contour plots representing the effects of extraction time and temperature on the bioactivities of water-extracted needle-rich fractions: (a) FRAP, (b) ORAC, and (c) antibacterial activity against *S. aureus*.

5.3.4 Optimization

Using the RSM, it was possible to generate models for the extraction of phenolics, tannins, and bioactivities from needle-rich extracts, and, subsequently, find the best extraction conditions through simultaneous optimization based on desirability function. The optimization suggested 120 °C and 10 min for water and 125 °C and 68 min for aqueous ethanol extraction to obtain an extract with the desired properties. These statistical models presented a d-value of 0.816 for water and 0.891 for aqueous ethanol, indicating that approximately 82% and 89%, respectively, of the desirability criteria for optimizing the responses were met. The extraction process involved a complex biological matrix of needles, wood, bark, and twigs. Despite this, the extraction optimization was successfully achieved.

While theoretical verification was obtained for both solvents under optimized conditions, experimental verification was conducted for aqueous ethanol at 110 °C and 60 min in order to better preserve the phenolics, especially tannins (**Table 7**). Milder extraction parameters are also preferred for industrially friendly processes. The aqueous ethanol verification indicated that within a 95% tolerance interval for a 99% population, the TDS, yield, ORAC, TPC, and *E. coli* results were within the predicted value range. However, FRAP and *S. aureus* values did not align with these tolerance intervals. This observation further supports the hypothesis that the FRAP assay is more sensitive to potential variations in sampling, assortment, and handling procedures, resulting in inconsistencies within the heterogeneous biomass composition.

Under the optimized conditions (**Table 7**), aqueous ethanol showed a higher theoretical extraction yield than water. This can be attributed to the solvent's ability to release both polar and non-polar compounds, including waxes. The hydrophobic epicuticular waxes present in the needles may inhibit water permeability during extraction, leading to reduced TDS values. Aqueous ethanol extracts exhibited higher antibacterial activity, while water would provide a slightly higher yield for obtaining condensed tannins. In the aqueous ethanol extraction (Study **II**, Figure 6A), the optimized conditions were close to the maximum temperature and time values, highlighting the distinctions between solvents and their specific applications. The process successfully extracted antioxidant and antibacterial products from starting materials that are industrially feasible. The only exception was the anticipated antibacterial activity in water extraction, which was low for *S. aureus*, likely due to the presence of carbohydrates in the extracts that could serve as nutrients for the bacteria.

In the present study, the relatively low temperature ranges from 40 to 135 °C and short extraction times from 10 to 70 min were chosen for energy preservation purposes, as less energy is used for heating, and extraction could be performed under the solvent boiling point to avoid the need for a pressurized vessel. The aim was to obtain bioactive compounds mainly present in the cellular matrix. However, results indicated that the optimized temperatures surpassed the boiling points for both water and aqueous ethanol solvents. Generally, temperatures under 100 °C are preferable to avoid hydrolysis, oxidation, and isomerization reactions that can degrade the bioactive compounds (Che Sulaiman et al., 2017).

Significant volumes of logging residues are generated as a byproduct during stemwood harvesting. However, there is a lack of industrially viable processing methods for utilizing these residues beyond energy production. The data indicated that it is feasible to extract antioxidant and antibacterial fractions containing condensed tannins from industrially assorted spruce logging residues, and the process can be effectively optimized. These findings offer valuable

insights into the potential for repurposing logging residues for higher-value applications.

Table 7. Theoretical optimization solutions obtained using the Design Expert software. The responses used in the optimization are bolded. Water with Na_2CO_3+$NaHSO_3$ and limonene models were excluded.

Aqueous ethanol (observed)				
Response variables	***Predicted mean***	***95% CI low for Mean***	***95% CI high for Mean***	***Observed mean***
TDS, Wt %	3.4	3.0	3.7	3.6
TDS, mg/g	230.1	205.4	254.8	247.7
FRAP,µM Fe(II) eq./g	**399.3**	**338.7**	**459.8**	**504.8**
ORAC, µM TE/g	**2376.7**	**1266.9**	**3486.6**	**2740.3**
TPC, mg GAE/g	**12.5**	**8.2**	**16.8**	**13.2**
E. coli, inhibition %	**33.5**	**7.2**	**59.7**	**10.6**
S. aureus, inhibition %	54.2	45.1	63.2	43.9
Aqueous ethanol (theoretical)				
Response variables	***Predicted mean***	***95% CI low for Mean***	***95% CI high for Mean***	***Theoretical mean***
TDS, Wt %	3	3	3	3
TDS, mg/g	183	180	187	241
FRAP,µM Fe(II) eq./g	**319**	**311**	**328**	**412**
ORAC, µM TE/g	**1755**	**1618**	**1893**	**2664**
TPC, mg GAE/g	**9**	**8**	**9**	**14**
E. coli, inhibition %	**20**	**15**	**25**	**36**
S. aureus, inhibition %	54	53	55	54
Water (theoretical)				
Response variables	***Predicted mean***	***95% CI low for Mean***	***95% CI high for Mean***	***Theoretical mean***
TDS, Wt %	2	2	2	2
TDS, mg/g	139	136	142	162
FRAP,µM Fe(II) eq./g	243	234	252	303
ORAC, µM TE/g	**1417**	**1302**	**1532**	**1933**
TPC, mg GAE/g	**8**	**8**	**9**	**11**
E. coli, inhibition %	4	3	5	12
S. aureus, inhibition %	**12**	**12**	**13**	**16**
CT (g/100g)	**4**	**4**	**4**	**4.6**
DP	**3**	**3**	**4**	**3.8**

5.4 Bread fortified with green needle and twigs: characterization and quality assessment (Study III)

Study **III** investigated the impact of replacing water with NT aqueous extracts from Norway spruce (*P. abies*), Japanese red pine (*P. densiflora*), and silver fir (*A. alba*) on the stability of secondary metabolites, functionality, nutritional value (proximate composition and mineral profile), as well as technological quality (texture, color, and sensory evaluation) of whole wheat bread.

5.4.1 Effects of extraction stages on total phenolic content and bioactivities of the extracts

A semi-industrial-scale HC pilot device was chosen for its flexibility and proven scalability in the extraction process, which contribute to the feasibility and affordability of the entire process chain (Meneguzzo et al., 2019). The aim was to examine the preliminary bioactivity of all liquid extracts and select the most bioactive ones of each NT source for further fortification in bread. Each conifer NT was collected at specific extraction temperatures and times (stages): NT-1 (25 ± 3 °C; 10 min), NT-2 (30 ± 2 °C for 20 min), NT-3 (35 ± 2 °C for 30 min), and NT-4 (47 ± 2 °C for 55 ± 3 min). Higher extraction temperatures and durations, especially NT-4, enhanced the antioxidant properties, while milder conditions favored the inhibition of *E. coli* and *S. aureus* strains (Study **III**, Figure 1).

The Pearson correlation analysis revealed a significant correlation between TPC and antioxidant activities measured by FRAP ($r=0.81$; $p=0.0014$) and ORAC ($r=0.822$; $p=0.0010$). However, no significant correlation was found between *E. coli* and antioxidant activity or *S. aureus*. Similarly, a non-significant correlation was found for *S. aureus* with TPC, FRAP, ORAC, and TDS, indicating that other antimicrobial metabolites, possibly thermolabile or degraded by prolonged cavitation, may be responsible for the observed activities. Potential sources of antibacterial activity include antimicrobial peptides and terpenes (Antonelli et al., 2020; Lee et al., 2021). Previous research found that conifer monoterpenes exhibited significant antibacterial effects but limited protection against hydrogen peroxide (Muilu-Mäkelä et al., 2022). This may explain the contrasting effects observed: while antioxidant activity increased with prolonged extraction times and higher temperatures, antibacterial activity against *E. coli* decreased in these conditions. In contrast, activity against *S. aureus* remained mostly unchanged, except in the case of PNT.

5.4.2 Total phenolic content and antioxidant activity of bread samples during storage

Based on the TDS, TPC, and antioxidant activity values, the aqueous extracts from the fourth extraction stage (NT-4, 47 °C for 55 min) were selected for further investigation. Whole wheat bread models were enriched at 0% (control), 35% (S/F/PNT35), and 70% (S/F/PNT70) NT levels (w/w). The initial solid-to-liquid extraction ratios were 100 g/L for SNT, 53.5 g/L for SFT, and 46.5 g/L for PNT (Study **III**, Supplementary Table 2). Consequently, the higher extraction ratio of SNT made SNT35 results more comparable to PNT70 and FNT70. Incorporating these extracts as a partial water substitute in whole wheat bread at 35% and 70% significantly enhanced the antioxidant potential and TPC over time at room temperature, showing consistent trends across all NT-fortified bread samples (**Figure 16**).

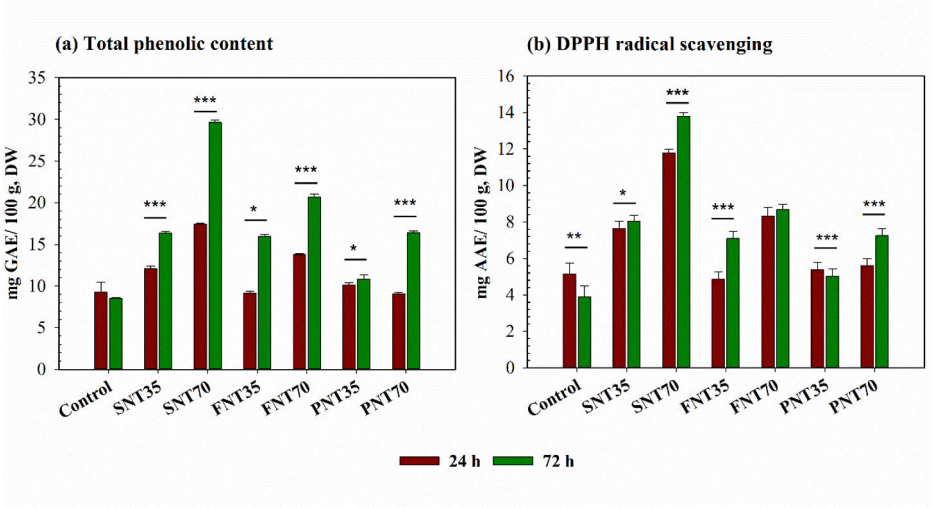

Figure 16. TPC (a) and antioxidant activity (b) of control and bread fortified with NT after 24 h and 72 h of storage. Data were expressed as mg/100 g DW of bread sample. *,**, and *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Control: dough sample with the addition of 0% NT, S/F/PNT35: dough sample with the addition of 35% spruce/pine/fir NT (w/w), S/F/PNT70: dough sample with the addition of 70% spruce/pine/fir NT (w/w). Adapted from the original publication (Study **III**).

5.4.3 Chemical composition of the extracts and bread samples

A total of 115 compounds were identified or tentatively identified from spruce, pine and fir NT samples, including flavonoids (12 compounds), hydroxycinnamic acids (6), hydroxybenzoic acids (3), alkaloids (10), stilbenes (10), lignans (14), resin acids (11), gibberellins (4), and others (29). In the control

bread, 16 identified compounds could have originated from the wheat, potentially arising from the yeast or its activity, or resulted from the heat treatment during the baking phase. These compounds included ferulic acid, tryptophan, pinellic acid, N1,N10-Bis(4-hydroxycinnamoyl)spermidine, apigenin-C-pentosyl-C-hexoside, apigenin diC-pent, luteolin-CC (carlinoside-5), and four unknown compounds. Detailed information of individual compound identification are provided in Study **III** (Supplementary Table 8). While not all major compounds identified by HPLC-DAD (Study **III**, Table 2 and 3, Supplementary Figures 3 – 19) could be confirmed by UHPLC-QTOF-MS/MS, several compounds, initially undetected due to low concentrations or lack of UV response, were putatively identified through MS and MS/MS data (Study **III**, Supplementary Table 8).

Alkaloids, including epihydropinidine, euphococcine, dehydropinidinol, pinidinol, were found in all extracts, while 1,6-dehydropinidine, pinidine, and bonvalotidine A were found only in SNT-4. Phenolic acids were characterized mainly as hydroxycinnamic acid conjugates, such as 3-p-coumaroylquinic acid, caffeic acid hexoside, caffeoylquinic acid, feruloylquinic acid, and p-coumaroyl-xyloside-hexoside. Identified flavonoids or flavonoid derivatives included luteolin, dihydrokaempferol, kaempferol glucoside, catechin, taxifolin, epigallocatechin, isorhamnetin, vitexin, quercetin-galactoside, and procyanidin B1/2. The condensed tannins were essentially procyanidins in PNT extract, while SNT and FNT extracts contained a mix of procyanidins and prodelphinidins. Across all samples, the tannins exhibited a relatively low average degree of polymerization (DP).

The negative MS data revealed the presence of stilbenes, including resveratrol, astringin, piceatannol, rhapontin and isorhapontigen, isorhapontin, and dimeric isorhapontin which were found in SNT-4 and piceid found in PNT-4. Lignans, such as secoisolariciresinol, lariciresinol and hydroxymatairesinol, along with their conjugates were identified in all samples. Resin acids primarily consisted of abietic acid and its derivatives (hydroxydehydroabietic acid and dehydroabietic acid). SNT-4 also contained smaller molecules like vanillin and piceol (4'-hydroxyacetophenone). The presence of umbelliferone in SNT-4, initially identified by its UV spectrum, was confirmed by both negative and positive MS data. In fact, umbelliferone-glucoside has been previously reported in spruce needles (Strack et al., 1989).

The bread results suggested that the identified compounds remain stable during both the baking process and subsequent storage (Study **III**, Table 3). While the total compound content in the control bread decreased over time, the fortified breads generally showed a slight increase after 3 days of storage. The addition of 70% SNT and PNT led to a reduction in wheat- and yeast-derived compounds compared to the control and 35% S/PNT breads, possibly due to

antagonistic interactions. Otherwise, the concentrations of compounds from wheat flour and yeast remained largely unchanged. Due to the initial solid-to-liquid ratios in the extractions, a higher compound content was expected in SNT70 compared to the other extracts. In all models, the tannins exhibited a relatively low average DP. The content of condensed tannins was below the quantification limit (10 mg/100 g) in FNT- and PNT-fortified breads and was detectable only in the SNT variant (Study **III**, Supplementary Table 4). The baking process might have induced some polymerization, as the tannins' average DP was slightly higher in SNT-fortified breads than in the original extract. Prodelphinidins were undetectable due to their low content, with storage time showing no significant impact on tannin levels.

The relationship between the total sum of secondary metabolites, total phenolic content, and antioxidant activity during bread storage was examined (Study **III**, Supplementary Table 5). Overall, the total phytochemical content, quantified by HPLC-DAD in all fortified bread models, was significantly correlated ($p < 0.05$) with DPPH ($r = 0.816$) and TPC ($r = 0.647$). Similarly, a strong positive correlation was observed between the sum compound content in bread and DPPH after 24 h ($r = 0.813$) and 72 h ($r = 0.818$) of storage.

HC-based extraction in water effectively released a considerable amount of bioactive compounds. In fact, incorporating NT into the dough may enhance its functionality due to the presence of bioactive compounds. The mechanisms behind the increased antioxidant activity in enriched bread can vary, depending on factors such as the extraction process, extract composition, and baking conditions. The improved availability of phenols may result from the stability of polyphenols in the extracts or the release of new bioactive compounds, influenced by thermal, enzymatic, microbial, or chemical reactions during or after baking (Meral and Köse, 2019). Synergistic effects and interactions with other organic compounds during heat treatment, along with structural changes and increased stability, may contribute to this effect (Larrosa and Otero, 2021). Other factors influencing antioxidant activity are pro-oxidation and nutrient degradation can also impact the antioxidant activity (Jensen et al., 2011).

5.4.4 Impact of needle and twig addition on the proximate composition and mineral profile of bread

A representative sample was chosen for specific analyses to allow an in-depth evaluation of quality and technological characteristics, emphasizing the formulation with the most promising functionality. The SNT70 formulation, in particular, was selected for initial assessments of proximate composition, mineral content, and texture profile, as it exhibited the highest concentrations of bioactive compounds according to LC-MS analysis and demonstrated

considerable antioxidant activity. Regarding proximate composition, the SNT70-fortified bread displayed values similar to those of the control, with only minor differences (**Figure 17**). A small rise in carbohydrate content in the fortified bread, calculated by difference, can be linked to a reduction in moisture content (41.7 g/100 g for control vs 39.4 g/100 g for SNT70), likely due to the solid content of the NT extract, which does not hydrate as effectively as water. This decrease in moisture is consistent with findings from other studies incorporating plant-based ingredients, such as potato peel, into bread formulations (Soltan et al., 2023). Additionally, energy values between the control and fortified bread were comparable, with a slight increase (245 kcal/100 g for SNT70 vs 236 kcal/100 g for control), indicating that fortification does not notably change the bread's caloric density, thereby maintaining its energy contribution.

The higher fructose and maltose levels in SNT-fortified bread compared to the control (Study **III**, Supplementary Table 6) correspond with the elevated Brix values observed in the SNT extract, contributing to the increased carbohydrate content in the fortified bread (Study **III**, Supplementary Figure 2). Although protein content showed a slight yet statistically significant decrease (from 10.3 g/100 g in the control to 10 g/100 g in SNT70), this reduction is minimal and remains within an acceptable range for bread products. Overall, these small changes in protein, carbohydrate, and moisture levels highlight the typical adjustments involved in incorporating plant-based ingredients into bread formulations, suggesting that the addition of SNT70 effectively supports the preservation of technological quality.

Data showed that incorporating SNT70 into the bread increased key minerals, particularly Na (3700 mg/kg), Mg (670 mg/kg), Fe (25 mg/kg), and Ca (270 mg/kg), consistent with findings that highlight spruce needles as a rich mineral source (Ivanov et al., 2022; Jyske et al., 2020). The presence of these components enhances the bread's functional and nutritional value by contributing to essential mineral intake. Moreover, conifer needles are known to be sources of vitamins such as vitamin C, folates, and beta-carotene, adding further nutritional benefits to the product (Jyske et al., 2020).

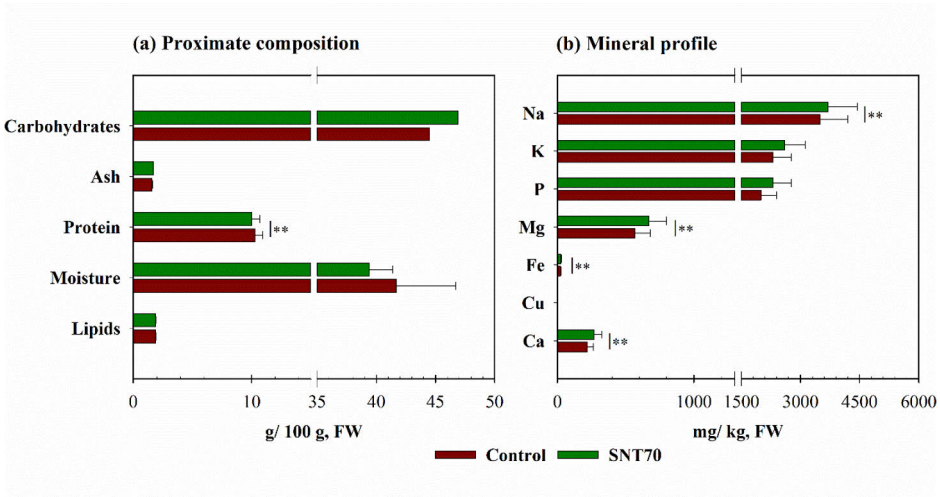

Figure 17. Proximate composition (a) and mineral profile (b) of control bread and bread fortified with 70% spruce needles-twigs extract (SNT70). The t-test ($p < 0.05$) was used to compare mean differences, with ** indicating significant differences at $p < 0.01$. Adapted from the original publication (Study **III**).

5.4.5 Impact of needle and twig addition on physical properties and sensory quality of bread

Bread fortified with NT showed differences in color intensity compared to the control, with notable variations in L* (lightness), a* (red to green), and b* (yellow to blue) value (**Table 8**). Higher extract concentrations (NT70) generally led to lower L*, a*, and b* values, resulting in darker bread. Among the tested samples, PNT35 displayed the lightest crumb color, with balanced redness and yellowness, making it potentially the most visually appealing. This was further supported by its favorable appearance rating in sensory evaluations.

As previously noted, SNT70 was selected as a representative bread model to facilitate a thorough assessment of quality and technological characteristics, focusing on the formulation with the most promising bioactivity and metabolite profile. TPA results showed that adding SNT70 significantly ($p < 0.05$) decreased bread hardness by 16% and increased springiness by 19% (Study **III**, Table 5). This reduction in hardness, an essential factor for bread quality, may positively impact dough handling and shelf-life stability. On the other hand, fracturability, chewiness, cohesiveness, and resilience remained comparable to the control, suggesting that the NT extract maintained several key textural properties. Overall, the preliminary TPA assessment indicated that a 70% substitution with NT extracts had minimal impact on texture, suggesting that NT can be used effectively to fortify whole wheat bread without compromising its quality.

The bread models were assessed for consumer acceptability using a seven-point hedonic scale. Significant differences were observed among the bread samples in terms of appearance and taste/texture attributes (**Table 8**). For the appearance attribute, the scores increased in the following order: control > PNT35 > FNT35 > FNT35 > FNT70 and PNT70 > SNT70. No significant differences were noted in smell/odor. Taste and texture ratings followed this order: PNT35 > control > PNT70 > FNT35 > SNT35 > FNT70 > SNT70. Consequently, the overall acceptability rankings followed a similar trend: control and PNT35 > SNT35 and FNT35 > PNT70 > FNT70 > SNT70. Moreover, seven out of eight panelists expressed their willingness to purchase SNT35 and PNT35. Overall, panelists favored the PNT35 formula the most, as it provided an adequate forest-like flavor without compromising the bread's organoleptic properties.

The addition of 70% NT, especially in the SNT70 model, impacted its texture and sensory qualities, resulting in reduced hardness relative to the control bread despite its reduced moisture content. This effect may be attributed to the interactions between NT polyphenols, polysaccharides and gluten during dough formation, potentially weakening the gluten network, softening the bread’s texture, and altering moisture distribution (Sivam et al., 2010; Xu et al., 2019; Zhu et al., 2016). Phenolics may also delay starch gelatinization and retrogradation due to competition for water, which could help maintain the bread’s softness over time (Xu et al., 2019). The lower acceptability of SNT70 could also result from perceived dryness, increased chewiness, and off-flavors introduced by the extract. As a result, it was confirmed that reducing the extract substitution level led to a formulation with improved overall acceptability. As previous studies suggest, a 35% extract substitution allows the bread to retain its original sensory attributes while enhancing its nutritional profile (Parenti et al., 2022).

Table 8. Effect of substituting water with needle and twig extracts (NT) at 0%, 35%, and 70% mass levels on the color of whole wheat bread crumb and sensory evaluation of bread samples. The mass levels correspond to the amount of water used in the control formulation.

Bread	***Crumb***			***Appearance***	***Smell/ odor***	***Taste and texture***	***Overall liking***	***Willingness to purchase (yes)***
	L*	***a****	***b****					
Control	54.7±7.2^{bc}	6.6±3.1^{a}	22.6±3.7^{ab}	5.9±0.4^{a}	4.9±1.0	5.0±1.1^{a}	5.3	5/8
SNT35	55.6±1.7^{c}	5.6±0.8^{a}	21.3±1.0^{ab}	5.3±0.5^{ab}	4.1±1.2	3.5±1.1^{ab}	4.3	7/8
FNT35	57.5±0.9^{ab}	4.5±0.5^{b}	20.5±1.0^{bc}	4.5±0.9^{bc}	4.4±0.7	3.9±0.6^{ab}	4.3	3/8
PNT35	58.6±1.0^{a}	6.0±0.7^{a}	22.5±1.1^{a}	5.6±0.5^{ab}	5.1±0.6	5.1±0.6^{a}	5.3	7/8
SNT70	53.3±0.6^{d}	4.2±0.3^{b}	18.5±1.2^{d}	3.5±0.5^{c}	3.8±1.3	3.0±1.1^{b}	3.4	1/8
FNT70	50.3±2.1^{e}	4.6±1.3^{b}	19.1±2.3^{cd}	3.8±1.0^{c}	4.1±1.0	3.4±1.5^{ab}	3.8	1/8
PNT70	56.9±1.3^{bc}	4.3±0.5^{b}	19.1±1.2^{cd}	3.8±1.2^{c}	4.5±1.2	4.0±1.6^{ab}	4.1	2/8
p-value (*one-way* ANOVA)	**	**	**	***	ns	**	-	-

Data is presented as mean ± standard deviation. **, and *** indicate significant differences at $p < 0.01$, and $p < 0.001$ respectively. Abbreviations of bread samples can be found in Figure 16.

6 SUMMARY AND CONCLUSION

This thesis highlighted three valorization approaches, including a fractionation of reed and hemp biomasses followed by a detailed clarification of their chemical heterogeneity (Study **I**), subsequent extraction optimization of green needles (Study **II**), and technological application of green needle and twig fractions (Study **III**). Additionally, the research provided new insights into the effects of extraction techniques on the properties of side streams from plant species relevant to Finland. An improved understanding of fundamental processing aspects was expected to open possibilities for utilizing these biomasses in biorefinery and food systems, provide knowledge for further purification/conversion, and identify a desired extraction-based biorefining approach.

Study **I** investigated the influence of plant fraction, harvesting time, and treatment parameters on extractives, TPC, and bioactivities of reed and hemp biomasses. Overall, results showed that oil hemp and reed canary grass were the most promising biomasses when exploring the lipophilic and hydrophilic fractions. As expected, utilizing a two-stage PHWE allowed for the isolation of extractives at lower temperatures, followed by the sequential extraction of hemicelluloses at higher temperatures. Oil hemp and reed canary grass showed overall potential regarding chemical composition; common reed and reed canary grass had higher antioxidant activity, while all the biomasses showed promising antibacterial properties. Each extract showed unique carbohydrate profiles in both composition and concentration.

This study is the first to provide a detailed foundation of the extractive content and composition of biomass fractions obtained from marginal lands and peatlands, forming the basis for future research. The potential for increasing the recovery yields of valuable compounds was also explored by bringing the harvest time forward. From a biorefining perspective, the harvest time indeed affects the extractable substances. Since compounds such as sugars, sterols, and fatty acids are present in higher concentrations during summer and hold significant value for potential commercial applications, harvesting immature plants may be a worthwhile strategy to capitalize on these elevated levels. From an economic point of view, valorization of hemicelluloses and other key constituents offers an opportunity for forest industries to create new revenue streams by enabling their extraction as by-products from existing pulp and paper processes. Insights from this study can support pulp and paper mills in transitioning to biorefineries by optimizing recovery methods that are aligned with their specific applications and existing operational setups.

In Study **II**, RSM was used to model the effects of extraction time and temperature on chemical composition and bioactivity of spruce needle-rich

fraction extracted with water and aqueous ethanol. The best condition was achieved at 120 °C and 10 min when using water, and 125 °C and 68 min for ethanol/water. This study provides a basis for future studies concentrated on isolating specific components from green needles and logging residue. The optimized extraction conditions can also be scaled up for industrial use. However, the large-scale production of biomass and the purification steps present challenges that need to be addressed before the efficient commercial-scale use of bioactive compounds becomes feasible.

In Finland, conifer-based side streams are the most abundantly available biomass compared to reed and hemp, offering substantial potential for food applications. After a thorough evaluation to identify the most promising raw materials, spruce, pine, and fir green needle and twig extracts were selected as water substitutes in a bread formulation. Study **III** provided a theoretical and practical foundation for product development, highlighting the innovative, value-added use of underutilized forest resources. A total of 115 compounds were identified in the NT extracts and bread samples, with selected compounds remaining stable throughout baking and storage. The NT-bread significantly increased antioxidant activity, total phenolic content, and the total sum of several compounds after three days of storage. Among the tentatively identified compounds were flavonoids, phenolic acids, alkaloids, stilbenes, lignans, resin acids, and gibberellins. Among the substitution levels, a 35% fortification was sufficient to improve functionality, extend shelf-life, and maintain nutritional and textural properties, while also enhancing overall acceptability and purchase intent. Of all formulations, PNT35 exhibited the best overall balance of these qualities, positioning it as a strong candidate for further development as a bioactive, consumer-friendly product. Beyond enhancing bread quality, the extractives in these conifer extracts may serve as natural preservatives. Additionally, a semi-industrial-scale HC pilot device was chosen for its flexibility and proven scalability in the extraction process, contributing to the process chain's feasibility and affordability and making it valuable for food production.

This thesis demonstrated the potential for scaling up and commercializing the valorized plant extracts, which could contribute significant industrial and societal benefits. Future work should focus on further evaluating technical feasibility, sustainability, and business modeling for practical applications of the derived fractions. Building on the demonstrated bioactivity, future research should explore the specific applications of polyphenol-rich complexes and assess their potential for functional ingredient development, including safety, gut microbiota modulation, and cellular antioxidant activity and protection.

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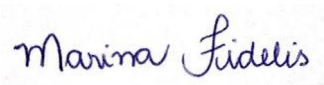
Espoo, July 2024



Marina Fidelis

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APPENDIX: ORIGINAL PUBLICATIONS

I. Reprinted from *Sustainability*, 1, 2202-2223, with permission from Royal Society of Chemistry, an open access article published under the terms of the Creative Commons (CC-BY 3.0) licence.

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DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

1. **REINO R. LINKO (1967)** Fatty acids and other components of Baltic herring flesh lipids. (Organic chemistry).
2. **HEIKKI KALLIO (1975)** Identification of volatile aroma compounds in arctic bramble, *Rubus arcticus* L. and their development during ripening of the berry, with special reference to *Rubus stellatus* SM.
3. **JUKKA KAITARANTA (1981)** Fish roe lipids and lipid hydrolysis in processed roe of certain *Salmonidae* fish as studied by novel chromatographic techniques.
4. **TIMO HIRVI (1983)** Aromas of some strawberry and blueberry species and varieties studied by gas liquid chromatographic and selected ion monitoring techniques.
5. **RAINER HUOPALAHTI (1985)** Composition and content of aroma compounds in the dill herb, *Anethum graveolens* L., affected by different factors.
6. **MARKKU HONKAVAARA (1989)** Effect of porcine stress on the development of PSE meat, its characteristics and influence on the economics of meat products manufacture.
7. **PÄIVI LAAKSO (1992)** Triacylglycerols – approaching the molecular composition of natural mixtures.
8. **MERJA LEINO (1993)** Application of the headspace gas chromatography complemented with sensory evaluation to analysis of various foods.
9. **KAISLI KERROLA (1994)** Essential oils from herbs and spices: isolation by carbon dioxide extraction and characterization by gas chromatography and sensory evaluation.
10. **ANJA LAPVETELÄINEN (1994)** Barley and oat protein products from wet processes: food use potential.
11. **RAIJA TAHVONEN (1995)** Contents of lead and cadmium in foods in Finland.
12. **MAIJA SAXELIN (1995)** Development of dietary probiotics: estimation of optimal *Lactobacillus* GG concentrations.
13. **PIRJO-LIISA PENTTILÄ (1995)** Estimation of food additive and pesticide intakes by means of a stepwise method.
14. **SIRKKA PLAAMI (1996)** Contents of dietary fiber and inositol phosphates in some foods consumed in Finland.
15. **SUSANNA EEROLA (1997)** Biologically active amines: analytics, occurrence and formation in dry sausages.
16. **PEKKA MANNINEN (1997)** Utilization of supercritical carbon dioxide in the analysis of triacylglycerols and isolation of berry oils.
17. **TUULA VESA (1997)** Symptoms of lactose intolerance: influence of milk composition, gastric emptying, and irritable bowel syndrome.
18. **EILA JÄRVENPÄÄ (1998)** Strategies for supercritical fluid extraction of analytes in trace amounts from food matrices.
19. **ELINA TUOMOLA (1999)** *In vitro* adhesion of probiotic lactic acid bacteria.
20. **ANU JOHANSSON (1999)** Availability of seed oils from Finnish berries with special reference to compositional, geographical and nutritional aspects.
21. **ANNE PIHLANTO-LEPPÄLÄ (1999)** Isolation and characteristics of milk-derived bioactive peptides.
22. **MIKA TUOMOLA (2000)** New methods for the measurement of androstenone and skatole – compounds associated with boar taint problem. (Biotechnology).
23. **LEEA PELTO (2000)** Milk hypersensitivity in adults: studies on diagnosis, prevalence and nutritional management.
24. **ANNE NYKÄNEN (2001)** Use of nisin and lactic acid/lactate to improve the microbial and sensory quality of rainbow trout products.
25. **BAORU YANG (2001)** Lipophilic components of sea buckthorn (*Hippophaë rhamnoides*) seeds and berries and physiological effects of sea buckthorn oils.
26. **MINNA KAHALA (2001)** Lactobacillar S-layers: Use of *Lactobacillus brevis* S-layer signals for heterologous protein production.
27. **OLLI SJÖVALL (2002)** Chromatographic and mass spectrometric analysis of non-volatile oxidation products of triacylglycerols with emphasis on core aldehydes.
28. **JUHA-PEKKA KURVINEN (2002)** Automatic data processing as an aid to mass spectrometry of dietary triacylglycerols and tissue glycerophospholipids.
29. **MARI HAKALA (2002)** Factors affecting the internal quality of strawberry (*Fragaria x ananassa* Duch.) fruit.
30. **PIRKKA KIRJAVAINEN (2003)** The intestinal microbiota – a target for treatment in infant atopic eczema?
31. **TARJA ARO (2003)** Chemical composition of Baltic herring: effects of processing and storage on fatty acids, mineral elements and volatile compounds.
32. **SAMI NIKOSKELAINEN (2003)** Innate immunity of rainbow trout: effects of opsonins, temperature and probiotics on phagocytic and complement activity as well as on disease resistance.
33. **KAISA YLI-JOKIPII (2004)** Effect of triacylglycerol fatty acid positional distribution on postprandial lipid metabolism.
34. **MARIKA JESTOI (2005)** Emerging *Fusarium*-mycotoxins in Finland.
35. **KATJA TIITINEN (2006)** Factors contributing to sea buckthorn (*Hippophaë rhamnoides* L.) flavour.

36. **SATU VESTERLUND (2006)** Methods to determine the safety and influence of probiotics on the adherence and viability of pathogens.
37. **FANDI FAWAZ ALI IBRAHIM (2006)** Lactic acid bacteria: an approach for heavy metal detoxification.
38. **JUKKA-PEKKA SUOMELA (2006)** Effects of dietary fat oxidation products and flavonols on lipoprotein oxidation.
39. **SAMPO LAHTINEN (2007)** New insights into the viability of probiotic bacteria.
40. **SASKA TUOMASJUKKA (2007)** Strategies for reducing postprandial triacylglycerolemia.
41. **HARRI MÄKIVUOKKO (2007)** Simulating the human colon microbiota: studies on polydextrose, lactose and cocoa mass.
42. **RENATA ADAMI (2007)** Micronization of pharmaceuticals and food ingredients using supercritical fluid techniques.
43. **TEEMU HALTTUNEN (2008)** Removal of cadmium, lead and arsenic from water by lactic acid bacteria.
44. **SUSANNA ROKKA (2008)** Bovine colostral antibodies and selected lactobacilli as means to control gastrointestinal infections.
45. **ANU LÄHTEENMÄKI-UUTELA (2009)** Foodstuffs and medicines as legal categories in the EU and China. Functional foods as a borderline case. (Law).
46. **TARJA SUOMALAINEN (2009)** Characterizing *Propionibacterium freudenreichii* ssp. *shermanii* JS and *Lactobacillus rhamnosus* LC705 as a new probiotic combination: basic properties of JS and pilot *in vivo* assessment of the combination.
47. **HEIDI LESKINEN (2010)** Positional distribution of fatty acids in plant triacylglycerols: contributing factors and chromatographic/mass spectrometric analysis.
48. **TERHI POHJANHEIMO (2010)** Sensory and non-sensory factors behind the liking and choice of healthy food products.
49. **RIIKKA JÄRVINEN (2010)** Cuticular and suberin polymers of edible plants – analysis by gas chromatographic-mass spectrometric and solid state spectroscopic methods.
50. **HENNA-MARIA LEHTONEN (2010)** Berry polyphenol absorption and the effect of northern berries on metabolism, ectopic fat accumulation, and associated diseases.
51. **PASI KANKAANPÄÄ (2010)** Interactions between polyunsaturated fatty acids and probiotics.
52. **PETRA LARMO (2011)** The health effects of sea buckthorn berries and oil.
53. **HENNA RÖYTIÖ (2011)** Identifying and characterizing new ingredients *in vitro* for prebiotic and synbiotic use.
54. **RITVA REPO-CARRASCO-VALENCIA (2011)** Andean indigenous food crops: nutritional value and bioactive compounds.
55. **OSKAR LAAKSONEN (2011)** Astringent food compounds and their interactions with taste properties.
56. **ŁUKASZ MARCIN GRZEŚKOWIAK (2012)** Gut microbiota in early infancy: effect of environment, diet and probiotics.
57. **PENGZHAN LIU (2012)** Composition of hawthorn (*Crataegus* spp.) fruits and leaves and emblic leafflower (*Phyllanthus emblica*) fruits.
58. **HEIKKI ARO (2012)** Fractionation of hen egg and oat lipids with supercritical fluids. Chemical and functional properties of fractions.
59. **SOILI ALANNE (2012)** An infant with food allergy and eczema in the family – the mental and economic burden of caring.
60. **MARKO TARVAINEN (2013)** Analysis of lipid oxidation during digestion by liquid chromatography-mass spectrometric and nuclear magnetic resonance spectroscopic techniques.
61. **JIE ZHENG (2013)** Sugars, acids and phenolic compounds in currants and sea buckthorn in relation to the effects of environmental factors.
62. **SARI MÄKINEN (2014)** Production, isolation and characterization of bioactive peptides with antihypertensive properties from potato and rapeseed proteins.
63. **MIKA KAIMAINEN (2014)** Stability of natural colorants of plant origin.
64. **LOTTA NYLUND (2015)** Early life intestinal microbiota in health and in atopic eczema.
65. **JAAKKO HIIDENHOVI (2015)** Isolation and characterization of ovomucin – a bioactive agent of egg white.
66. **HANNA-LEENA HIETARANTA-LUOMA (2016)** Promoting healthy lifestyles with personalized, *APOE* genotype based health information: The effects on psychological-, health behavioral and clinical factors.
67. **VELI HIETANIEMI (2016)** The *Fusarium* mycotoxins in Finnish cereal grains: How to control and manage the risk.
68. **MAARIA KORTESNIEMI (2016)** NMR metabolomics of foods – Investigating the influence of origin on sea buckthorn berries, *Brassica* oilseeds and honey.
69. **JUHANI AAKKO (2016)** New insights into human gut microbiota development in early infancy: influence of diet, environment and mother's microbiota.
70. **WEI YANG (2017)** Effects of genetic and environmental factors on proanthocyanidins in sea buckthorn (*Hippophaë rhamnoides*) and flavonol glycosides in leaves of currants (*Ribes* spp.).
71. **LEENAMAIJA MÄKILÄ (2017)** Effect of processing technologies on phenolic compounds in berry products.
72. **JUHA-MATTI PIHLAVA (2017)** Selected bioactive compounds in cereals and cereal products – their role and analysis by chromatographic methods.

73. **TOMMI KUMPULAINEN (2018)** The complexity of freshness and locality in a food consumption context
74. **XUEYING MA (2018)** Non-volatile bioactive and sensory compounds in berries and leaves of sea buckthorn (*Hippophaë rhamnoides*)
75. **ANU NUORA (2018)** Postprandial lipid metabolism resulting from heated beef, homogenized milk and interesterified palm oil.
76. **HEIKKI AISALA (2019)** Sensory properties and underlying chemistry of Finnish edible wild mushrooms.
77. **YE TIAN (2019)** Phenolic compounds from Finnish berry species to enhance food safety.
78. **MAIJA PAAKKI (2020)** The importance of natural colors in food for the visual attractiveness of everyday lunch.
79. **SHUXUN LIU (2020**) Fermentation with non-*Saccharomyces* yeasts as a novel biotechnology for berry wine production.
80. **MARIKA KALPIO (2020)** Strategies for analyzing the regio- and stereospecific structures of individual triacylglycerols in natural fats and oils.
81. **JOHANNA JOKIOJA (2020)** Postprandial effects and metabolism of acylated anthocyanins originating from purple potatoes.
82. **NIINA KELANNE (2021)** Novel bioprocessing for increasing consumption of Nordic berries.
83. **NIKO MARKKINEN (2021)** Bioprocessing of berry materials with malolactic fermentation.
84. **GABRIELE BELTRAME (2021)** Polysaccharides from Finnish fungal resources.
85. **SALLA LAITO (2022)** Bioactive compounds in oats and gut health.
86. **KANG CHEN (2022)** Multi-omics study on the effects of anthocyanin extracts from bilberries and purple potatoes on type 2 diabetes in Zucker diabetic fatty rats.
87. **WENJIA HE (2022)** Bioprocessing of alcoholic beverages from apples and pears: Effects of raw materials and processes on quality.
88. **TANJA KAKKO (2023)** Alternative approaches to improve the processing and quality of under-utilized fish.
89. **MIKAEL FABRITIUS (2023)** Mass spectrometric methodologies for analysis of triacylglycerol and phospholipid regioisomers in natural fats and oils.
90. **ELLA AITTA (2023)** Green technologies for the extraction of oil and protein from Baltic herring (*Clupea harengus membras*).
91. **AMRUTA KULKARNI (2023)** Effect of omega-3 deficiency and positional distribution of docosahexaenoic acid in triacylglycerols on tissue lipids in rats.
92. **LIZ A. GUTIÉRREZ QUEQUEZANA (2023)** Effect of cultivar, growth environment and developmental stage on phenolic compounds and ascorbic acid in potato tubers grown in Finland.
93. **MINNA ROTOLA-PUKKILA (2024)** The umami compounds in Nordic food raw materials and the effect of cooking.
94. **EIJA AHONEN (2024)** Impact of lipid structure and selected antioxidants on the oxidation of docosahexaenoic acid.
95. **MARINA FIDELIS (2024)** Valorization of underutilized biomass for biorefinery and food applications: Exploring the processing, plant material composition, bioactivity, and fortified bread models.

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