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Extractives of Tree Biomass of Scots Pine (*Pinus sylvestris* L.) for Biorefining in Four Climatic Regions in Finland—Lipophilic Compounds, Stilbenes, and Lignans

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Abstract: The aim of the study was to quantify total extractive contents and lipophilic compounds, stilbenes, and lignans in Scots pine stem wood, stem bark, branch biomass, and sawmill residues in four climatic regions of Finland to evaluate the most optimal sources of extractives for bio-based chemical biorefining and bioenergy products. Data were derived from 78 chip samples from the before-mentioned raw materials, the samples being pooled by tree height position from the sample trees of 42 experimental forest stands, and sawdust lots from 10 log stands. Accelerated solvent extraction (ASE) was employed to determine total extractive contents, followed by gas chromatography with flame ionization detection (GC–FID) to quantify extractive groups and gas chromatography-mass spectrometry (GC–MS) to analyse individual extractive compounds. Resin acids and triglycerides followed by fatty acids were the dominant extractive groups. Resin acids were most abundant in stem wood from final fellings and in sawdust, fatty acids in bark and branch biomass, and triglycerides also in stem wood from thinnings and the top parts of trees. Of the minor extractive groups, stilbenes were the most abundant in stem wood from final fellings and in sawdust, and steryl esters, sterols, and lignans in bark and branch biomass, the two last groups almost missing from other biomass components. Regional differences in the contents of extractive groups were generally small, $1.0-1.5$ percentage points at the maximum, but factor analysis distinguished northern and southern regions into their own groups. Bark was the most potential source of fatty acids and sterols in southern Finland, and triglycerides and steryl esters in northern Finland. In stem wood, steryl esters, triglycerides, and lignans decreased and stilbenes increased from north to south. Certain fatty acids and resin acids were more frequent in the north. The results highlighted the importance of focused procurement and efficient sorting of raw materials, purity, unique properties, and feasible isolation techniques for competitive ability as well as large raw material volumes or well-defined value-added products.

Keywords: *Pinus* sp.; wood extractives; biorefining; regional effects; climatic effects; stem wood; bark; branch biomass; sawmill residues

1. Introduction

The current industrial policies in Finland and Europe aim toward sustainable circular bioeconomy, including novel technologies and value-added products to forest biomass valorisation with eco-design, technoeconomic, or bioactive functionalities [1,2]. Forest

biomass is an important feedstock to fulfil the demand of novel biomaterials, biofuels, and biorefinery products, along with the more traditional solid wood products, wood composite panels, pulp and paper, and forest energy [3–5].

While the structural high molecular weight biopolymers (cellulose, hemicelluloses, lignin) overwhelmingly dominate the chemical composition of forest biomass, the other polymeric components (pectins, starch, proteins) and numerous non-structural low-molecular weight components occur in limited amounts [6,7]. In the latter group, wood extractives (secondary metabolites), which are commonly divided to phenolic, aliphatic and other compounds are the most abundant, their composition varying between tree species, individuals and parts (stem, root, branches, bark), wood type (sapwood or heartwood), as well as environmental growth conditions and genetic background [8–17]. Most extractives of coniferous species are lipophilic that dissolve in fats, oils, lipids, and non-polar solvents, such as hexane or toluene, whereas hydrophilic compounds that dissolve in water and other hydrophilic substances occur in small amounts [8]. The lipophilic portion contains fats and fatty acids, sterols, and steryl esters, terpenes, terpenoids, resin acids, and waxes [6–8]. Different compounds can be extracted from different lignocellulosic materials using different solvents depending on their nature of polarity [5,9].

Extractive components of wood raw materials have not been in the core of chemical wood processing in the past, mostly for economic reasons [9,10,18], but now their potential for novel value-added products of biorefining is commonly acknowledged [3,4,14,19]. Accordingly, a diversity of applications was suggested during the 2000s, as listed by the recent studies [3,14]. However, efficient sourcing of the interesting wood biomass fractions for extractives has remained an open question, as it was indicated by Verkasalo et al. [19].

In coniferous species, the highest extractive contents have been observed in internal knots, branch biomass and stumps followed by bark, and the last, in stem wood [8,13,14,16]. Wood type has been observed as the most significant source of variation, heartwood having a larger total extractive content than sapwood [8,14,19,20]. Therefore, the content should decrease from the butt toward the top and horizontally from the pith toward the bark in coniferous trees, and it should stay between the values of sapwood and heartwood in sawmill and veneer mill residues [19,20].

For the supply of extractives, the roles of climatic region and geographic location are still unclear, although at general level they have been demonstrated as factors for wood chemistry along with soil, stand, and tree attributes, as well as forest regeneration and management practices [12,15,20–22]. Total extractive contents of coniferous species have been observed to grow toward north in northern Europe [20–22] and Canada [23]. However, the geographic trends are not uniform as regards the different groups of extractives. For most phenolic and resin acid compounds of Scots pine in Finland, the highest mean and maximum contents were observed in the northern regions, the highest contents of lignans, neolignans, and pinosylvin monomethyl ether glycosides in Lapland, but those of pinosylvins and vanillic acid in southern Finland [19]. No general statistically significant trend in the north–south axis or significant latitudinal effects connected to effective thermal sum were shown, but northern and southern regions could be statistically distinguished to their own geographic groups. Benefits for northern locations were observed also in the studies on other plant species regarding the biosynthesis of some secondary metabolites [24–27].

Scots pine (*Pinus sylvestris* L.) is the most abundant natural tree species in Finland with the generally highest extractive content. According to different reviews, the total extractive percentage in Scots pine biomass is typically as follows: stem wood 3%–5%, stump 19%, roots 13%, bark 25%, branches 17%, needles 40% [12–16]. As high a variation as 1.0%–6.8% in the average values has been shown in different studies [28]. In a European context, the average value of Scots pine is between the values of typical low-extractive species of Norway spruce (1.7%) and high-extractive species of Douglas fir (5.3%).

Accordingly, there is a long history of large-volume extractive products based on pine tar since the Middle Ages and tall oil and turpentine since the industrialization era,

but also a high current and future interest for exploitation of a multitude of extractives for a variety of novel biorefinery products [4–6,12,19]. In addition to using tall oil in liquid fuels, tall oil fatty acids are processed into alkyd resins, adhesives, soaps, protective coatings, and detergents, whereas resin acids have been used in synthetic rubber and in printability enhancers, for example [29–32]. Coniferous resins have been extracted for industrial purposes back to the 1960s, and for traditional products from the 4th to 5th century [9,10].

Resin acids and thereafter sterols, stilbenes, lignans, and flavonoids are the main extractive compounds of Scots pine with known bioactive effects [9,14–16,33–35]. Resin acids are used for perfumed compounds for cosmetics, additives for food and beverages, antimicrobial food protection, and biomedical applications [14,33–35]. Sterols are used for nutraceutical foods (cholesterol lowering, antioxidative effects) and selected cosmetics applications [5,33–35].

Tree biomass components that are rich in stilbenes or lignans have been proposed for industrial utilization for a wide variety of preservatives due to their antioxidative, antifungal, and antibacterial properties [5,16,21,36]. Stilbenes of pine species have been introduced as effective agents in wood treatment and processing, but they have so far been used only in a limited scale (surface treatment, impregnation, gluing) [15,16,36,37]. Lignans are already in use for medical purposes, such as cancer treatments, weight control and prevention of cardiological diseases [15,16,38].

The objective of this study was to quantify the total extractive content and examine systematically the composition and variation of lipophilic extractives, stilbenes, and lignans in the above-ground biomass (stem wood at three height positions; stem bark; branch biomass) and sawmill residues (sawdust) of Scots pine in four climatic regions in Finland. The differences between final-felling and thinning stands and forest site types were considered as well. The purpose was to identify and quantify the before-mentioned extractives in the raw materials to find the most optimal sources, support the procurement, and provide the basis for product and technology development toward utilization in bio-based chemical biorefining and bioenergy generation. The study continued the general mapping of extractive compounds and complemented the analysis of phenolic and resin acid extractives of Verkasalo et al. [19], based on the same raw material sampling.

2. Materials and Methods

2.1. Tree Biomass Sampling

The experimental design consisted of four geographic regions from north to south in Finland: 1. Lapland North (LN), 2. Lapland South (LS), 3. Middle Finland (MF), 4. South Finland (SF) (Figure 1). The design was based on increasing gradients of effective thermal sum and annual precipitation from north to south, with the range of individual forest stands from 537 to 1564 d.d. and 527 to 664 mm, respectively. The experimental stands were located the lower above sea level the more southerly the region was, the range being from 81 to 320 m. A detailed description of the climatic and environmental characteristics of the study regions and experimental stands was presented by Verkasalo et al. [19].

In each region, eight experimental stands were sampled (exception: four stands in Middle Finland), four of them being final-felling stands (mature development stage) and four thinning stands (productive development stage) each. The stand types represented different sources and harvesting conditions of Scots pine, with typically different tree age and heartwood content that have effects on the occurrence and composition of extractives [12,14,19,28]. The tree stock in all stands was of natural origin. Forest site types (fertility levels) were typical for Scots pine in each region, ranging from poor sites (*Calluna* type, CT) to fertile sites (*Oxalis-Myrtillus* type, OMT) [39].

In each stand, five study trees were selected and cut for the study from February to March 2014. The aim was to catch the sample during the dormancy period to provide a uniform physiological stage for all sample trees. For the trees representing the final-felling stage, the age criterion was ca. 90 years or older in Lapland and ca. 70 years or older

in Middle and South Finland, respectively. For the trees in thinning stage, the age was aimed at ca. 30 to 80 years in Lapland and ca. 20 to 60 years in Middle and South Finland, respectively. In each stand, the sample trees were selected to cover the full diameter range of saw-log sized trees that provided conventional and/or small-diameter logs (diameter at breast height, $d_{1.3} \geq 14$ cm).
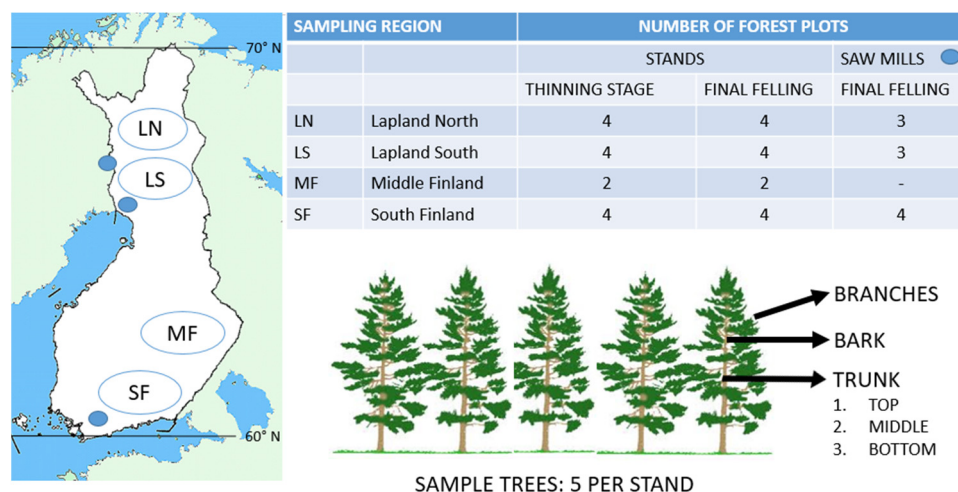

SAMPLING REGION		NUMBER OF FOREST PLOTS		
		STANDS		SAW MILLS
		THINNING STAGE	FINAL FELLING	FINAL FELLING
LN	Lapland North	4	4	3
LS	Lapland South	4	4	3
MF	Middle Finland	2	2	-
SF	South Finland	4	4	4

BRANCHES
BARK
TRUNK
1. TOP
2. MIDDLE
3. BOTTOM

SAMPLE TREES: 5 PER STAND

Figure 1. Experimental design, location, and the number of the sampling sites in Finland are shown in the graph. Location of the sawmills is indicated with blue spots on the map.

Tree age, diameter at 1.3 m height and total height were measured of each sample tree in the forest. Basic physical characteristics of stem wood were determined in the laboratory from disc samples cut from the cross-cut points of the stem: growth rate and latewood proportion, heartwood proportion, moisture content, basic density. The measurement methods are described, and the basic statistics of tree and wood properties of sample trees are shown by region and stand development stage in Verkasalo et al. [19].

In the final cutting stands, bolts of 1.5-m length in the butt and two-meter length in the middle and top of the merchantable stem part were cross-cut from each sample tree. Thus, three vertical stem parts per tree were taken to the study material. In the thinning-stage stands, only one 3-m butt bolt was cut from each tree. The bolts were debarked manually and immediately crushed to wood chips in outdoor conditions by mobile truck-based chippers within 2–4 weeks from the cross-cutting of bolts, however, during the period when the temperature stayed above zero Celsius.

Branch biomass without needles was collected from the living crown of each sample tree and crushed to chips by garden shredders at the same time as the bolts. We aimed at totals of 400 litres of stem wood chips and branch biomass chips each, from each forest plot. During debarking, the bark was collected separately from the bolts of all sample trees and pooled together by experimental stand, aiming at the samples of 40–50 litres each.

All chip and bark samples were packed immediately after crushing into airproof plastic bags (80 litres). All materials were stored during a period of 1–3 months in a freezer at −20 °C before further measurement and analysis in the research laboratories of Finnish Forest Research Institute (Metla), Vantaa Research Unit (nowadays a part of Natural Resources Institute Finland Luke).

Sawmill residues were sampled from similar regions as the sample trees, in collaboration with three sawmills (Figure 1). Sawdust samples of ca. 10 litres and sawmill chip samples of ca. 50 litres were taken from the flow of wood residues after primary sawing of fresh logs from 10 final-felling stands, their origin being individually identified and location, forest site type, and tree age recorded before cutting. The samples were packed and stored in the same way as those collected from the forest. Descriptive data on the origin of sawmill residue samples are available in Verkasalo et al. [19].

2.2. Chemical Analyses

For the laboratory analyses, wood chip samples from the final-felling stands were pooled by tree height position from the sample trees of each stand. The pooled samples, each 2.5 litres, consisted of 0.5 litres of wood chips from the respective height position in each sample tree, accordingly, three wood chip samples were obtained per stand, "butt", "middle", and "top". In the thinning-stage stands, wood chip samples were pooled from the five sample trees of each stand, resulting also in the samples of 2.5 litres. Of the sawmill residue samples, only saw dust of 2.5 litres per each sample was used in this study, whereas sawmill chips were allocated for further studies.

Before extractive analyses, 30 g of each freeze-dried sample were ground using a one mm sieve. The ground samples were freeze-dried again after milling and 1 g of each sample was taken for accelerated solvent extraction (ASE-350, Dionex Corporation, Sunnyvale, CA, USA). Samples were extracted with acetone/water (95/5, *v/v*) 3 × 15 min at 100 °C. Extracts were collected and diluted to 100 mL. Samples were transferred to closed storage bottles that were further sealed with parafilm and stored in a freezer at −21 °C. Extracted sample was collected, freeze-dried, and weighted to calculate mass loss and determine total extractive contents.

The amount of extract used in the further analyses was dependent on the sample material. A sample of 1.0 mL was taken from stem wood and sawmill chip samples, and a sample of 0.5 mL from branch biomass and bark samples. Internal standards (ISTD) were diluted into methyl-*tert*-butyl ether (MTBE) and 2 mL of it was added to the samples. The internal standard contained 0.02 mg/mL of heneicosanoic acid, betulin, cholesterol heptadecanoate, and 1,3-dipalmitoyl-2-oleoylglycerol. Samples were then dried in nitrogen flow at 50 °C until the solvent was evaporated. Samples were further dried in a vacuum oven for 15 min at 40 °C. After drying, samples were silylated for GC analyses by adding 100 μL of pyridine, 100 μL of bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 50 μL of trimethylchlorosilane (TMCS). Silylated samples were then heated at 70 °C for 20 min and samples were transferred to GC vials.

Extractive groups were quantified using GC–FID short column gas chromatography (Shimadzu GC-2010, Kyoto, Japan) with wide bore HP-1 column (length 7 m, 0.53 mm i.d., 0.1 μm film thickness), according to the protocol of Örså and Holmbom [40]. Samples were injected by temperature-programmed splitless PTV (1.0 μL) injection (50 °C for 0.5 min, 50 °C → 200 °C/min and 200 °C → 340 °C), where the temperature was kept for 18 min. Hydrogen was used as carrier gas and during the run, temperature was initially 100 °C for 1.5 min, temperature increased 12 °C/min → 325 °C and kept there for 6 min. FID detector temperature was 325 °C.

Individual extractive compounds were analysed on the silylated samples using GC–MS gas chromatography mass spectrometry instrument (HP 6890-5973, Palo Alto, CA, USA) as described by Raitanen et al. [34]. The GC column was a HP-5 column (Agilent Technologies, Inc., Santa Clara, CA, USA; 30 m × 0.25 mm i.d., film thickness 0.25 μm). The injector and MS interface temperatures were kept at 280 and 300 °C, respectively. Helium was used as carrier gas and the injection was made in a splitless mode. Mass spectra were obtained in EI mode (70 eV), and the fragmentation pattern was compared to the standards in commercial libraries (Wiley11) as well as to the standards available in our own MS libraries.

2.3. Statistical Analyses

Statistical analyses of the data were performed using the IBM® SPSS® Statistics program, Version 26 (2019). Significance levels of 0.01 (**) and 0.05 (*) were used in all statistical tests. Principal component analysis (PCA) was used to identify the common dimensions of pattern and structure of the occurrence of extractive compound groups both in stem wood samples and in all tree biomass components together. Linear relationships between the studied variables were analysed with Pearson's correlation coefficients.

The normality of distribution of the content of each compound was tested using Kolmogorov–Smirnov (K–S) test. According to the K–S test, the assumption of a normal distribution was realized only for triglycerides (Sig. > 0.05). Therefore, log transformation was used for all but triglycerides to normalize the skewed distributions. Contents of compound groups and individual compounds were compared between material sources and geographic regions with GLM multivariate analysis. The pairwise comparisons between different geographic regions or tree biomass components were made via Tukey's test.

3. Results

3.1. Amounts of Extractives

3.1.1. Totals of Extractives

Total amount of extractives and thereafter compound groups and individual compounds of resin acids, triglycerides, fatty acids, lignans, sterols, steryl esters, and stilbenes were analysed on the above-ground biomass components of Scots pine. Total extractive content covered both lipophilic and hydrophilic extractives within the detection accuracy and extraction capacity of the accelerated solvent extraction technology (ASE).

The total extractive content of Scots pine wood was, on average, higher in the butt and in three regions in the top than in the middle of the trees in the final-felling stands, or in the stem wood in thinning stands, where it was the lowest (Figure 2). The total extractive content of sawdust was almost equal to that of stem wood from the middle parts of the trees in final-felling stands. The highest total extractive content was observed in bark, while in branch biomass it did not differ from stem wood of final-felling stands.
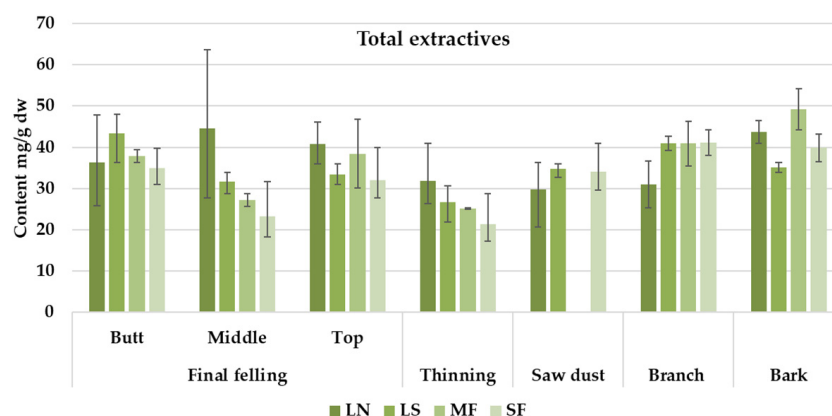

Figure 2. Total contents of extractives in the tree biomass components in the four regions: Lapland North (LN), Lapland South (LS), Middle Finland (MF), South Finland (SF). Means and ranges of variation. dw: dry weight.

Regional differences were observed in the total extractive content of stem wood from both final-felling and thinning stands, with the contents generally decreasing from north to south. No regional differences in total extractive content were observed in sawdust or branch biomass. In bark, clear differences were found between the regions, the extractive content being the largest in Middle Finland and the smallest in Lapland South.

3.1.2. Compound Groups and Individual Compounds

The resin acid content was higher in the stem wood of final-felling trees than in other types of biomass material (Figure 3). Within the final-felling trees the amount of resin acids decreased from the butt to the top. Similar to the total extractive content, regional differences were observed in the first hand in the middle of the stems in final-felling stands, showing a decreasing trend from north to south. In addition, a small increase was observed in bark and branch biomass in the same geographic direction. The most common resin acids were dehydroabietic acid and abietic acid, other resin acids being palustric, pimaric, isopimaric, sandaracopimaric, neoabietic, and levopimaric acids (Table 1).
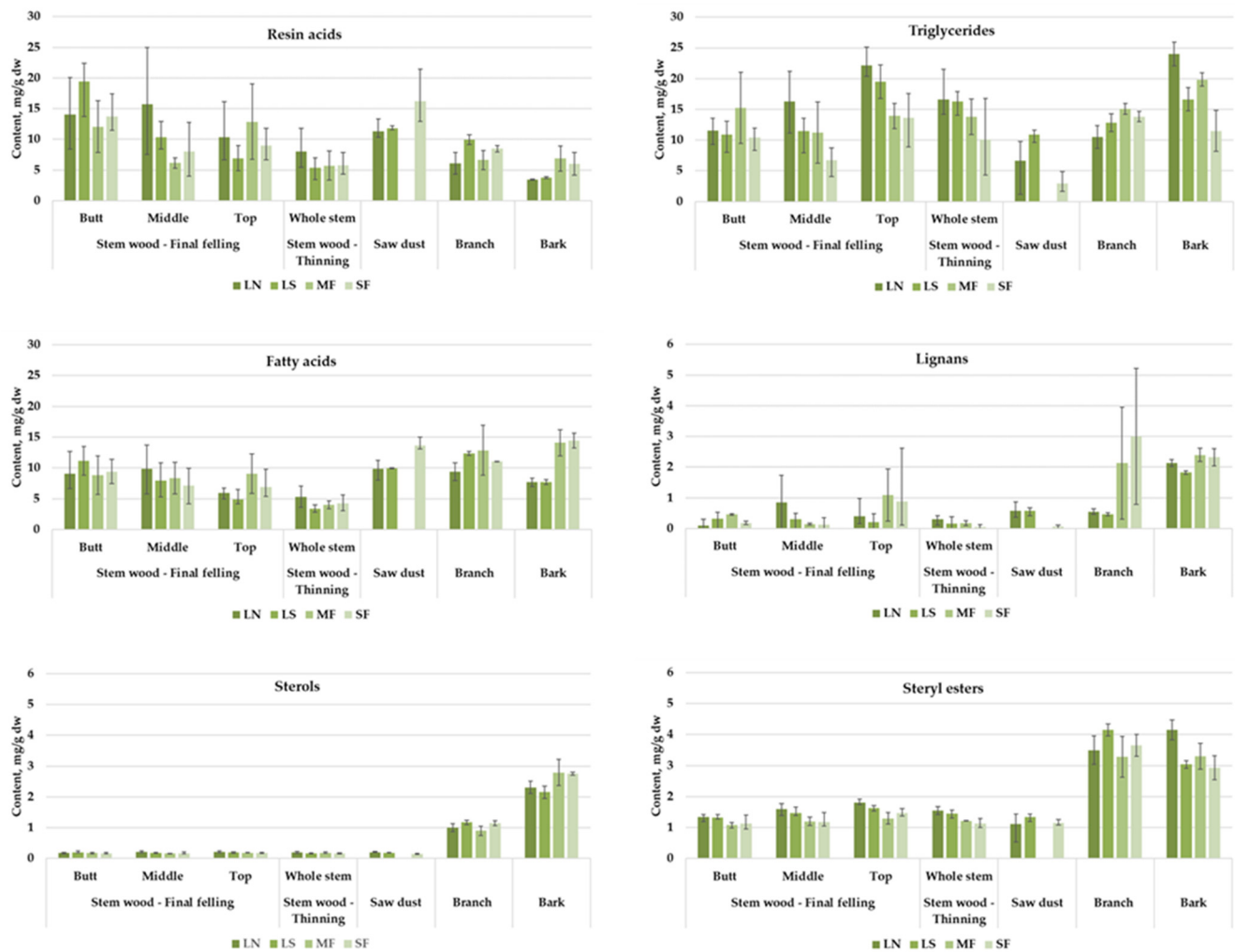
Figure 3. Contents of six major extractive groups in the tree biomass components in the four regions: Lapland North (LN), Lapland South (LS), Middle Finland (MF), South Finland (SF). Means and ranges of variation. dw: dry weight.

Table 1. Average contents of individual extractive compounds in the tree biomass components in the four regions: Lapland North (LN), Lapland South (LS), Middle Finland (MF), and South Finland (SF). dw: dry weight.

Compound mg/g dw		Stem Wood—Final Felling			Stem Wood—Thinning	Sawdust	Branch Biomass	Bark
		Butt	Middle	Top				
FATTY ACIDS								
Palmitic acid 16:0	LN	0.660	0.542	0.514	0.342	0.227	1.137	1.104
	LS	0.441	0.437	0.505	0.339	0.265	0.563	0.908
	MF	0.384	0.319	0.371	0.376		0.525	0.955
	SF	0.226	0.144	0.194	0.216	0.350	0.527	0.866
Margaric acid 17:0	LN	0.162	0.143	0.149	0.098	0.030	0.275	0.260
	LS	0.129	0.152	0.151	0.120	0.044	0.111	0.232
	MF	0.121	0.097	0.165	0.183		0.103	0.178
	SF	0.041	0.028	0.036	0.078	0.104	0.127	0.211

Table 1. *Cont.*

Compound mg/g dw		Stem Wood—Final Felling			Stem Wood—Thinning	Sawdust	Branch Biomass	Bark
		Butt	Middle	Top				
Linolenic acid 18:3	LN	0.350	0.338	0.274	0.272	0.390	0.288	0.608
	LS	0.373	0.436	0.256	0.167	0.379	0.345	0.429
	MF	0.300	0.572	0.254	0.143		0.256	0.559
	SF	0.304	0.313	0.169	0.089	0.628	0.207	0.409
Oleic acid 18:1	LN	1.045	0.998	1.010	1.025	1.715	1.622	1.342
	LS	1.050	1.149	1.188	1.042	1.464	1.529	1.103
	MF	1.568	1.797	1.584	1.392		1.297	1.104
	SF	1.389	0.952	0.701	0.780	3.013	0.834	0.703
Stearic acid 18:0	LN	0.509	0.444	0.714	0.331	0.298	1.881	1.201
	LS	0.747	0.667	0.992	0.407	0.503	0.330	1.018
	MF	0.577	0.395	0.458	0.393		0.179	1.629
	SF	0.281	0.143	0.289	0.255	0.357	0.350	1.006
Linoleic acid 18:2	LN	1.052	1.021	0.836	0.771	1.322	0.945	0.795
	LS	1.168	1.195	0.860	0.568	1.266	0.976	0.576
	MF	1.097	1.706	0.902	0.587		0.833	0.590
	SF	1.232	1.002	0.556	0.356	2.634	0.559	0.362
STILBENES								
Pinosylvin	LN	1.464	1.314	0.409	0.664	1.067	0.377	0.043
	LS	2.037	1.407	0.626	0.200	1.227	0.761	0.063
	MF	1.991	1.311	1.326	0.091		0.350	0.000
	SF	2.202	1.167	0.945	0.374	1.741	0.260	0.037
Pinosylvin monomethyl ether	LN	1.706	2.039	0.973	1.128	1.530	1.243	0.278
	LS	2.726	1.930	1.041	0.283	1.665	1.759	0.294
	MF	2.431	1.369	2.774	0.289		1.029	0.000
	SF	2.021	1.341	1.779	0.301	1.711	0.975	0.106
RESIN ACIDS								
Dehydroabietic acid	LN	2.108	1.888	1.018	1.073	2.706	2.178	1.313
	LS	2.171	1.286	0.913	0.707	2.616	3.013	1.588
	MF	1.955	1.516	2.176	1.269		2.340	2.336
	SF	2.666	1.587	1.649	1.367	3.137	3.290	2.503
Abietic acid	LN	1.882	2.674	1.905	1.568	2.721	0.899	0.404
	LS	3.994	1.987	1.216	0.905	3.153	1.682	0.398
	MF	3.146	1.094	3.654	1.318		1.549	1.286
	SF	3.222	1.741	2.190	0.920	3.758	2.291	1.097
Palustric acid	LN	1.085	1.585	1.024	1.004	1.419	0.059	0.000
	LS	2.707	1.248	0.893	0.635	1.845	0.031	0.000
	MF	1.506	0.603	1.682	0.572		0.000	0.000
	SF	2.094	1.059	1.333	0.768	1.998	0.000	0.000
Pimaric acid	LN	1.074	1.274	0.853	0.864	1.380	0.744	0.373
	LS	1.731	1.073	0.888	0.582	1.494	1.206	0.583
	MF	1.500	0.955	1.750	0.562		1.053	0.835
	SF	1.544	0.887	1.212	0.782	1.687	1.316	0.746
Sandaracopimaric acid	LN	0.186	0.218	0.150	0.139	0.202	0.111	0.091
	LS	0.307	0.162	0.112	0.095	0.209	0.217	0.095
	MF	0.245	0.111	0.267	0.088		0.198	0.175
	SF	0.234	0.123	0.159	0.085	0.281	0.265	0.160
Isopimaric acid	LN	0.512	0.636	0.341	0.239	0.721	0.670	0.312
	LS	0.960	0.503	0.351	0.259	0.792	1.013	0.270
	MF	0.502	0.243	0.486	0.106		0.865	0.499
	SF	0.751	0.383	0.623	0.264	0.896	1.135	0.433

Table 1. *Cont.*

Compound mg/g dw		Stem Wood—Final Felling			Stem Wood— Thinning	Sawdust	Branch Biomass	Bark
		Butt	Middle	Top				
Levopimaric acid	LN	1.402	2.072	1.543	1.660	1.185	0.820	0.070
	LS	2.796	1.721	1.699	1.273	1.878	0.180	0.012
	MF	1.418	0.857	1.391	0.951		0.127	0.116
	SF	2.256	1.022	1.560	1.320	1.892	0.104	0.046
Neoabietic acid	LN	1.161	1.741	1.226	1.093	1.188	0.000	0.000
	LS	2.868	1.375	0.976	0.700	1.636	0.239	0.000
	MF	1.765	0.528	2.135	0.565		0.144	0.044
	SF	1.836	0.882	1.133	0.599	1.823	0.124	0.042
STEROLS								
Sitosterol	LN	0.104	0.116	0.129	0.065	0.068	0.450	1.291
	LS	0.084	0.077	0.097	0.052	0.040	0.620	1.215
	MF	0.064	0.046	0.058	0.044		0.326	1.653
	SF	0.035	0.034	0.048	0.027	0.038	0.460	1.420
Stigmasta-3.5-diene	LN	0.072	0.068	0.071	0.029	0.000	0.000	0.000
	LS	0.053	0.052	0.031	0.030	0.000	0.000	0.000
	MF	0.034	0.011	0.027	0.020		0.000	0.000
	SF	0.000	0.006	0.004	0.004	0.012	0.000	0.000
LIGNANS								
Nortrachelogenin	LN	0.029	0.596	0.245	0.051	0.207	0.155	0.000
	LS	0.000	0.041	0.064	0.011	0.152	0.224	0.000
	MF	0.130	0.000	0.669	0.000		0.229	0.000
	SF	0.000	0.005	0.604	0.000	0.000	0.131	0.000
Matairesinol	LN	0.033	0.164	0.020	0.015	0.083	0.031	0.000
	LS	0.072	0.011	0.028	0.016	0.023	0.061	0.076
	MF	0.011	0.000	0.085	0.000		0.000	0.000
	SF	0.000	0.005	0.020	0.004	0.000	0.044	0.000

Triglycerides were observed more in stem wood of thinning trees, stem wood in the top of final-felling trees and in bark than in the lower parts of final-felling trees, sawdust, or branch biomass (Figure 3). In parallel, decreasing contents from north to south were observed in the biomass components, which had the highest contents. Both results implied a positive effect of young age of wood material on the triglyceride contents.

Fatty acids had the lowest content in the stem wood of thinning trees, and the content was also slightly higher in the sawdust, branch biomass, and bark than in the stem wood of final-felling trees, where it decreased slightly from the butt to the top (Figure 3). We observed some decreasing trends in the contents from north to south as regards the middle part of final-felling trees, and the contents was higher in Southern and Middle Finland compared to Lapland North and South. We identified a total of six different fatty acids: stearic acid (18:0), oleic acid (18:1), palmitic acid (16:0), linolenic acid (18:3), linoleic acid (18:2), and margaric acid (17:0), the most abundant of them being oleic acid and linoleic acid. It is notable that also sawdust had high contents of oleic acid, linolenic acid, and linoleic acid, compared to other biomass components in the study (Table 1).

Lignans, sterols, and steryl esters were the most abundant in bark and branch biomass; bark was particularly rich in sterols compared to the other biomass components (Figure 3). Notable regional differences were observed only in lignans with a clear increase in branch biomass and a small increase in the top of final-felling trees from north to south, however, an opposite effect was found in the middle of final-felling trees. Among lignans, matairesinol, and nortrachelogenin were identified, and sterols included sitosterol and stigmasta-3,5-diene (Table 1). Sitosterols were observed especially in the bark, but stigmasta-3,5-diene in the stem wood samples only.

Stilbenes were clearly most abundant in the butt and middle parts of final-felling trees, thereafter in saw dust and branch biomass, but the content was very low in thinning wood and almost negligible in bark (Figure 4). Regional trends were unstable, although the lowest contents were observed in the northernmost region, with and exception of thinning trees and middle parts of final-felling trees. In the most biomass components, pinosylvin monomethyl ether was more abundant than pinosylvin (Table 1). Figure 4 with the average values and standard deviations shows the two stilbene compounds as examples of the level of variation in the contents of individual extractive compounds.
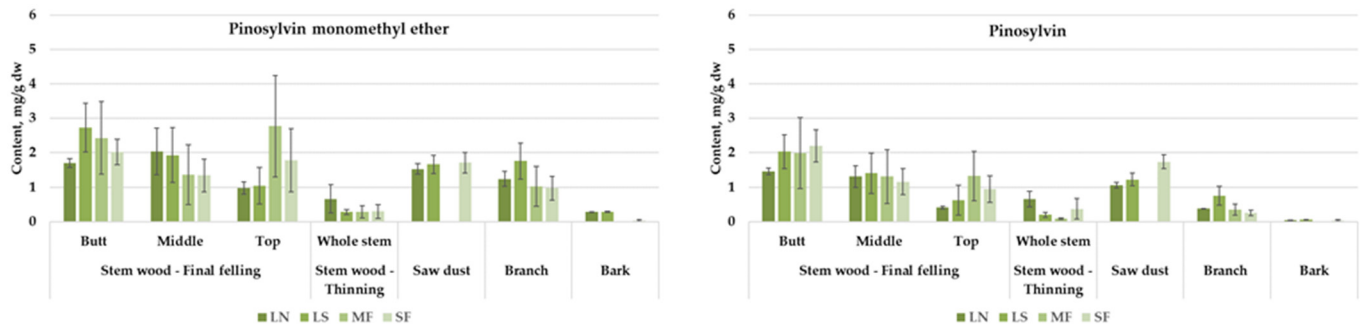
Figure 4. Contents of stilbene compounds pinosylvin monomethyl ether and pinosylvin (mean ±SD) in the tree biomass components in the four regions: Lapland North (LN), Lapland South (LS), Middle Finland (MF), and South Finland (SF). dw: dry weight.

3.2. Variation of Extractive Contents in Biomass Components

3.2.1. Stem Wood

GLM multivariate analysis of variance was used to examine the effects of geographic region and stem part on the contents of extractive compound groups in final-felling trees (Table 2). We found some differences in the contents of steryl esters and triglycerides. The most divergent regions were South Finland vs. Lapland North and Lapland South for steryl esters and South Finland vs. Lapland North for triglycerides. The most divergent differences between stem parts were butt logs vs. top logs for steryl esters and top logs vs. middle and butt logs for triglycerides. In addition, differences in the contents of fatty acids and resin acids were observed between stem parts (butt logs vs. top logs) and in the contents of sterols between the regions (Middle Finland vs. other regions). Regarding the total extractive contents, the region did affect but not the stem part (see also Figure 1). No interactions of the explanatory variables were observed in any of the groups.

Table 2. GLM multivariate analysis summary for the extractive compound groups in stem wood of final-felling trees. Significance levels: $\alpha = 0.01$ (**) and $\alpha = 0.05$ (*).

Compound	Source	df	MS	F	*p*
Resin acids	Region	3	24.400	1.173	0.34
	Part of stem	2	102.887	4.945	0.01 **
	Interaction	6	42.946	2.064	0.09
Triglycerides	Region	3	83.278	7.629	0.00 **
	Part of stem	2	134.581	12.329	0.00 **
	Interaction	6	25.998	2.382	0.05
Lignans	Region	3	0.125	0.425	0.74
	Part of stem	2	0.519	1.772	0.19
	Interaction	6	0.449	1.533	0.20
Sterols	Region	3	0.002	3.353	0.03 *
	Part of stem	2	0.001	1.675	0.20
	Interaction	6	0.001	1.100	0.39

Table 2. *Cont.*

Compound	Source	df	MS	F	p
Steryl esters	Region	3	0.321	15.042	0.00 **
	Part of stem	2	0.367	17.170	0.00 **
	Interaction	6	0.014	0.666	0.68
Fatty acids	Region	3	1.391	0.213	0.89
	Part of stem	2	27.875	4.259	0.02 *
	Interaction	6	8.155	1.246	0.31
Total	Region	3	222.208	4.062	0.02 *
	Part of stem	2	138.940	2.540	0.10
	Interaction	6	111.783	2.043	0.09

Of the individual extractive compounds, the content of sitosterol was lower in South Finland compared to Lapland North and Lapland South ($p < 0.01$) (Table 3). Only two of a total of eight resin acids, dehydroabietic acid, and levopimaric acid, were affected by the region. Contents of dehydroabietic acid tended to be higher in South Finland compared to Lapland South ($p < 0.05$) and that of levopimaric acid in South Lapland compared to Middle Finland, respectively. The butt parts of trees tended to have the highest contents of the most resin acids.

Table 3. GLM multivariate analysis summary for individual extractive compounds in stem wood of final-felling trees. Significance levels: $\alpha = 0.01$ (**) and $\alpha = 0.05$ (*).

Compound	Source	df	MS	F	p
FATTY ACIDS					
Palmitic acid 16:0	Region	3	0.546	88.613	0.00 **
	Part of stem	2	0.029	4.734	0.02 *
	Interaction	6	0.009	1.524	0.20
Margaric acid 17:0	Region	3	1.095	70.433	0.00 **
	Part of stem	2	0.024	1.542	0.23
	Interaction	6	0.011	0.737	0.62
Linolenic acid 18:3	Region	3	0.042	1.399	0.26
	Part of stem	2	0.145	4.867	0.02 *
	Interaction	6	0.016	0.522	0.79
Oleic acid 18:1	Region	3	0.080	6.175	0.00 **
	Part of stem	2	0.012	0.925	0.41
	Interaction	6	0.021	1.638	0.17
Stearic acid 18:0	Region	3	0.606	14.907	0.00 **
	Part of stem	2	0.178	4.389	0.02 *
	Interaction	6	0.014	0.337	0.68
Linoleic acid 18:2	Region	3	0.020	0.873	0.47
	Part of stem	2	0.104	4.516	0.02 *
	Interaction	6	0.017	0.732	0.63
STILBENES					
Pinosylvin	Region	3	0.028	0.224	0.88
	Part of stem	2	0.749	5.960	0.01 **
	Interaction	6	0.071	0.568	0.75
Pinosylvin monomethyl ether	Region	3	0.003	0.022	0.10
	Part of stem	2	0.263	2.091	0.14
	Interaction	6	0.098	0.775	0.60

Table 3. *Cont.*

Compound	Source	df	MS	F	*p*
RESIN ACIDS					
Dehydroabietic acid	Region	3	0.049	3.082	0.04 *
	Part of stem	2	0.151	9.497	0.00 **
	Interaction	6	0.038	2.360	0.06
Abietic acid	Region	3	0.007	0.122	0.95
	Part of stem	2	0.234	4.060	0.03 *
	Interaction	6	0.117	2.028	0.09
Palustric acid	Region	3	0.036	0.806	0.50
	Part of stem	2	0.181	4.107	0.03 *
	Interaction	6	0.105	2.374	0.05
Pimaric acid	Region	3	0.019	0.798	0.51
	Part of stem	2	0.087	3.572	0.04 *
	Interaction	6	0.048	1.968	0.10
Sandaracopimaric acid	Region	3	0.010	0.342	0.80
	Part of stem	2	0.178	5.958	0.01 **
	Interaction	6	0.068	2.272	0.06
Isopimaric acid	Region	3	0.054	1.518	0.23
	Part of stem	2	0.193	5.466	0.01 **
	Interaction	6	0.073	2.068	0.09
Levopimaric acid	Region	3	0.065	2.966	0.05 *
	Part of stem	2	0.073	3.301	0.05
	Interaction	6	0.057	2.578	0.04 *
Neoabietic acid	Region	3	0.040	0.872	0.47
	Part of stem	2	0.234	5.059	0.01 *
	Interaction	6	0.126	2.749	0.03 *
STEROLS					
Sitosterol	Region	3	0.534	22.381	0.00 **
	Part of stem	2	0.030	1.276	0.29
	Interaction	6	0.011	0.474	0.82
Stigmasta-3,5-diene	nd				
LIGNANS					
Nortrachelogenin	nd				
Matairesinol	nd				

Although the total content of fatty acids did not differ between the regions (Table 2), the contents of palmitic acid, stearic acid, margaric acid, and oleic acid were affected (Table 3). Oleic acid content was higher in Middle Finland compared to other regions ($p < 0.05$). Palmitic acid and margaric acid contents were lower in South Finland compared to other regions ($p < 0.001$). Similarly, stearic acid content was lower in South Finland compared to Lapland North and Lapland South ($p < 0.01$) and Middle Finland ($p < 0.05$).

Linolenic acid and linoleic acid contents did not differ between the regions. Stem part affected the palmitic acid, linolenic acid, stearic acid, and linoleic acid contents, the top part having the lowest contents of linolenic and linoleic acid.

The content of pinosylvin was higher in the butt part compared to the top part in final-felling trees, whereas the content of pinosylvin monomethyl ether did not significantly differ between the stem parts (Table 3, see also Figure 4). Region had no significant effect on either stilbene compound.

3.2.2. All Tree Biomass Components

Principal component analysis (PCA) was used to identify the common dimensions of pattern and structure of the occurrence of extractive compound groups, on the one hand, in stem wood samples, and on the other hand, in all tree biomass components together. The goal was to use PCA to reduce the large number of chemical compound variables to a few new variables, the principal components. Chemical compounds belonging to the same principal component indicate the likelihood of their co-occurrence in the biomass component in question. Finding one compound in a biomass component also indicates a strong probability of the other compounds of the same principal component to occur.

The Varimax orthogonal rotation method explained 76.0%–76.5% of the variance of the contents, showing that the compounds were charged to two main components which explained 42.0%–46.2% (PC1) and 29.8%–35.5% (PC2) (Table 4). For all biomass components, PC 1 consisted of five compounds, only resin acids not belonging to the same group, while PC 2 consisted of three compounds only, fatty acids, resin acids, and triglycerides (Figure 5). For stem wood, PC 1 consisted of three compounds, fatty acids, resin acids and lignans, while PC 2 covered also the remaining three compounds, sterols, steryl esters, and triglycerides. The analysis of all biomass components showed high factor loadings (>0.5) between fatty acids and resin acids, lignans and sterols, and sterols and steryl esters. When restricted to stem wood, high correlations were observed between fatty acids and resin acids, sterols and steryl esters, steryl esters and triglycerides, and resin acids and lignans.

Table 4. Factor loadings of the principal component analysis for extractive compound groups in Scots pine stem wood from final-felling and thinning trees and in all tree biomass, in all regions. Initial Eigenvalues (>1) and rotation sums of squared loadings SSL (after rotation) with the percent of variance explained by PCs. Only variable loadings > 0.3 listed to highlight the patterns revealed.

Total Variance Explained	PC 1	PC 2	PC 1	PC 2
	Stem wood		All biomass	
Initial Eigenvalue	2.52	2.07	2.77	1.79
% of variance	42.01	34.47	46.20	29.76
Rotation SSL	2.33	2.26	2.75	1.81
% of variance SSL	38.79	37.69	45.87	30.09
Rotated component matrix				
Fatty acids	0.955	-	0.509	0.820
Resin acids	0.930	-	-	0.856
Lignans	0.638	0.327	0.846	-
Sterols	-	0.788	0.928	-
Steryl esters	-	0.860	0.868	-
Triglycerides	-	0.875	0.376	−0.607
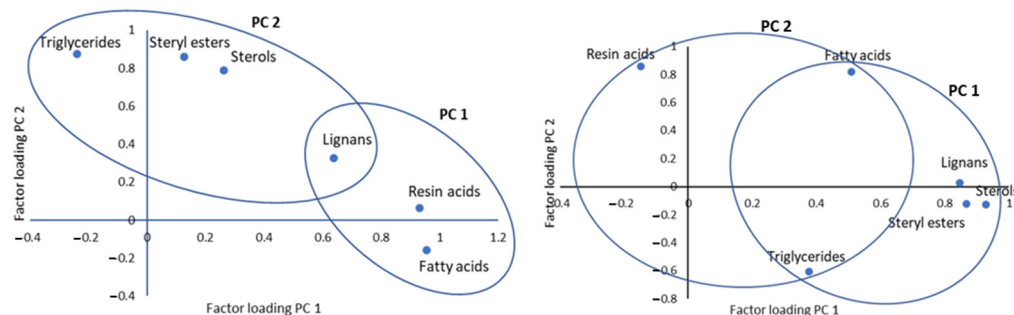
Figure 5. Factor loadings of compounds of Scots pine stem wood from final-felling and thinning trees (**left**) and all biomass components (**right**) generated by principal component analysis. Compounds belonging to PC 1 and 2 are marked by circles.

Pearson correlation coefficients between the extractive compound groups in the different biomass components were consistent with the results of principal component analysis (Table 5). In the stem wood from both final-felling and thinning stands, the correlations between fatty acids, resin acids, and lignans, on the one hand, and sterols, steryl esters, and triglycerides, on the other hand, mostly showed significant correlations, indicating their simultaneous presence in the respective biomass. In addition, triglycerides were negatively correlated especially with fatty acids and resin acids in these biomass components. In branch biomass, steryl esters, and sterols, triglycerides and fatty acids, and triglycerides, and resin acids showed significant correlations, whereas in bark the only highly significant correlation ($p < 0.01$) was found between triglycerides and steryl esters.

Table 5. Pearson correlation coefficients between the compounds in different Scots pine biomass components. Significance levels: $\alpha = 0.01$ (**) and $\alpha = 0.05$ (*).

	Resin acids	Triglycerides	Lignans	Sterols	Steryl esters
Stem Wood—Final Felling					
Triglycerides	−0.129				
Lignans	0.394 **	0.149			
Sterols	0.269	0.538 **	0.252		
Steryl esters	0.116	0.607 **	0.261	0.631 **	
Fatty acids	0.857 **	−0.429 **	0.419 **	0.054	−0.076
Stem Wood—Thinning					
Triglycerides	0.209				
Lignans	0.699 **	0.559 *			
Sterols	0.244	0.556 *	0.402		
Steryl esters	0.361	0.753 **	0.731 **	0.368	
Fatty acids	0.788 **	−0.098	0.578 *	0.192	0.190
Sawdust					
Triglycerides	−0.393				
Lignans	−0.669 *	0.684 *			
Sterols	−0.609	0.614	0.673 *		
Steryl esters	0.008	0.770 **	0.295	0.194	
Fatty acids	0.861 **	−0.420	−0.778 **	−0.677 *	0.178
Branch biomass					
Triglycerides	0.790 *				
Lignans	−0.382	−0.445			
Sterols	−0.411	−0.291	0.619		
Steryl esters	−0.127	−0.018	0.415	0.936 **	
Fatty acids	0.666	0.841 **	−0.576	−0.302	0.006
Bark					
Triglycerides	−0.363				
Lignans	0.029	0.026			
Sterols	0.252	−0.414	0.773 *		
Steryl esters	−0.264	0.884 **	−0.017	−0.513	
Fatty acids	0.728 *	−0.351	0.557	0.475	−0.207

4. Discussion

In this study, we hypothesized geographic regions in Finland to have impacts on the contents of wood extractives in Scots pine. Of the extractive groups, we focused on lipophilic compounds, stilbenes and lignans. By that way, the study complemented our previous analysis of phenolic and resin acid extractives [19], with an expansion from stem wood of final-felling and thinning stands and sawdust to other aboveground biomass

components, bark and branch biomass as well. An analysis of the total contents of wood extractives in each tree biomass component was included to the study as well.

The results from the stem wood analysis showed that the effects of the latitude (north vs. south) depend on the level of comparison: total extractives, compound groups or individual compounds. Total extractive content in stem wood generally decreased from north to south by one third as an average of the whole stem. As we expected, total extractive content was clearly highest in the butt parts of the final-felling trees and lowest in the thinning stand trees. The middle parts, however, showed higher extractive contents than the top parts only in Lapland North where the heartwood percentage clearly decreased from the middle to the top of the trees. Heartwood percentage has been shown to be a major factor for the variation in extractive content of Scots pine [19], see also [8,14,28,41–43].

In our previous study on stem wood, based on ^{1}H NMR spectroscopy, the latitude effect was opposite in heartwood, the amounts of extractable compounds being there approximately three-fold in South Finland compared to Lapland North, but the opposite difference in heartwood vs. sapwood percentage turned the conclusion slightly opposite in stem wood as a whole [19], see also [12,20]. Notably, in that study the geographic region showed effects on the total extractive content, but the vertical position in a stem did not.

During late 1960s, total percentages of wood extractives of 4.0 and 3.3 were reported as the average values for Scots pine pulpwood in northern and southern Finland, using acetone extraction in the analysis [20]. In a study on 9–13 cm young Scots pines where organic solvents and hot water extraction were used, total extractive contents of 6.2 and 5.0 were observed in the wood at 20% relative height in Inari (Lapland North) and Kannus (Middle Finland, west), the respective values being somewhat higher at 80% relative height, 8.7 and 6.2 [44]. In a parallel study on 28–38 cm mature Scots pines in Kannus, total extractive contents at the respective heights were a little higher than in 9–13 cm young Scots pines, 6.0% and 7.6% [45].

The level of total extractive percentage of 2.0 to 4.5 in our study was a little lower than the general values of three to five percent reported in the literature for Scots pine stem wood in northern Europe [6,7,12]. It should be noted that our material was collected in the forest and sawmills during boreal winter, implying the dormant period of trees, when the maximum of extractives should be present [8,14,28]. Sampling season, in addition to the location, site, and environment is probably one reason for the large variation in the results between the studies.

In bark, we observed distinct between-region differences in total extractive content, the percentage being the highest in Middle Finland and the lowest in Lapland South. However, the north–south effect was not clear. Our total extractive percentages of 3.5−5.0 were lower than the averages of 16−26 reported in textbooks for Scots pine bark in northern Europe [28]. We did not separate inner bark and outer bark, the extractive percentages of which being reported as 15−42 and 16−21 in the literature, respectively [28].

The extraction method used may explain a part of the differences between the studies, but obviously not all of them. For example, in a study in western Finland, inner bark and outer bark of young Scots pines at 20% relative height showed total extractive contents of 39.5 and 15.9 using organic solvents and hot water extraction, and corresponding values of 18.8 and 18.9 using alkalic extraction [44]. In Lapland North, these percentages were 5 and 8 percentage points higher than in western Finland using organic solvents and hot water extraction, but 2 and 9 percentage points lower using alkalic extraction. In mature Scots pines of western Finland, the extractive contents were 7 percentage points lower in inner bark but 10 percentage points higher in outer bark compared to young Scots pines when organic solvents and hot water extraction was used, but the corresponding differences were 3 and 6 percentage points when alkalic extraction was used [45]. Similar examples of the effect of extraction method or individual chemicals are largely available on other tree species, for example on the wood of poplar species [46].

No regional differences were demonstrated in the total extractive content of sawdust or branch biomass in this study. The stand wise mixture and small number of samples

may have affected the result. In our previous study, we found some small between-region differences for sawdust, but no general north–south trend [19]. Our branch biomass samples consisted of wood, bark, and some foliage; therefore, they were mixed compositions of tree biomass with different extractive contents. The average extractive percentage was 3.0−3.5 for sawdust and 3.0−4.0 for branch biomass, respectively.

At compound group level, we found differences between northern and southern regions in steryl esters and triglycerides in stem wood, and a decrease in their contents from the butt to the top of the trees. Stilbenes, resin acids, fatty acids, or lignans did not show any significant geographic effects, but the contents of resin acids, fatty acids and pinosylvin (of stilbenes) were higher in butt logs than in top logs. Our previous study showed higher contents of lignans and lower contents of stilbenes in stem wood in the north than in the south [19]. In former studies, environmental factors were suggested to influence especially the amounts of various fatty acids and sterols in stem wood, whereas stilbenes and resin acids seemed more strictly genetically determined [17]. For example, soil fertility and other factors of growth environment affecting tree growth were indicated to have an influence on pinosylvin and neolignans in southern Finland [47].

When the regions were compared at the level of individual compounds, we found differences in the contents of certain fatty acids and resin acids in stem wood. Previously, a higher content of unsaturated fatty acids, such as linoleic acid, in northern compared to southern Finland was connected to the cold temperature and the slow growth rate [48]. In our study, mainly saturated fatty acids, palmitic acids, and stearic acid showed higher contents at the northern latitudes compared to the southern ones. Sitosterol content was also higher in the north compared to the south. Dehydroabietic acid and levopimaric acid were affected by region, but no latitude effect was found. Thus, it is possible that different geographic provenances differ, but this does not solely come from environmental factors but are of genetic origin. This is in agreement with the finding that geographic provenance affects resin acids of Scots pine wood at a given growing site [49].

The latitude gradient in our study presented boreal locations only, which may partly explain the relatively few geographic differences. There is some evidence suggesting that Scandinavian Scots pine varieties have the highest total resin acid content in wood compared to other macro-regions in Europe [50]. As a reference, inner bark of Norway spruce was recently observed to have a higher total content of stilbene glycosides in northern origins of tree seeds (northern Finland) compared to more southern origins (Germany), but the result was opposite for the data of northern vs. southern tree provenances in Finland, the different growing sites having also significant effects on the contents [36].

Generally, studies evaluating the effect of latitude on the chemical composition of coniferous species are scarce, their number of replicates is low, and they are largely limited to stem wood. Observations on individual compounds are few and they mostly apply to deciduous tree species or flowering plants. However, they showed responses to local light and temperature conditions [24–26,51,52].

Principal component analysis on the six extractive compound groups covering all biomass components revealed high multiple correlations between fatty acids and resin acids, lignans and sterols, and sterols and steryl esters. Restricted to stem wood, high correlations were observed between fatty acids and resin acids, sterols and steryl esters, steryl esters and triglycerides, and resin acids and lignans. The results indicated the presence of these compounds in wood to be dependent on the same background factors. Pearson correlation analysis on stem wood confirmed the simultaneous presence of fatty acids, resin acids and lignans, on the one hand, and sterols, steryl esters and triglycerides on the other hand, and the lack of compatibility of triglycerides with fatty acids and resin acids. In branch biomass, steryl esters and sterols, triglycerides and fatty acids, and triglycerides and resin acids belonged together, respectively. In bark, only triglycerides and steryl esters occurred significantly together.

An important aspect is the shelf life of the wood extractives during the logistic chain of utilization because harvesting of the logs for commercial uses takes place throughout

the year and there are periods of raw material storage and often delays before processing. As we stated before, we aimed to minimize the loss of chemical compounds by collecting the materials in winter during the dormancy of trees, preparing the chip, crush, and bark samples in outdoor conditions during the periods of temperature below 0 °C and storing the samples with a minimum time delay in freezer (−20 °C) until grinding and extraction.

During growing season higher temperature and air humidity may enhance degradation of the compounds of the harvested trees and change the chemical composition of the extractives [53–55]. For example, stilbene glycosides and tannins in spruce bark have been shown to reduce more in summer compared to winter in a log storage experiment in Finland [54]. In addition, compounds that are most sensitive to UV radiation and hydrolysis have been shown to degrade fastest in spruce bark in summer [53]. Industrial bark of spruce from sawmills showed an as high as a tenfold stilbenoid content in winter than in summer [36]. Moreover, Scots pine bark was found to rapidly lose extractives already in two weeks in a buffer pile storage in late summer/autumn, leading to a decrease from 9.9% to 5.5% in eight weeks [55].

Our study considered both lipophilic compounds (resin and fatty acids) and hydrophilic compounds (stilbenes), which may differ in the sensitivity to degrade during the storage. According to a study, although the total phenolic content in spruce bark degraded at the same rate during winter and summer storage, the content of a specific group of phenolics, monomeric stilbenoids, was 61% higher during the winter storage period [54].

The findings of our study and former studies both highlighted careful timing of harvesting and processing of logs to maximize the yield of the targeted chemical compounds and sustain the composition of the extractives. In addition, to confirm the stability of the bark quality for biorefining, it is recommended not to debark logs before storage [53,54]. For example, the inherent variation in the stilbenoid content of Norway spruce bark was offset by the variation caused by the debarking process and experimental pilot-scale processing [36]. In our study, we debarked the bolts rather soon after felling of sample trees, which may have decreased the yield of bark extractives.

Scots pine is generally classified as a moderate species for sourcing resin acids (heartwood), fatty acids (sapwood), stilbenes (especially knot wood, also stem wood), or sterols (stem wood) [14]. Similar to all wood extractives, these differ considerably in the utilization potential for biorefining as regards the easiness or difficulty in fractionation, conversion, processing technology, production costs, and expected unit price of products [4,5]. Fractionation routes are therefore different for individual extractives or their groups.

Purity is an important requirement in the industrial uses, creating challenges for the isolation in wood processing. In this respect, fatty acids, terpenes, and resin acids are the easiest, being collected along with pulping process and in this way providing basis for a multitude of distillates, chemical processes and conversion processes, thermodynamic or enzymatic [56]. In pine species, fatty acids and resin acids make the basis for crude tall oil, along with a minor part of sterols, diterpenic aldehydes and alcohols; this is the raw material for many chemical products and biodiesel fuels [4,15].

Practically all fatty acids that we identified are industrially interesting, for example for food and cosmetics products, if they can be isolated in a pure form. Fatty acids are available in large volumes from vegetative oils, thereby, there is no shortage of them in the raw material market in a normal political situation of global trading. Instead, the competitiveness of wood as base material may come from the fact that its production does not need any lands that are suitable for food production, and from the unique properties, good stability and no seasonal composition drift. Some fatty acids, such as pinolenic, occur only in wood, albeit the drawback of their low content in wood.

Mixtures of tall resin acids have wide industrial applications, then, purity is not the biggest challenge [56,57]. They are not currently used in a pure form of individual resin acids, and their uniqueness for processes or products or technical and commercial potential for extracting have not been shown. In addition to liquid biofuels, the most important application areas as industry chemicals are printing inks, adhesives and sealants, paper

size, and emulsifiers and coatings [3,5,56,57]. The value-added applications of resin acids based on bioactive effects that may expand cover special products of cosmetics, food and beverage, nutraceutical and biomedical applications [14,33–35]. Sterols are already now separated from tall oil after pulping for bioactive raw materials as well as for selected nutraceutical and cosmetics applications [5,33–35]. For more applications of sterols from Scots pine, a competitive extraction method and new markets should be developed.

Polyphenolic compounds, such as stilbenes, lignans, and tannins, are more difficult to process than fatty acids and resin acids, being either dissolving or non-dissolving, often unstable, and challenging in chemical modification [33,37,58]. In addition to resin acids and sterols, stilbenes, lignans, and flavonoids are the main extractive compounds of Scots pine which provide bioactive benefits based on antioxidative, antifungal, and antibacterial properties [5,21,36]. Potential uses have been shown for stilbenes in wood product industries (wood protection and gluing) [5,35,37], and for lignans in medical and well-being purposes [15,16,38]. New uses of tannin after leather tanning and adhesives for wood panels and solid wood products have been scanned [37,58,59], but an actual industrial break-through is pending [35].

Sugars and monosaccharides are technically easier, in principle, but they have not proven yet any significant market potential [35]. Instead, it is notable that the active fractionation research during 2000s on lignin resulted in bio-adhesives and nanoparticle products with growing markets albeit the technical difficulties of processing [60].

Economy of scale has been usually the basis for utilizing chemical compounds of biomass materials. Accordingly, wood extractives are typically managed as a whole entity without aiming to individual compounds in the process [3,35]. Here, large industry companies typically utilize the wood-based materials, and incentives from small and medium scale industries have been few. Recently, chemical forest industry companies have started to attract spin-offs to utilize their findings and patents for value-added niche products and novel manufacturing processes.

Moreover, the global trend of cascading pursues to a gradual shift from energy use of raw materials to material uses with a large diversity. Many wood-based materials are nowadays used in large quantities for energy, but they provide simultaneously a significant potential for material uses. For example, the energy use of bark totalled to 7.7 million cubic meters in Finland in 2021, compared to 3.3. and 1.5 million cubic meters of saw dust and industrial wood chips, respectively (data from Luke). In all, as holistic and full-scale utilization of chemical fractions as possible could bring profitability and competitiveness for the utilization of wood extractives as well [3–5,35,36,56,61].

5. Conclusions

Resin acids, triglycerides, and fatty acids proved out dominant of the extractive groups that we focused on in this study on Scots pine. Resin acids and stilbenes are the most abundant in stem wood of mature trees and in sawdust, whereas triglycerides occur the most in bark and branch biomass. Of the minor extractive groups, stilbenes (pinosylvin and pinosylvin monomethyl ether) are the most abundant in stem wood of mature trees and in sawdust, and lignans, sterols, and steryl esters in bark and branch biomass. Stem wood of thinning trees is between the stem wood of mature trees and bark or branch material for all extractive groups of this study. Resin acids and fatty acids decrease from the butt toward the top of the trees, but triglycerides show a reverse longitudinal effect.

Of those industrial side streams that are mainly used for bioenergy nowadays, bark has the largest quantitative potential as a raw material. Bark in southern Finland would be the most potential source of fatty acids and sterols. For triglycerides and steryl esters, bark in northern Finland would have more potential. In stem wood, steryl esters and triglycerides seem to decrease from north to south, and there is evidence of decreasing lignans and increasing stilbenes in the same geographic direction. Certain fatty acids and resin acids are more frequent in the north than in the south.

The opportunities to increase and diversify the use of extractives for value-added products are based on finding the unique properties and functionalities and developing processes to achieve competitive advantages, such as stability and renewability of the materials, substitution of fossil or synthetic materials and saving land for food production. The results indicate the need to study further and use extractive fractions to direct materials or products, isolate and functionalize them. The biggest challenges in research and development are related to isolation/extraction, purity and quality, raw material volumes, and identification, procurement and sorting from forest or wood processing plants.

Market acceptance, customer benefits, and proof-of-concepts for investors and industries should be the ultimate goals to achieve, both for larger-volume bulk production and smaller-volume niche production, among BtoB and BtoC customers. Moreover, it is necessary to pay more attention in research to the availability of the desired chemical compounds of tree biomass in the forest and wood-based raw materials at the mills, prediction models for the occurrence of the most important compounds, and links between genetic factors and other attributes of forest management, tree breeding, and nursery production.

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