JYU DISSERTATIONS 737

Eelis Halmemies

Chemical Changes in the Industrial Extractive-Containing Sidestreams of Norway Spruce (*Picea abies*) during Storage

UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF MATHEMATICS
AND SCIENCE

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ABSTRACT

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This dissertation focuses on the changes in the chemical composition of Norway spruce (*Picea abies)* industrial sidestreams during outside storage, with a particular focus on the behavior of hydrophilic and lipophilic extractive groups as well as the selected individual extractives. Detailed chromatographic analyses of extracted bark, needles, and stumps stored in several setups revealed the degradation patterns of various extractive groups. Generally, hydrophilic extractives showed more significant disruption and losses during storage than more stable lipophilic extractives. Stilbenes, tannins, and lignans were particularly interesting compound groups that could feasibly be used for added-value purposes. By revealing how storage affects wood degradation, the data gathered and presented aim to assist in the handling and processing of wood-derived raw material to increase its potential. Industries focused on developing wood extractive applications may particularly benefit from this information.

Keywords: Norway spruce (*Picea abies)*, wood extractives, storage

TIIVISTELMÄ

Halmemies, Eelis
Kuusen *(Picea abies)* uuteaineita sisältävien sivuvirtojen kemialliset muutokset varastoinnin aikana
Jyväskylä: Jyväskylän yliopisto, 2024, 84 s.
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Tämä väitöskirja käsittelee kuusen (*Picea abies*) teollisissa sivuvirroissa ulkovarastoinnin aikana tapahtuvia kemiallisia muutoksia keskittyen erityisesti hydrofiilisten ja lipofiilisten uuteaineryhmien (kuten stilbeenien, tanniinien ja lignaanien) käyttäytymiseen, mutta myös yksittäisiin uuteaineisiin. Yksityiskohtaiset kromatografiset analyysit kuoresta, neulasista ja kannoista uutetuista uuteaineista, jotka varastoitiin erilaisissa varastointiolosuhteissa, osoittivat eri uuteaineryhmien tyypillisiä hajoamismalleja. Työssä kootulla ja esitetyllä aineistolla on mahdollista edistää puuperäisen uuteainerikkaan raaka-aineen kokonaisvaltaista teollista hyödyntämistä. Osoittamalla, miten varastointi vaikuttaa puun hajoamiseen, voidaan ennakolta arvioida ja suunnitella raaka-aineen käsittelyvaiheet niin, että sen kemiallinen koostumus säilyy lopullista käyttökohdetta silmällä pitäen mahdollisimman edullisena.

Avainsanat: kuusi (*Picea abies)*, puun uuteaineet, varastointi

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LIST OF PUBLICATIONS

This thesis is based on the following papers, which are referenced in the main text by their Roman numerals. The papers are appended at the end of the thesis.

I Halmemies, E.S., Brännström, H.E., Nurmi, J., Läspä, O., Alén, R. Effect of seasonal storage on single-stem bark extractives of Norway spruce (*Picea abies*). *Forests,* 2021, 12(6): 736. https://doi.org/10.3390/f12060736

II Halmemies, E.S., Alén, R., Hellström, J., Läspä, O., Nurmi, J., Hujala, M., Brännström, H.E. Behaviour of extractives in Norway spruce (*Picea abies*) bark during pile storage. *Molecules,* 2022, 27(4): 1186–1216. https://doi.org/10.3390/molecules27041186

III Halmemies, E.S., Brännström, H.E., Karjalainen, M., Nurmi, J., Alén, R. Availability of extractives from various Norway spruce (*Picea abies*) stumps assortments. *Journal of Wood Chemistry and Technology*, 2023, 43(1): 13–27. https://doi.org/10.1080/02773813.2022.2152049

AUTHOR CONTRIBUTIONS

I I participated in the fieldwork of organizing the sawlog storage setups described in the article as well as sampling, prepared the original draft for the article, performed all the chemical analyses, developed several chemical analysis methods, handled the data analysis, interpreted the results, and did all the visualizations for the paper.

II I participated in the fieldwork of organizing the bark pile storage setups described in the article as well as sampling, prepared the original draft for the article, participated in developing analysis methods, performed most of the actual chemical analyses, interpreted the results, and did all the visualizations for the paper.

III I participated in the fieldwork of organizing the stump storage setups described in the article as well as sampling, prepared the original draft for the article, developed analysis methods, performed all the chemical analyses, interpreted the results, and did all the visualizations for the paper.

SUPPORTING PUBLICATIONS

I have additionally contributed to the following subject-related papers, which may be viewed as supporting publications but are not discussed in detail in this thesis:

1. Jyske, T., Brännström, H., Halmemies, E., Laakso, T., Kilpeläinen, P., Hyvönen, J., Kärkkäinen, K., Saranpää, P. Stilbenoids of Norway spruce bark: does the variability caused by raw-material processing offset the biological variability? *Biomass Conversion and Biorefinery,* 2022. doi:10.1007/s13399-022-02624-9

2. Jylhä, P., Halmemies, E., Hellström, J., Hujala, M., Kilpeläinen, P., Brännström, H. The effect of thermal drying on the contents of condensed tannins and stilbenes in Norway spruce (*Picea abies* [L.] Karst.) sawmill bark. *Industrial Crops and Products*, 2021, 173: 114090. doi:10.1016/j.indcrop.2021.114090

3. Carlqvist, K., Arshadi, M., Mossing, T., Östman, U. B., Brännström, H., Halmemies, E., Nurmi, J., Lidén, G., Börjesson, P. Life-cycle assessment of the production of cationized tannins from Norway spruce bark as flocculants in wastewater treatment. *Biofuels, Bioproducts and Biorefining*, 2020, 14(6): 1270–1285. https://doi.org/10.1002/bbb.2139

4. Jyske, T., Brännström, H., Sarjala, T., Hellström, J., Halmemies, E., Raitanen, J-E., Kaseva, J., Lagerquist, L., Eklund, P., Nurmi, J. Fate of antioxidative compounds within bark during storage: a case of Norway spruce logs. *Molecules*, 2020, 25(18): 4228. doi:10.3390/molecules25184228

FOREWORD

This work has been in preparation for a long time alongside other research, studying, and work and has now finally been finished. It is mainly based on the research data from the EU-funded Bio-Hub project of the Natural Research Institute Finland (Luke) regarding the chemical changes in the sidestreams of Norway spruce (*Picea abies)* during storage. Initially, I ended up studying chemistry because I wanted to know how to evaluate the material structure of compounds. After specializing in NMR technology and organic chemistry, I was introduced to applied and wood chemistry through university-internship. I enjoyed that the research I got to be part of had not only a theoretical and futuristic (and one might say eschatological) but also a very practical and immediate application and usefulness. I have enjoyed the study of the properties of trees with chromatographical methods. And although I have only focused on studying the components of one major tree, spruce, this work has increased my appreciation and understanding of all trees, as well as material and analytical chemistry as a whole.

There is a real temptation to start to worship the works of one's own hands – in this case, the sawlogs and the potentially prosperous chemicals that can be harvested from them. The man who was called the wisest of all men – king Solomon, was interestingly also a man who "spoke of trees" but he also taught that "fear of the Lord is the beginning of wisdom". Sharing this conviction, I want from all the spruce and extractives (how multidimensional and fascinating research objects they may be) to regard my first and foremost thanks to my God and Saviour, Lord Jesus Christ. Through the works of my hands, he has elevated my thoughts to appreciate the wisdom, intricate detail, and beauty with which he has bound up everything in the created order into a harmonious and functional whole.

A remarkable thanks for finishing this work is also due to my loving wife and children and the support of my church family, who have encouraged me to continue the work through more difficult times. A monumental thanks are also due to my mentors in wood chemistry, professor Raimo Alén and Dr. Hanna Brännström for their wise counsel and proper reflection over the years for the furthering of this research as well as their extensive knowhow regarding all things wood chemistry related. Large thanks are also due to the excellent grammar editors who have helped edit this work. I am also very thankful to Hannu Pakkanen, Hannu Salo, Jarmo Louhelainen, and Jukka Pekka Isoaho for their practical help in many aspects of my work, without forgetting the laboratory technicians Maria, Arja, and Kaisa, who have maintained order in the laboratories needed for my work to succeed. I am also thankful for all the discussions in the coffeeroom with friends over the years.

Jyväskylä 10.7.2023
Eelis Halmemies

CONTENTS

LIST OF ABBREVIATIONS

ASE	accelerated solvent extraction
ATP	adenosine triphosphate
CT	condensed tannin
DAD	diode array detection
DP	degree of polymerization
FID	flame ionization detection
FLD	fluorescence detection
GC	gas chromatography
GGM	galactoglucomannan
HMR	7-hydroxymatairesinol
HPAEC	high-performance anion exchange chromatography
HPLC	high-performance liquid chromatography
MaxTDS	maximum amount of total dissolved solids
MS	mass spectrometry
PAHs	polycyclic aromatic hydrocarbons
TDS	total dissolved solids
TPC	total phenolic content
UHQ	ultra high quality
UV	ultraviolet
Vis	visible

1 INTRODUCTION

In the current age, more than previously, the sustainability of forest industry practices is being questioned. Similarly to how fears and wars create political pressures to develop weapons, political pressures related to climate concerns have motivated the discovery of more sustainable solutions to using forest-derived raw materials. Are the ways wood is handled as a raw material as efficient and holistic as possible while ensuring that no unnecessary burden is placed on the environment? For example, is the simple incineration of bark and logging residues for energy production a wise use of the raw material? Alternatively, do ways exist to increase their value both economically and functionally? These questions have motivated the search for potential sources of biochemicals from what was previously considered waste material, as well as the development of methods to increase the functionalities of given raw materials.

Furthermore, the sidestreams of the forest industry (such as conifer bark) can contain significant amounts of valuable extractives, lignin, and carbohydrates for various potential applications. Using simple drying, extraction, and purification steps, one can multiply the worth of otherwise mundane material. For refining industries, the prospect of harnessing wood extractives concerning their beneficial functionalities in trees has increased general interest in studying their properties.

The holistic use of various plants and trees is not new. Human civilizations have consistently been involved in woodworking and have been interested in the practical study of the various properties of trees. Generally, it is reasonable to claim that (through trial and error) ancient peoples knew the functionalities of many plants and their extracts better than modern man. Often it is much easier to understand the functionality of something better than its essence. However, today's researchers have the advantage of scientific methods of studying trees' properties on a molecular level. If ancient man had, generally, a better grasp of how a particular plant extract functions regarding humans/animals (whether in beneficial or harmful ways), today's researchers can determine why a given mixture of chemicals functions as it does.

Despite the collective scientific literature on wood chemistry, much remains to be learned. For past generations, wood chemistry focused strongly on discovering the chemical compositions of various tree species and how they differ. Moreover, the chemical composition of wood is evidently the foundation for determining the best applications. However, considering today's demand for maximizing the gained value from a given raw material, an in-depth understanding is required of not only the chemical/physical state of a given material, but also how that state changes over time and under various circumstances. Thus, regarding wood-derived matter, storage matters.

Storage is fundamental to the handling of any raw material. The question is not whether to store raw materials but how and for what period. Knowing how a particular storage method and time can affect the quality of the raw material is essential to its proper handling.

1.1 Aims and outline

This thesis aims to increase the general understanding of the degradation of Norway spruce (*Picea abies*) sidestreams during storage, focusing mainly on the changes in the extractive fractions of bark and stump raw material. The research questions addressed in this study are as follows:

1) How quickly and to what extent does the chemical composition of spruce sidestreams change during conventional means of storing raw material?
2) What can be learned from the seasonal effect of storage on the same raw materials with otherwise identical storage setups except for the season (winter or summer)?
3) How can storage be improved to ensure optimal preservation of important extractives for added-value applications?

1.2 Chemical constituents of wood

Appreciating these questions regarding wood and its extractives, requires first locating them in a larger context of the general chemical composition of wood. All woody biomass primarily comprises cellulose, hemicelluloses, lignin, and extractives.

1.2.1 Cellulose

Cellulose is the Earth's most essential biopolymer (Alén 2000; Thomas et al. 2013). It is formed by hundreds to thousands of interlinked D-glucose units via β-(1→4) glycosidic bonds and generally comprises 40–50% of woody biomass. While its

primary industrial application is in papermaking, modified forms of cellulose, such as cellulose ethers and esters, can be produced for diverse uses, including films, gels, and food additives.

The primary distinction between cellulose and starch is the type of linkage between the glucose units, with starch being α-linked (Alén 2018). While humans can break down starch, they lack the necessary enzymes to digest cellulose, unlike animals that feed on biomass.

Cellulose is a crucial constituent of plant cell walls, as demonstrated in Figure 1. Cellulose molecules readily interact with each other via hydrogen bonding and form bundles known as micelles. Cellulose molecules bundle together to form almost crystalline microfibrils due to their strong intermolecular hydrogen bonding. These microfibrils interlock to form macrofibrils, with lignin, pectin, and hemicelluloses filling the remaining spaces between the micro- and macrofibrils to establish a firm and supportive structure for the fiber cell.

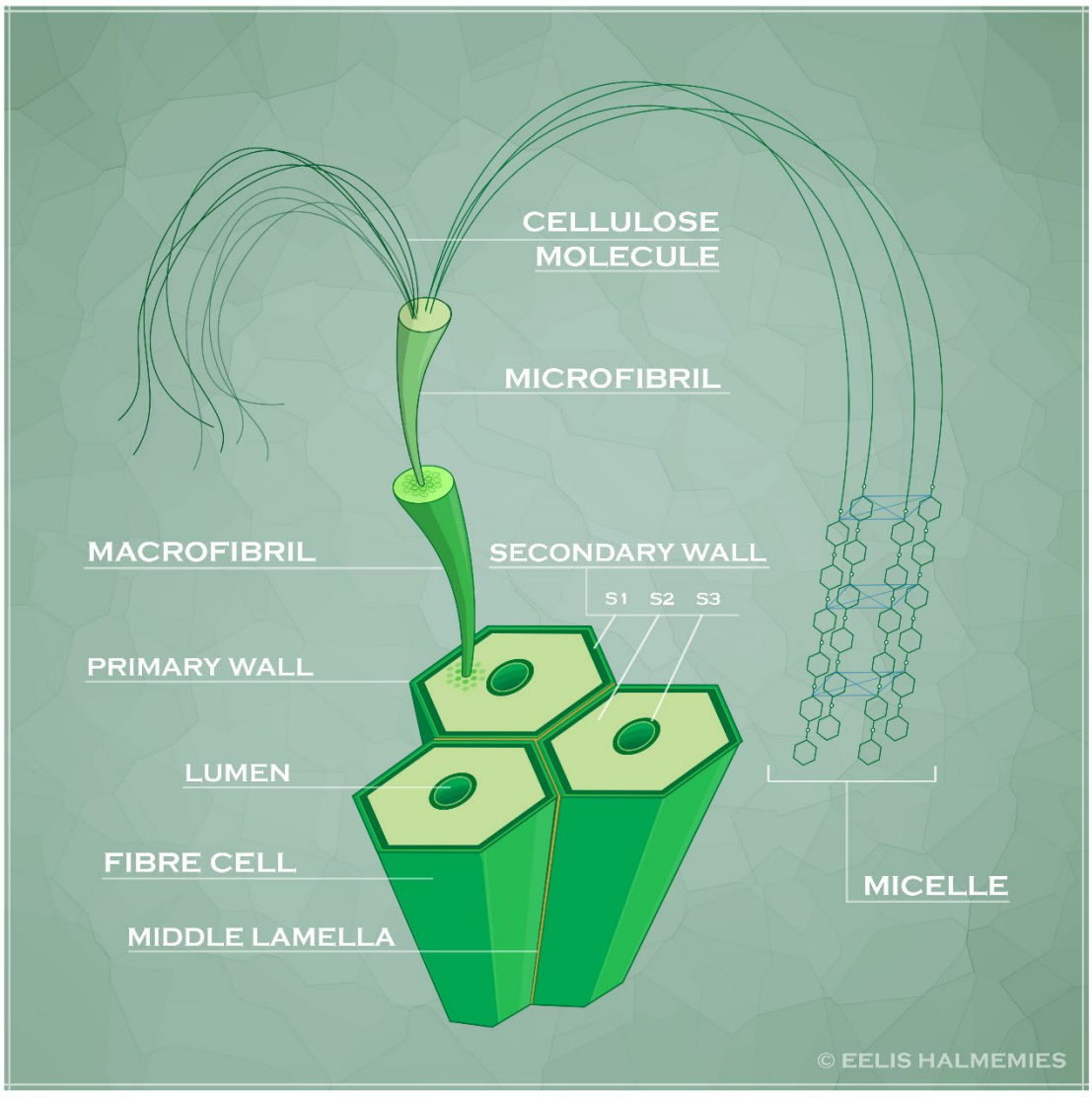


FIGURE 1. Morphology of a plant fiber cell.

1.2.2 Hemicelluloses

Approximately 20–35% of the woody biomass is comprised of hemicelluloses, mainly various heteropolysaccharides (Ek et al. 2009). Hemicelluloses are the primary constituents of plant cell walls. However, the native structure of hemicelluloses is less well-defined or well-understood than that of cellulose.
Hemicelluloses comprise various carbohydrates, such as hexoses (D-glucose, D-mannose, and D-galactose), pentoses (D-xylose, L-arabinose, and D-arabinose), or deoxyhexoses (L-rhamnose or 6-deoxy-L-mannose, and occasionally L-fucose or 6-deoxy-L-galactose). Additionally, certain uronic acids (including 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid, and D-glucuronic acid) have been detected in hemicelluloses.
Variations exist in the hemicelluloses of softwoods and hardwoods regarding the frequency and composition of sugar units. For example, the hemicelluloses of softwoods generally have a higher concentration of mannose and galactose units (Jones et al. 2017). On the other hand, hardwoods contain more xylose units and acetylated hydroxyl groups. In addition, softwoods contain primarily glucomannans or galactoglucomannans, while the primary hemicellulose in hardwoods is xylan.
Hemicelluloses are less thermally stable than cellulose due to their lower degree of polymerization and lack of crystallinity (Patel and Parsania 2018). Many hemicelluloses are also more readily hydrolyzed and solubilized by alkali and acidic solutions than cellulose (Penfield & Cambell 1990). Some hemicellulose-derived sugars (mainly mono- and disaccharides) are typically fully soluble in water, such as galactoglucomannan from Norway Spruce.

1.2.3 Lignin

Lignin accounts for 15–35% of softwood biomass. Lignin is a complex, amorphous, polyphenolic polymer with a distinct chemical structure and a high degree of heterogeneity and entropy that distinguishes it from the other macromolecules in wood. Much of the trees' structural rigidity is due to lignin. Due to its amorphous nature and irregular chemical structure, it is challenging to characterize lignin based on its morphology (Ek et al. 2009).
Although the precise chemical structure of lignin has not been fully elucidated, it is widely recognized that lignin predominantly comprises three phenylpropane monolignols: *p*-coumaryl, coniferyl, and sinapyl alcohols (Nasrullah et al. 2017). The central part of the lignin polymer comprises the apparently random interlocking of these three monolignol units, resulting in a condensed, amorphous framework that encapsulates and encompasses the secondary cell walls of plants (Figure 1). Lignin may also be found in the middle lamella, composed mostly of pectin and joining fibre cells to each other. However, non-core lignin is formed when the monolignol units bond to some of the hemicellulosic species via benzyl ether, benzyl ester, or phenyl glycoside linkages. Ether or ester bonds can also link the core and non-core lignin. The bonding between cellulose, hemicellulose, and lignin molecules primarily relies on

hydrogen bonds. Conversely, the chemical linkages between hemicelluloses and lignin are established through covalent bonds.
The composition of lignin varies depending on the plant source, particularly in the relative amounts of its monolignol subunits. Three major groups of lignins exist based on their plant origin: softwood, hardwood, and grass lignins (Gellerstedt and Henriksson 2008). Lignin derived from wood sources primarily comprises coniferyl and sinapyl alcohol units, while grass and herbaceous plants also contain *p*-coumaryl alcohol.
Lignin is a versatile additive that finds application in various fields due to several functional groups, including phenolic and aliphatic hydroxyl, methoxy, carbonyl, and carboxyl groups. These functional groups make lignin an ideal choice for use in dispersants, resins, surfactants, absorbents, and other purposes. The antioxidant activity of lignin is mainly attributed to its free phenolic hydroxyl groups.

1.2.4 Extractives

As their name suggests, extractives are chemical compounds extractable from woody biomass via organic solvents or water. Differentiation between any *extractable compounds* and *wood extractives* has sometimes been made. Some studies have treated the term *extractives* as synonymous to *resinous wood pitch*. This is most likely due to the fact that pulping industries have needed to focus on solving practical problems proposed by lipophilic extractives. One key difference between any extractable compound and wood extractive is that wood extractives are relatively small and naturally non-structural. They give trees many of their characteristics (e.g., color, odor, and taste) while also functioning as protective agents against external threats, such as insects, lesions, bacteria, and fungi (Fengel and Wegner 1984; Dorado et al. 2001). However, using an appropriate extraction procedure, structural compounds are also extractable. Thus, the composition of extracts strongly depends on the harshness of the extraction procedure used. For instance, if high temperatures and pressures or mechanical stirring are involved in the extraction, structural disintegration is likely. With woody material, this may increase cellulosic and hemicellulosic materials in the produced extracts (Leppänen et al. 2011).

Extractives can be categorized based on their respective functionalities in trees, functional groups, or polarity (Hillis 1971). While some solvents, such as acetone-water mixtures, can be used to extract both polar and nonpolar extractives, a more holistic approach to extracting the extractives of a given species usually includes a fractionation by applying multiple solvents of increasing or decreasing polarities.

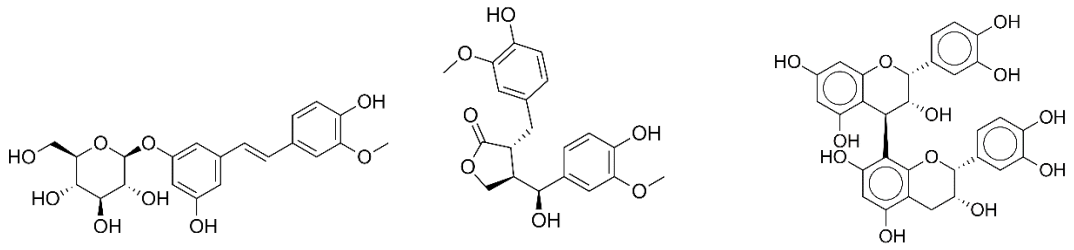
Extractives are not distributed evenly in trees. Generally, bark and pith contain the highest amounts of extractives, even up to 30–40% of the dry weight, while inner sapwood contains only a few percent. However, this generalization has exceptions; such as the Brazilian *quebracho* tree's high tannin yields in the sapwood (Gominho et al. 2007; Via et al. 2007). Generally, the extractives content is more abundant in older trees and decreases from the stump-root system to the

crown and from the bark to the pith (Jyske et al. 2014). Tree foliage also contains a significant amount of extractives, which may differ from those in wood. Similarly to how the type of flowers honeybees use to produce honey affects its quality, the tree species and environment greatly affect the quality (and quantity) of wood extractives. Chapters 3.1 and 3.2 discuss the factors affecting the extractives.

The distribution of lipophilic and hydrophilic extractives varies according to the biomass assortment. The bark and foliage of *Picea abies* contain more hydrophilic extractives in comparison to sapwood, while lipophilic extractives are primarily contained in the sapwood. Contrary to hardwoods, many softwoods, such as conifers, have resin ducts used by the tree to direct extractives from parenchyma cells to areas of lesions to protect the tree. The living parenchyma cells in the sapwood are used to synthesize and store widely varying extractives, whereas the dead heartwood cells of many conifers (such as *Pinus sylvestris)* are mainly saturated with resinous and lipophilic extractives. For instance, it is well-known that in *Pinus* trees, the pith of the stump may be concentrated with resinous extractives even long after felling, eventually producing what is called *tervaskanto* in the Finnish language, translating to tar-stump in English. The name is derived from using such stumps to produce tar in tar pits – an ancient practice known in Finland at least since 1500 A.D. and reaching its pinnacle in the 1860s (Hennius 2018).

1.2.4.1 Hydrophilic compounds

Hydrophilic extractives are extractable via water or polar organic solvents and cover a large group of molecules with different sizes and functional groups. For instance, the hydrophilic extractive groups contain, for instance, sugars, sugar alcohols, organic acids, lignans, stilbenoids, tannins, flavonoids, flavanols, and other phenolics (Holmbom 2011). Many hydrophilic compounds can readily form larger oligomers and polymers. For example, condensed tannins are polymers formed from the flavonoid proanthocyanidin. Hydrophilic compounds are also highly saturated with sugars, making them a potent source of nutrients for microbial organisms. Although the antioxidative and antifungal capacities of many hydrophilic extractives also prevent fungal invasion and degradation, the addition of glycosidic bonds increases the polarity of molecules. For instance, some stilbene aglycones are more soluble in acetone than water, while their glycosidic counterparts are readily water-soluble (Gabaston et al. 2017). High hopes for the valorization and commercialization of hydrophilic extractives have motivated the increasing study of these compounds. Figure 2 presents some of the more interesting hydrophilic extractives of *Picea abies.*

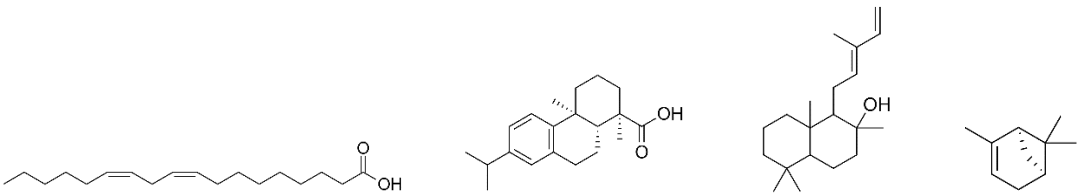


a. b. c.

FIGURE 2. Examples of commercially interesting hydrophilic *Picea abies* extractives: a) isorhapontin (among other stilbenoids), b) 7-hydroxymatairesinol, and c) proanthocyanidin B2 (belonging to condensed tannins).

1.2.4.2 Lipophilic compounds

Lipophilic extractives include those that are nonpolar and may be solvated via nonpolar solvents, such as *n*-hexane or dichloromethane. Many natural exudates of trees (aka pitch/resin) are primarily formed of lipophilic compounds. This is also the case with *Picea abies* and *Pinus sylvestris.* Common lipophilic extractive groups are glycerides, fatty acids, resin acids, steryl esters, sterols, terpenoids, fatty alcohols, and waxes (Back 2000; Ekman 2000). Figure 3 presents some of the prominent lipophilic extractives in *Picea abies.*



a. b. c. d.

FIGURE 3. Examples of prominent lipophilic extractives in *Picea abies:* a) linoleic acid (18:2), b) dehydroabietic acid, c) *trans*-neoabienol, and d) *a*-pinene.

1.3 Industrial sidestreams of *Picea abies*

This subchapter shortly describes the generally underutilized industrial sidestreams/leftovers of *Picea abies* and their potential for extractive utilization.

1.3.1 Bark

Bark is the most prominent of the sidestreams produced by the forest industry. In Finland, the annual bark production was 6.8 million m^3 in 2020 (Statistics

Finland [OSF] 2020). Most of this bark is ultimately incinerated for heat and energy. However, compared to stemwood, bark is an abundant resource for valuable extractives, such as stilbenes and tannins. Thus, much of the interest regarding added-value chemicals from forestry sidestreams has centered around bark.

Bark is a complex morphological unit comprising various tissues. It is challenging to determine the exact chemical composition of spruce bark since, even within the same species, it varies considerably between trees of different ages, heights, and environments, among other factors. It is also known that inner and outer bark compositions differ considerably. For example, the amounts of lignin and lipophilic extractives are greater in the outer bark, while the amounts of carbohydrates and hydrophilic extractives are greater in the inner bark (Krogell et al. 2012). However, industrially there is no practical differentiation between inner and outer bark, and because the main purpose of this study was to provide a helpful overview of the changing chemistry of *Picea abies* sidestreams specifically concerning their industrial utilization; thus, no differentiation between inner and outer bark was made in this study either.

Due to the high content of extractable materials in *Picea abies* bark (as well as those in many other softwoods and plants), it has been used since antiquity for many traditional purposes, such as natural remedies, food supplements, and dyes (especially tannins). Today, due to more developed simple extraction and purification methods, the extracts can be refined further to yield cosmetic ingredients, platform chemicals, resins, adhesives, and anticancer and antiviral compounds. Various types of gel filtration have been applied to purify *Picea abies* bark extracts, for instance, with Sephadex-L-20 or XAD7HP (Granato et al. 2022).

1.3.2 Harvesting residues and needles

A large proportion of the harvesting residues of *Picea abies* comprises their needles. Needles of *Picea abies* are covered with a lipophilic layer but primarily comprise carbohydrates, proteins, and hydrophilic extractives. They also contain a relatively high proportion of proteins, which, besides their vitamin and antioxidative contents, has increased interest in using them as food additives. It is known that traditionally in Finland, *Picea abies* needles were sometimes fed to livestock and horses (Lönnrot 1860). Juvenile *Picea abies* needle sprouts are also known to be edible to humans as they contain fewer extractives and 2.7 times more protein than more mature needles, as demonstrated by the study of Jyske et al. (2020). Ascertaining the feasibility of large-scale use of forest residue for biochemical applications, requires determining its chemical durability during storage. This has been studied relatively little.

1.3.3 Stumps and knotwood

Interestingly, both the stumps of *Picea abies* and inner branches, that is, knotwood, contain relatively high levels of specific hydrophilic extractives, namely lignans,

compared to stemwood or bark. According to Willför et al. (2003), seven *Picea abies* knotwood samples contained 6–24% of dry weight lignans. Methods have also been developed to efficiently harvest knotwood for lignan extraction, which has increased the potential for knotwood to be used as a raw material for producing lignan-based products, such as HMRlignan™.

Stump material has similarly high lignan content. For example, Latva-Mäenpää (2017) reported that the root neck of *Picea abies* stump contains 10% 7-hydroxymatairesinol (HMR). Despite this, stumps have generally not been utilized for their extractive content – instead, they are burned for energy. The difficulty of harvesting stumps, for example, compared to knotwood, may be the primary explanation for their comparatively limited study and use of their extractives. On a large scale, whether it is feasible to extract valuable compounds from already harvested stumps before their incineration for energy remains unanswered. However, it seems worthwhile to investigate this option further.

2 STORAGE OF WOOD-DERIVED FEEDSTOCKS

Although the ideal method of using raw material would be sufficiently efficient to omit any waiting period and direct the collected raw material immediately into the next phase of the procurement chain, this does not occur in practice. Storage appears inevitable. Despite the methods of harvesting and transporting raw material (in this case, the sidestreams of the forest industry), a distinctive period exists before the extractives can be extracted from the raw material. Furthermore, regardless of how short that period is, some alteration to the native chemical state of the raw material ought to be expected.

Harvested trees are usually stored in harvesting sites, windrows, terminals, or factory yards, sometimes covered, sometimes exposed to the elements, sometimes elevated from the ground and kept dry, and sometimes intentionally stored in water. The storage method can drastically influence the raw material's chemical composition.

2.1 Pile storage

Pile storage is standard for shredded and comminuted materials produced as side-products from the forest industry, such as chipped (debarked) wood, sawdust, bark, harvesting residue (needles), and uprooted and split stumps.

Chipped wood is commonly stored in piles in factory yards or silos for fuel for energy production or pulping. Chipped wood is known to undergo monthly material losses of 0.5–1% during storage, depending on the raw material properties and storage pile parameters (Thörnqvist 1985; Jirjis 1995). Finland and Sweden produce huge volumes of bark material from pulp and sawmills yearly. Much of this bark material is shredded and contains a widely varying materials with different particle sizes.

The pile storage of harvesting residues (treetops and branches with needles or leaves) is common practice near harvesting sites. These piles also undergo

material losses. However, according to some reports, their dry matter losses are less significant than in the storage of chips (Thörnqvist 1985; Jirjis 1995).

Bark piles are also commonly stored in factory yards or storage hubs. Material losses in the bark are known to be significant, and they commonly undergo fungal degradation. Bark piles also contain many free sugars in the inner bark, making them prone to microbial degradation (Krigstin and Wetzel 2016).

Pile storage of stumps in windrows is commonly observed where clear-cuts of forests are made or on sites being prepared for infrastructure. From the perspective of using stumps as raw material for bioenergy, pile storage of stumps can help remove the inorganic material stuck in the root-stump system and dry the material since stumps are not easily rewetted (Anerud 2012). However, from the perspective of extracting the valuable polyphenolic extractives of stumps, few studies concern the impact of pile storage.

It is noteworthy that, overall, the mechanics of pile storage differ significantly from other types of storage and may, if poorly planned, lead to catastrophic material losses (even fires). Furthermore, due to unequal exposure to degradation factors in the storage piles, heterogeneous degradation of the stored material is fundamental to pile storage. For example, how the storage piles release heat impacts the piled material differently depending on their physical location inside the pile. Chapter 3.2 provides a more in-depth discussion concerning degradation factors during pile storage.

2.2 Sawlog storage

The practices of storing sawlogs vary considerably. Sawlogs are sometimes intentionally stored wet, while dry storage is preferred at other times. While each type of storage has benefits, in terms of maintaining the original chemical composition of wood, especially regarding the hydrophilic extractive content, dry storage of sawlogs is the preferred option.

2.2.1 Wet storage

Wet storage of sawlogs can be accomplished in two primary ways: on land or in water. Storage of timbers under water is one of the oldest practices of wood storage and remains extremely common for many industries and mills. Scandinavian sawmills and pulp mills have used this storage method for timber and pulpwood during the warmest summer months. However, storage of sawlogs wet on land (by sprinkling) is the most common storage method worldwide for land-stored timber (Olsson 2005). The main goal of wet storage is to prevent timber wood from drying and thus cracking and reduce the extent of oxygenation and influence and accumulation of some fungi, molds, and insects that would otherwise present an issue.

The problem with this method is that due to constant contact with water, hydrophilic extractives, such as tannins and stilbenes, are readily and gradually

leached from the material into the storage water (Malan 2004). Wet storage is also known to accelerate hydrolysis reactions, such as glycerides releasing free fatty acids and glycerol. Depending on the pool of water where the storage is conducted, this may cause serious environmental concerns as the acidified water from extractives alone may significantly alter the pH of the pool, attracting various fungi and microorganisms and negatively affecting the ecosystem. Often minimal thought has been invested in the environmentally detrimental leachate produced by such a method (Hedmark and Scholz 2008). Under optimum conditions, a leachate rich in extractives could be collected and processed further. However, without such qualifications, while wet storage may be suitable for preventing some sources of degradation, overall, it is a poor method for preserving wood extractives and the material's initial chemical state.

2.2.2 Dry storage

Dry storage of sawlogs is the best option for maintaining the original chemical state of wood and wood derivatives. The chemical changes in bark and wood are minimal compared to pile or wet storage.

However, some degradation still occurs due to outside influences, as discussed in Chapter 3.2. The bark of *Picea abies* is much better preserved intact on sawlogs than bark stored in piles. Storing the sawlogs uncovered may expose them to more ultraviolet (UV) radiation, rain, and other degrading effects that increase material losses. Thus, a common method of shielding the raw material is using coverings.

Semipermeable coverings are often used in sawlog storage, as they shield from ultraviolet (UV) light and rain but can still pass on moisture from the trees without leading to the rewetting of the material. Covering can increase the shelf-life of woody raw material and preservation of extractives. Attempts have even been made to completely cover raw material with a sort of bag to reduce oxygenation-related issues.

3 EFFECTS OF STORAGE ON WOOD DEGRADATION

3.1 External factors

In a study of the chemical changes of any given raw material derived from trees, more factors can affect the degradation or changes in the material than can be listed here. However, the following are some of the more influential degradation factors whose impact should be considered in storage planning.

3.1.1 Time and space

It is important to understand that wood and raw materials derived from trees have no fixed chemical state. While a tree is alive and receiving nutrients, it constantly grows, produces extractives, and exhibits locational and seasonal variability in its chemical composition. Specifically, trees do not exist in a timeless vacuum. Instead, they are constantly in flux, experiencing changes caused by their surroundings while influencing the environment around them. Moreover, as time passes, the overall effect of storage will increase, for better or worse, depending on the end purpose of the stored material.

Regarding the storage of wood raw material, the effect of storage time is usually more significant for hydrophilic extractives and hemicelluloses than lipophilic extractives and lignin. Therefore, the degradation may not follow a linear path depending on the storage setup and raw material. Instead, significant and sudden losses may occur due to sporadic changes in the storage conditions, such as a large rainstorm or heat wave or the breaking of the natural defenses trees use against outside threats.

Naturally, the storage site also plays a significant role. The storage may occur in a hot, windy open field, in a damp, shadowy forest with many microorganisms, or between these extremes. Appropriate planning should precede determining the optimal choice for any given situation.

3.1.2 UV light

One of the most apparent degradation factors is the one that ultimately degrades everything on the planet: the sun and the UV light it emits. Hydrophilic compounds (mainly phenolics) are especially vulnerable to modification under UV light. While UV light may increase the calorific value of woody material by reducing moisture, it also causes photocatalytic degradation of photosensitive chemicals, such as stilbenoids and flavonoids, transforming them into hydrocarbons, as well as radical oxidation and polymerization. These reactions are often observed through color changes in the raw material. Polycyclic aromatic hydrocarbons (PAHs) are also harmful compounds that may be formed through photodegradation (Moore et al. 1963). The impact of UV light may be averted using coverings and choosing a shadowy storage site.

3.1.3 Rainfall and precipitation

Increased humidity and rain increase the moisture content of the raw material, reducing its heating value. Additionally, rainfall may cause the leaching of hydrophilic compounds, leading to problems common with wet storage (Olsson 2005). Apart from good ventilation and impermeable coverings, material may be rewetted through precipitation of escaping moisture. Generally, this facilitates the fungal growth and microbe activity leading to enzymatic degradation.

3.1.4 Thermal degradation in pile storage

Pile storage is known for the tendency of the piled material to generate heat during storage (e.g., from cell respiration and microbial activity; Krigstin and Wetzel 2016). Generally, increased temperatures facilitate chemical reactions. Hence, high storage temperatures also lead to significant extractive losses.
Notably, degradation in storage piles due to increased temperature occurs in steps and intensifies when each consequent step introduces other chemical reactions. A shift from biological to chemical degradation also occurs as the temperature increases. For example, enzymatic degradation occurs at up to 60–70 °C. The cellulosic acetyl groups are cleaved at these temperatures, releasing acetic acid and heat, further increasing the temperature to 80–90 °C (Fuller 1985). Given that many extractives are also volatile and flammable, in appropriate circumstances, the heat combined with a steady flow of oxygen can lead to thermo-oxidative chemical reactions resulting in the self-ignition of piles (Krigstin et al. 2020). This occasionally occurs in large and compact piles with otherwise minimal access to oxygen and the capacity to release heat. Pyrolysis reactions occur inside such piles; thus, ignition may occur after a sudden oxygen flow (e.g., when the pile is disturbed by an excavator). Small particle sizes can also markedly increase the self-heating and compactness of storage piles. Thus, mixing very fine particles, such as sawdust, into storage piles of larger particles is generally inadvisable.

3.1.5 Season

Evidently, seasonal variation in storage conditions significantly affects the degradation rate of wood material and the natural saturation level of certain extractives in wood tissues. Winter storage is more suitable for preserving the initial chemical state of the stored material due reduced UV light and lower ambient temperatures facilitating chemical reactions.

3.1.6 Microbial degradation

Many of the extractives of trees (primarily of the softwood family) are produced as a reaction to external stress, such as lesions and microbial activity. They are, thus, by design, antimicrobial, antifungal, and antioxidative. In some softwood species, new traumatic resin canals are formed to address outside threats, while other species, such as the *Pinaceae* family, have fixed resin canals (Wu and Hu 1997). Despite the extractives being used as a defensive measure, they are not immune to microbial degradation. Particularly, extractives with increased levels of glycosidic bonds are a good source of nutrients for microbes.

Enzymatic degradation by fungi, such as brown-rot, white-rot, and sapstain fungi, is the primary cause of initial degradation in freshly felled trees (Kallioinen et al. 2003). Fungal activity presupposes adequate temperature and moisture. Enzymes effectively catalyze the transformation of larger chemical species into smaller fragments. For example, the cell respiration of living parenchyma cells enzymatically catalyzes the breakdown of carbohydrates into carbon dioxide, water, and adenosine triphosphate (ATP):

$$C_6H_{12}O_6\,(s) + 6O_2(g) \xrightarrow{\substack{enzymatic \\ catalysis}} 6CO_2\,(g) + 6H_2O\,(g) + 25ATP \qquad (1)$$

Bacterial degradation by thermotolerant bacteria is more common in piled material with higher temperatures (Routa et al. 2017). The bacterial colonies can vary significantly depending on the species, storage setup, and raw material assortment. For example, water-stored and dry-stored sawlogs are attacked by different microorganisms.

3.2 Internal factors – material properties

Examining internal raw material properties is as important as observing the outside influencers of raw material degradation. The following section lists some expectations of different chemical and physical aspects of woody raw materials regarding storage.

3.2.1 Particle size and bulk density

Material with a smaller particle size has a greater reactive surface area. Generally, this means that storing material with a smaller particle-size leads to more material losses. Large-scale storage of chipped and debarked wood has functioned as a standard backup reserve for pulping industries, ensuring an undisturbed flow of biomass in times of low supply (Jirjis 1995). Nevertheless, chipping is also linked to significant extractive losses (Fuller 1985). Research indicates, for instance, that the extent of triglyceride degradation in wood chips stored outside for 2 months is comparable to that observed in roundwood stored for a whole year (Assarsson 1969). Whether the tree bark remains intact significantly affects wood quality maintenance. Provided the bark remains intact, it will function as a natural defense, which is lost if the material is debarked, exposing it to more attacks. This factor, the intactness of the bark after harvesting, alone can account for much of the variation observed in the chemical composition of bark from different sawlogs.

3.2.2 Heating value, moisture, and ash content

The moisture content of raw material is known to alter during storage. Moisture is generally higher during fall and winter and lower during summer, depending largely on the amount of rain and air humidity. A high moisture content increases the risk of microbial and fungal infestation over time. In *Picea abies* bark, the average moisture content is 40–50% in sawlogs and approximately 60% in piled bark. Some inhomogeneity in moisture content may occur due to differences in the exposure to UV light, humidity, and rain, for example, between the material inside and on top of a pile. Shielding materials are commonly used to help dry woody raw materials (Anerud et al. 2018).

The heating value of woody raw materials is closely linked to their moisture content but varies slightly between tree species and assortments. Storage is not known to considerably affect the heating value of woody raw materials. However, the heating value may increase if the raw material is dried.

The ash content of woody raw material is not generally known to be affected by the storage. However, the relative ash content during storage may increase somewhat due to the loss of other components, such as extractives or hemicelluloses.

3.3 Effect of storage on wood constituents

3.3.1 Cellulose

Fungal enzymatic hydrolysis, as well as bacterial consumption of cellulose, occurs during the storage of woody raw materials. In wet conditions, more significant cellulose losses due to hydrolysis are expected. Due to the hydrolysis

of cellulose, the degree of polymerization in the cellulose polymers decreases; consequently, various smaller (water-soluble or insoluble) oligosaccharides and glucose are formed (Ek et al. 2009). The degradation of cellulose also depends heavily on the raw-material properties.

3.3.2 Hemicelluloses

Similar changes in hemicellulose compared to cellulose are expected due to microbial/enzymatic degradation. Generally, some enzymes target the side chains and some of the main chains of the hemicellulose polymers. For instance, hemicellulases, such as endomannase and endoxylanase, release mannose and xylose moieties, respectively (Ek et al. 2009).
The main hemicellulose in spruce bark is the hydrophilic galactoglucomannan (GGM), mainly comprising interlinked glucose and mannose units with galactose and acetyl moieties. The accumulating acidity during storage conditions could hydrolyze GGM yielding fermentable sugars. Hemicelluloses are more readily hydrolyzed and broken down by acidification than cellulose (Sjöström 1993).

3.3.3 Extractives

Based on the total amount of extractives in any woody raw material that has been stored for weeks, it is noticeable that a marked difference exists in the degradation rate between hydrophilic and lipophilic species. Hydrophilic extractives are typically more exposed to factors that remove or leach them from the raw material. Conversely, depending on the extractive group, lipophilic and resinous compounds exhibit only minor changes in their total amount, sometimes even after a considerable time. However, this does not mean that lipophilic extractives do not change their chemical composition. The most significant chemical changes occurring during wood storage are i) the rapid hydrolysis of triglycerides and simultaneous slower hydrolysis of waxes and steryl esters, ii) the oxidation/polymerization of resin acids, unsaturated fatty acids, and iii) evaporation of volatile terpenoids (Ekman 2000).

Concerning hydrophilic extractives, phenolic extractives undergo both oxidation/polymerization and radical-induced photodegradation (Zahri et al. 2007). As many hydrophilic extractives are also glycosylated, microbes can target them more easily. Furthermore, due to moisture and rain, hydrophilic extractives are prone to leaching from raw materials. Therefore, if the storage continues for prolonged periods, the changes in hydrophilic extractives due to storage are expected to be substantial unless protective measures are applied.

3.3.4 Lignin

Lignin gives plants much of their structural rigidity. Lignin is also known for exhibiting relatively high antioxidant capabilities and has raised much interest in spruce-derived compounds with interesting applications (Zhang et al. 2019).

However, lignin is generally challenging to study precisely due to its complex nature.

With storage, lignin appears to resist degradation well. It is known that some compounds included among the extractives could be bound to lignin and conceivably be released after long storage. Rencoret et al. (2019) demonstrated that the stilbenoids of *Picea abies* could also be incorporated into the lignin mass as monolignols during the lignification process. This raises the question of how much of the extractives deemed lost during storage are actually being naturally modified and incorporated in the lignin complex. Within the scope of this study, this question is left mainly unanswered, and further research is needed to probe the question further. However, some evidence suggests the rapid loss of sugars is correlated with increasing lignin content in the dry bark.

4 MATERIALS AND METHODS

The chemical composition of the *Picea abies* samples in this study (mainly bark, but also stumps and needles) was studied primarily regarding the content of extractives but also those of hemicelluloses, cellulose, and lignin. Thus, the raw material needed to be separated into fractions. Extractives were extracted by accelerated solvent extraction (ASE) and studied qualitatively and quantitatively by gas chromatography-flame ionization/mass spectrometry (GC-FID/MS) and high-performance liquid chromatography (HPLC) methods. Extractive-free material was further subjected to acidic methanolysis and acid hydrolysis methods to determine the amounts of hemicellulosic and cellulosic carbohydrates as well as that of acid-insoluble lignin.

4.1 Extraction via accelerated solvent extraction (ASE)

Soxhlet extraction is traditionally used for extracting plant extractives by refluxing of boiled solvent for several hours. However, ASE was chosen for this study rather than Soxhlet extraction for several reasons: i) it is faster and, thus, suitable for the extraction of multiple samples; ii) it has a high level of repeatability; iii) it can be performed at temperatures and pressures above the boiling point of the solvent used, making the extraction more efficient as the solvent more easily penetrates the raw-material; iv) it is simple to switch solvents of different polarity between extractions; v) extracts are automatically collected in collection vessels; and vi) it enables precise control over the extraction temperature used.

ASE extractions were performed in tandem for a single sample: first, with *n*-hexane to extract the lipophilic extractives from the sample material and then with hot ultra-high-quality (UHQ) water to extract the hydrophilic extractives.

After several preliminary tests with the bark material to be studied, the ASE extraction method selected was as follows: a temperature of 120 °C, a pressure of

1500 psi (unalterable), an extraction cell flush of 60%, a single extraction cycle, and a nitrogen purge for 70 s.

The efficiency of the chosen extraction method was evaluated as follows: successive ASE UHQ extractions at 120 °C were performed with the fresh bark zero-samples to determine the maximum amount of extractable hydrophilics (MaxTDS); the MaxTDS was then contrasted with the total dissolved solids (TDS) from a single ASE extraction with the chosen parameters.

4.2 Gravimetric analysis of total dissolved solids (TDS)

The amount of TDS in a given extract was evaluated gravimetrically. In lipophilic *n*-hexane extracts, the TDS was measured from the whole sample by evaporating the solvent under nitrogen flow until a constant weight was achieved. In hydrophilic water/water-acetone extracts, a stock solution of 100 mL was first prepared, then 10–20 mL of the solvent was lyophilized and weighed, and the total amount of extractives was determined from the weight of the smaller fraction.

4.3 Chromatographic methods for extractive analysis

The extractives and extractive-free wood material were evaluated both qualitatively and quantitatively. The qualitative analysis of extractives was performed by GC-MS and the quantitative analysis by GC-FID with internal standards. Additionally, high-performance anion exchange chromatography (HPAEC) was used to both qualitatively and quantitatively evaluate the hemicellulose fraction of the extractive-free samples, while HPLC was used to identify and evaluate the amounts of certain hydrophilic extractives.

4.3.1 GC-FID/MS

The water and *n*-hexane extracts produced from the storage samples were analyzed qualitatively by GC-MS and quantitatively (with internal standards) by GC-FID using a modification of the method by Örså and Holmbom (1994). For details regarding the method, see **Ref. I.**

4.3.2 HPLC method for stilbene analysis

The water extracts of the winter stored sawlog bark samples (zero-sample and 24-weeks-stored sample) were analyzed quantitatively and qualitatively for their stilbene content using a modification of the method by Gabaston et al. (2017). For details regarding the method, see **Ref. I.**

4.3.3 Thiolysis and HPLC method for tannin analysis

The condensed tannin (CT, or proanthocyanidin) content of the lyophilized bark samples was analyzed using an HPLC method after a thiolytic degradation method described by Korkalo et al. (2020). The HPLC method used diode array detection (DAD) and fluorescence detection (FLD) to detect the tannin species. For details regarding the method, see **Ref. II**.

4.3.4 Folin-Ciocalteu determination of phenolic content

The total phenolic content (TPC) was evaluated from the sawlog bark samples using the Folin-Ciocalteu method described by Singleton and Rossi (1965). For details regarding the method, see **Ref. I.**

4.4 Carbohydrate and lignin analyses

4.4.1 Acid hydrolysis

The holocellulose (cellulose together with hemicelluloses) content of the extractive-free bark samples from sawlog and pile storage was evaluated using an acid hydrolysis method. For details of the method, see **Ref. I**.

4.4.2 Acidic methanolysis

The hemicellulose content of the extractive-free bark samples from sawlog and pile storage was evaluated using a modification of the methanolysis method by Bertaud et al. (2002). For details regarding the method, see **Ref. I.**

4.4.3 HPAEC method for monosaccharides

The amounts of monosaccharides in the holocellulose of the sawlog and pile storage bark samples were evaluated from the UHQ filtrate produced during acid hydrolysis using HPAEC. For details regarding the method, see **Ref. II**.

4.4.4 The evaluation of acid-insoluble/soluble lignin

The amount of acid-insoluble lignin in sawlog and pile storage bark samples was determined gravimetrically as the acid-insoluble residue from the acid hydrolysis. However, the amount of acid-soluble lignin was determined via ultraviolet-visible (UV-Vis) spectrometry from the UHQ filtrate produced during acid hydrolysis. For details regarding the methods, see **Ref. I**.

4.5 Kjeldahl analysis of total nitrogen

The protein content of the harvesting residue samples (needles) was accomplished via the Kjeldahl analysis of the total nitrogen content using a Kjeldahl apparatus with digestion and distillation units. First, 0.1 g of needle samples was weighed in glass tubes, where 15 mL of 98.3% H_2SO_4 and a single Kjeldahl tablet were added. The tubes were then placed in the digestion unit and maintained at 430 °C for 1 h 30 min. For distillation, 1 L of 40% NaOH solution and 4% boric acid were prepared in UHQ water. An indicator solution was also prepared by mixing 10 mL of bromocresol green and 2 mL of methyl red in ethanol. Then, 20 mL of UHQ water was added to sample tubes, and the tubes were placed in the distillation unit. The NaOH solution was added in excess during the distillation. The distillate was received in a mixture of 50 mL of boric acid with 20 drops of indicator solution until the volume was 200 mL. Subsequently, the distillate mixture was titrated with 0.1 M HCl until a color change from turquoise to yellow/orange occurred. The amount of titrant utilized (mL) was used to calculate the amount of solvated nitrogen. The raw protein content was calculated using a correlation factor of 6.25.

4.6 Raw materials and pretreatments

The sawlogs used in the sawlog bark studies were from *Picea abies* trees felled in 2017 in Kälviä, Western Finland. At sampling, the bark from the sawlogs was peeled off by hand, comminuted, lyophilized, and ground to bark powder (stored frozen below -20 °C) before the extraction and chemical analysis. For more details regarding the sawlog samples, see **Ref. I**.

The bark samples in the bark pile storage setup originated from the UPM-Kymmene Oyj sawmill in Ostrobothnia in 2017. The bark was freshly debarked from *Picea abies* trees and stored outside in the factory yard in Pietarsaari. Before extraction and chemical analysis, the bark samples were comminuted, lyophilized, and ground to powder (stored frozen below -20 °C). For more details regarding the bark pile samples, see **Ref. II**.

The stump samples were produced in 2017 in forests in Kälviä, Western Finland, from clear-cut and were split and lifted in piles immediately after felling. Various stump assortments were cut out via chainsaw without bar oil from the stumps, and some were crushed as a whole with a horizontal grinder. The stumps were comminuted, lyophilized, and ground to powder (stored frozen below -20 °C) before the extraction and chemical analysis. For more details regarding the stump samples, see **Ref. III**.

The studied harvesting residue samples (needles) originated from mature *Picea abies* trees. The needles were manually removed from other harvesting residues, lyophilized (for at least 3 days), and ground to powder with a Retsch

SM 100 cutting laboratory mill with <1.0 mm sieve. The resulting powders were stored frozen (below -20 °C).

4.7 Storage setups

4.7.1 Sawlog bark

Picea abies bark intact on sawlogs was stored identically both during winter and summer. Sawlogs were cut to length, elevated from ground level, and placed side by side without touching one another. The storage lasted 24 weeks, and sampling was conducted at the initiation of the storage and after 4, 12, and 24 weeks. During sampling, six discs were cut: three each from two sawlogs. For more details regarding the storage setup, see **Ref. I**.

4.7.2 Piled bark

Picea abies debarked bark was pile stored in two large piles during winter. One of the piles was left uncovered, while the other was covered with snow. Thermocouple sensors monitored the pile's temperature in different locations (top, side, middle, and bottom). The storage lasted 24 weeks, and sampling was conducted at the initiation of the storage and after 4, 12, and 24 weeks. Samples were taken from the pile's top, middle, and side during each sampling. For more details regarding the storage setup, see **Ref. II**.

4.7.3 Stump

Picea abies stump was stored in a large pile constructed from the stumps of freshly felled trees. The stumps were placed on top of older stumps to avoid contact with the soil. An excavator split the stumps into approximately three pieces during construction. The storage continued for 25 weeks, and sampling occurred at the initiation of the storage, and after 4, 12, and 25 weeks. For more details regarding the storage setup, see **Ref. III**.

4.7.4 Needles

Picea abies harvesting residue (needles) was stored in five small piles during winter and summer from freshly felled trees. The piles were constructed in harvesting sites in the woods of Kälviä, Western Finland. During winter, the storage continued for 12 weeks, and the sampling occurred at the initiation of the storage and after 4 and 12 weeks. During summer, the storage continued for 12 weeks, and the sampling occurred at the initiation of the storage and after 1, 2, 4, and 12 weeks.

5 RESULTS AND DISCUSSION

5.1 Efficiency of ASE extraction

It was found that after 11 successive ASE hot-water extractions at 120 °C, the MaxTDS for the winter sawlog bark zero-sample was 35.6% of the dry matter. The TDS for a single ASE hot-water extraction at 120 °C for the same sample was approximately 84.0% of the MaxTDS. This demonstrated that the extractions used during these experiments were reasonably efficient. Similar studies of consecutive extractions on spruce bark have reported 41.7% of the MaxTDS while elevating the extraction temperature to 160 °C, consistent with the expectation that higher temperatures would further break down the hemicelluloses and increase the total amount of soluble carbohydrates in the extract (Le Normand et al. 2012). Therefore, it appears likely that further extractions after the initial one mainly serve to increase the soluble carbohydrates from the raw material.

It was previously shown that much hemicellulose is extracted during 140 °C water extraction (Krogell et al. 2012). Thus, it appears likely that much of the extractable carbohydrates in our samples originated from hemicelluloses. Based on this, it could be argued that our extraction method was too harsh since now the hemicellulose content may be skewed to appear less than it is, while the extractive fraction of soluble sugars may appear overly prominent. However, the purpose of this study was not to determine the exact amount of native hemicelluloses (which may indeed prove a formidable task). Instead, our purpose was, having first investigated and determined an efficient extraction method, to present the chemical status of all the studied fractions (cellulose, hemicelluloses, and extractives) and their respective differences during storage.

The entire concept of efficiency of extraction is somewhat subjective and depends on the predetermined aim of extraction. For example, it could be claimed that certain acid mixtures can efficiently "extract" the whole sample – that is, render it in soluble form. However, while this would be an "efficient extraction" per se, it would not be helpful. This study aimed to efficiently extract the natural extractives of trees efficiently but in a way that does not significantly

alter their native state (although this represents a compromise). Some changes in the chemical composition of extractives (e.g., their degree of polymerization) must be expected due to extraction and further processing of the extracted material.

5.2 Chemical composition of *Picea abies* sidestreams

5.2.1 Bark

The general chemical composition of *Picea abies* bark before and after storage is shown in Table 1. It is noteworthy that initially, both in bark stored in sawlogs and in piles, hydrophilic extractives represented the most prominent chemical compound group at around 30% of dry bark. The only significant difference between the zero-samples of piled and sawlog bark was the higher cellulose and hemicellulose contents in piled bark and higher lignin content in sawlog bark. Interestingly, after the 24-week storage period, the hemicellulose content of sawlog bark significantly increased, while in piled bark, the amount of lignin increased markedly.

While the loss of extractives necessarily increased the relative amounts of other bark components, this alone appeared insufficient to account for the sudden increase in lignin in piled bark and hemicellulose in sawlog bark. It was more likely that the increase in lignin in piled bark was related to the rapid loss of extractives, primarily soluble sugars. It has been demonstrated that the phenylpropanoid pathway, which produces monolignols for lignin formation, is connected with sugar metabolism to the shikimate pathway (Herrmann and Weaver 1999). Liu et al. (2019) demonstrated a relationship between lignin accumulation and rapid sucrose degradation in pomelo fruits. Lignin accumulation in *Picea abies* bark certainly appeared to correlate with rapid sucrose depletion. In piled bark, the accumulation was noticeable, but in sawlog bark, where the decrease in sugars occurred more gradually (as is further demonstrated below), the amount of lignin was also less increased. Čabalová et al. (2021) also demonstrated a similar increase in the amount of lignin for 8-months-stored *Picea abies* bark. However, while they mainly related the increase in lignin to the hemicellulose degradation, it was concluded that the increase of lignin could be more simply linked to the decrease in the extractive fraction. This was observed in that even in only 2-months-stored *Picea abies* bark, although the hemicellulose fragment was not yet much degraded, however, the level of extractives was greatly decreased, and likewise, the amount of lignin much increased. Furthermore, a clearer dependency between the amount of remaining extractives and the amount of lignin is also observed in **Ref. II (Figure 1)**, based on the 24-weeks-stored bark pile sample. While the amount of hemicelluloses was minimally affected by the spatial location in a pile (whether the top, side, or middle of the pile), the amount of remaining extractives was affected, and where

the extractive content was decreased, the amount of acid-insoluble lignin was consistently increased.

TABLE 1. *Picea abies* bark chemical composition in sawlogs and bark pile before and after 24 weeks of storage (% of dry matter). The hydrophilic and lipophilic extractives were obtained with 120 °C hot water and *n*-hexane ASE extractions, respectively.

Constituent	***Piled bark***		***Sawlog bark***	
	Zero-sample	*24-weeks stored*	*Zero-sample*	*24-weeks stored*
Cellulose	17.2	16.0	15.5	8.7
Hemicelluloses	19.2	21.2	15.9	28.1
Lipophilic extract.	4.1	3.5	5.0	4.9
Hydrophilic extract.	33.5	10.3	30.0	14.5
Inorganics	8.5	9.0	8.1	14.5
Lignin	17.5	40	25.5	29.3

The chemical compositions of sawlog bark lipophilic and hydrophilic extractives, sorted by compound groups, are presented in Table 2. As is evident, a considerable proportion of the hydrophilic and lipophilic extractives remained unidentifiable via gas chromatography. This indicated the presence of low-volatility compounds. However, low volatility might indicate the presence of high-molecular-weight compounds, such as oligomeric derivatives from lignin or hemicelluloses. Nevertheless, low volatility is not always linked to high molecular weight. For example, polycyclic aromatic hydrocarbons such as phenanthrene (Figure 4) are compounds with relatively low molecular weight but very low volatility. Phenanthrene was probably among the unidentified compounds of bark extractives since it is readily formed as a photodegradation product from stilbenoids (Moore et al. 1963).

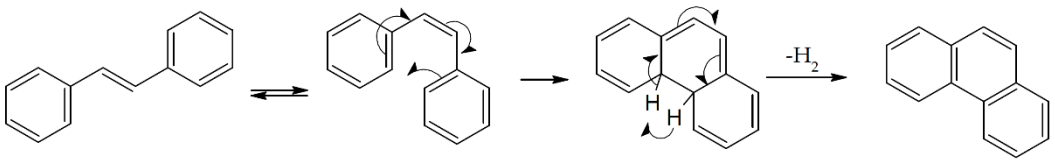


FIGURE 4. Phenanthrene is a photodegradation product of stilbenoids.

TABLE 2. *Picea abies* fresh sawlog bark lipophilic and hydrophilic extractive groups as analyzed by GC-FID/MS.

Hydrophilic extractives	***% of dry extract***	***% of dry bark***
Unidentified	48.6	14.6
Sugars	17.8	5.4
Organic acids	7.9	2.4
Stilbenes	5.5	1.6
Tannins	4.0	1.2
Distilbenes	3.9	1.2
Alcohols	3.9	1.2
Sesquistilbenes	3.1	0.9
Flavonoids	1.6	0.5
Other	3.7	1.1
Lipophilic extractives	***% of dry extract***	***% of dry bark***
Unidentified	35.3	1.6
Resin acids	18.3	0.8
Fatty acids	14.8	0.7
Triglycerides	10.3	0.5
Diterpenoids	6.3	0.3
Sterols	4.9	0.2
Steryl esters	3.9	0.2
Other	6.2	0.3

Since hot UHQ water was used as the solvent for extracting hydrophilic compounds, sugars were also a significant compound group in the hydrophilic extracts (~18% from 120 °C extractions). The amount of sugars was also found to be correlated with the extraction temperature used. Generally, the higher the extraction temperature, the higher the amount of extracted sugars. The degradation of hemicellulose species at more elevated temperatures primarily explained this result.

Ethanol or acetone mixtures could be used to target stilbenes and tannins more specifically to eliminate/reduce the amount of sugars in extracts. For example, ethanol/water (70/30, v/v) or acetone/water (95/5, v/v) mixtures were suitable alternatives to pure water. With the increase in water, the amount of unidentified compounds in extracts also increased markedly. For example, it was discovered that if *Picea abies* bark was extracted with hot water and then the extract was transferred to pure acetone, the compounds responsible for the darkest color in the solution (probably those with the highest molecular weight) were precipitated.

Organic acids, stilbenes, and tannins were also considerable compound groups in spruce bark, of which stilbenes and tannins have received the most interest due to their interesting properties and the prospect of purifying and using stilbene/tannin mixtures for added-value applications, such as coating materials, foams, and platform chemicals (Čop et al. 2015).

Lipophilic extractives totaled a relatively insignificant group of compounds as a % of dry matter compared to hydrophilic extractives. The amount of lipophilic compounds was approximately the same in actual wood samples as in

the bark (depending on tissue type and species). Among the lipophilic compounds, resin acids, fatty acids, and triglycerides comprised the majority of the free extractives. Most of the unidentified lipophilic extractives were esterified acids and sterols, which could be de-esterified and analyzed separately (**Ref. I**). Various of commercial applications are known for *Picea abies* lipophilic extractives.[1]

5.2.2 Harvesting residue (needles)

Figure 5 presents the general acetone-water (70/30, v/v) extracted chemical composition of *Picea abies* needles extractives groups. As shown in this figure, of the total mass of needles, approximately 7% were extractives, most of which (~60%) comprised sugars and organic acids. Other major component groups were sugar alcohols (11%), stilbenoids (9%), and flavonoids (6%). The lipophilic compounds comprised only approximately 6% of the extractives, mainly in the protective outer layer of needles. Bukhanko et al. (2020) reported a 3.6% ash content for *Picea abies* needles, which is relatively high.

[1] For instance, the *Picea abies* resin products of Havuka®: https://thearcticpure.com/collections/havuka.

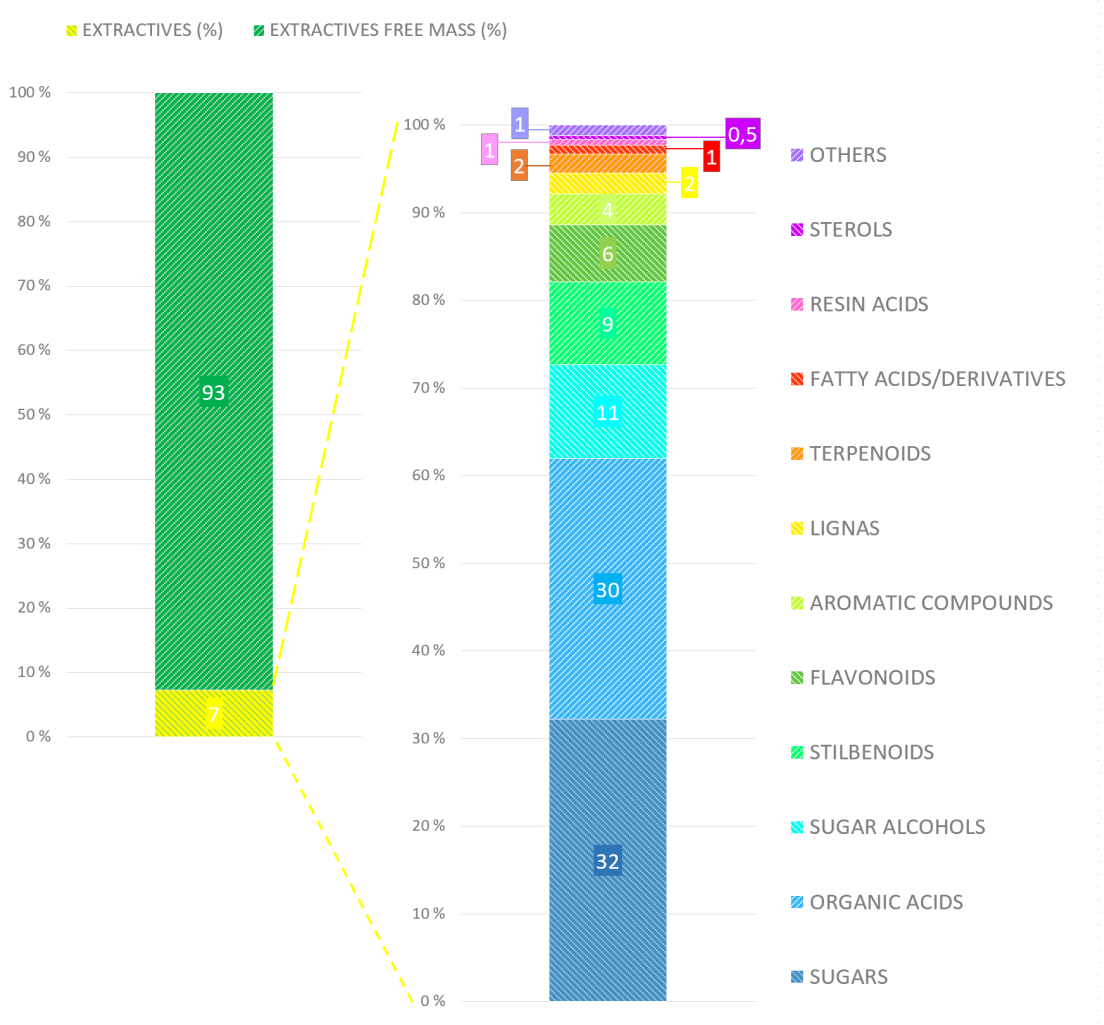


FIGURE 5. The general extractive groups of *Picea abies* needles as analyzed by GC-FID/MS from acetone-water (70/30; v/v) needle extract.

Based on the Kjeldahl results presented in Figure 6, it is evident that during wintertime, the protein content of stored needles was generally 4% higher than during the summer season. However, the effect of storing needles on the protein content was similar during winter and summer. From zero-samples to the end of the 12-week storage period, the protein content increased by 9.0% and 14.8% during winter and summer, respectively. The apparent increase in the amount of proteins was not caused by synthesizing more proteins but by reducing other naturally extractable/decaying/consumed material from the total needle mass. It is known from the literature that a large natural decrease occurs in the amounts of sugars and cyclitols in needles stored on the forest floor. One study demonstrated a 17.8% decrease in dry weight in 165 days (Theander 1982). Therefore, the increase in protein content during the storage should be viewed only as a coincidence of needle degradation and as relative rather than absolute. The results suggested that if needle material was used strictly for its protein

content, storage of 12 weeks or more could be a beneficial natural pretreatment of the raw material.

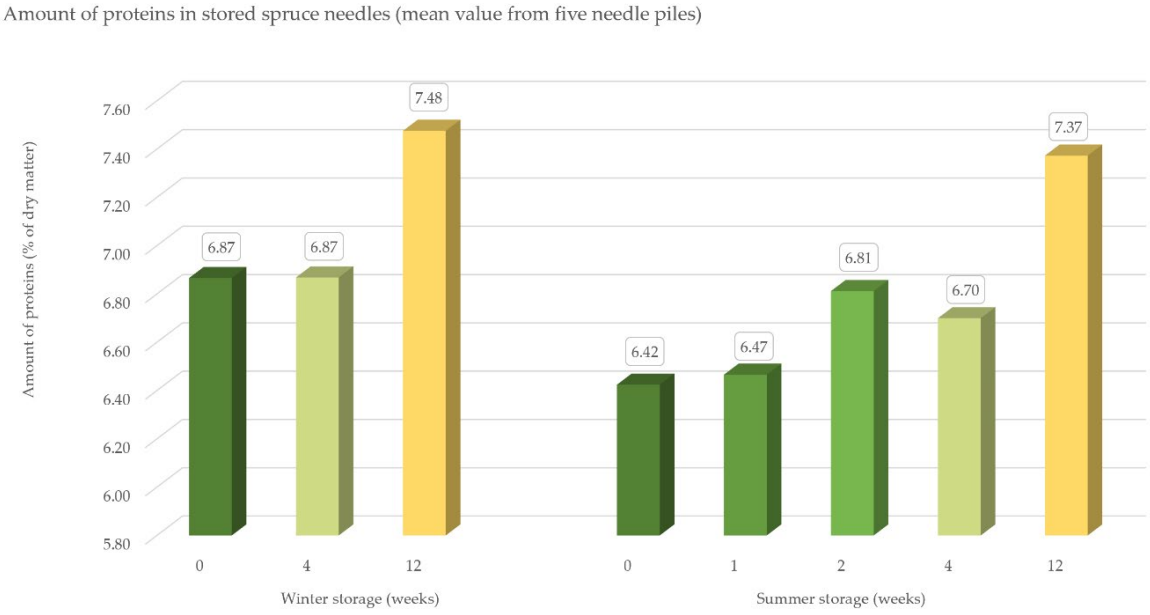


FIGURE 6. The amount of protein in *Picea abies* needles (% of dry matter) during summer and winter storage of 12 weeks as analyzed by the Kjeldahl method.

5.2.3 Stumps

The general chemical composition of *Picea abies* stumps is presented in Table 3. The results were obtained from crushed stump extractives analyzed by GC-FID. As with bark samples, a significant proportion of the stump extractives remained unidentified by GC-FID, indicating the presence of nonvolatile/polymeric species. Generally, the amount of extractives as a percentage of dry matter was relatively low compared to bark samples. Notably, however, lignans were the most prominent among the extractive groups. This increased the applicability of stump extractives due to the widely known health benefits of lignans (Saarinen et al. 2000; Špetík et al. 2022). The lipophilic compounds of *Picea abies* stumps generally appeared almost identical to bark samples based on their grouping. However, as their amount appeared very low, it was unlikely that spruce stumps would be used for their lipophilic content (a very different situation to the stumps of *Pinus sylvestris*, which may become highly resinous over time).

TABLE 3. Picea abies stump lipophilic and hydrophilic extractive groups as analyzed by GC-FID/MS.

Hydrophilic extractives	*% of dry extract*	*% of dry matter*
Unidentified	65.6	3.1
Lignans	10.5	0.5
Sugars	8.2	0.4
Stilbenes and other aromatics	5.3	0.2
Organic acids	2.5	0.1
Others	7.9	0.4
Lipophilic extractives	*% of dry extract*	*% of dry matter*
Unidentified	67.0	1.2
Resin acids	18.7	0.3
Fatty acids	4.1	0.1
Diterpenoids	2.6	0.05
Sterols	1.3	0.02
Lignans	0.9	0.02
Others	5.4	0.1

5.3 Storage setups versus TDS

The most significant aspect of this study focused on *Picea abies* bark. Various 24-week storage setups were planned and executed, after which the bark samples were similarly processed and extracted. The TDS results most clearly highlighted the differences in the storage setups. As shown in Figure 7, the hydrophilic extractives from hot-water extractives in the zero-samples constituted most of all extractives, approximately 31% of the total dry bark matter – a significant amount. However, lipophilic extractives were present in zero-samples at only approximately 4.6% of dry matter. Their total amount remained roughly constant during the storage periods.

The notable findings were as follows. i) A remarkable difference in the rate at which the TDS of water extracts was reduced in samples from pile and sawlog storage. The water extract TDS was 39% higher in sawlogs after 4 weeks, 49% higher after 12 weeks, and 36% higher after 24 weeks of storage. ii) Concerning the *n*-hexane extract TDS, a minimal difference existed between pile and sawlog storage. The pile-stored samples had a more uniform distribution of lipophilic compounds. iii) In pile and sawlog storage during summer, the decrease in water extract TDS was systematic. iv) During pile storage, the water extract TDS varied based on the sample location inside the pile (the middle, side, or top): the middle of the pile retained most of its original TDS value, the side less than the middle, and the top least of all. These differences could be primarily explained by the self-heating mechanisms of the storage piles and different exposure to outside influences in the sampling points. A more detailed analysis of the effect of the spatial location of bark material concerning its chemical composition was left out of this summary of the results and is presented in **Ref. 2.**

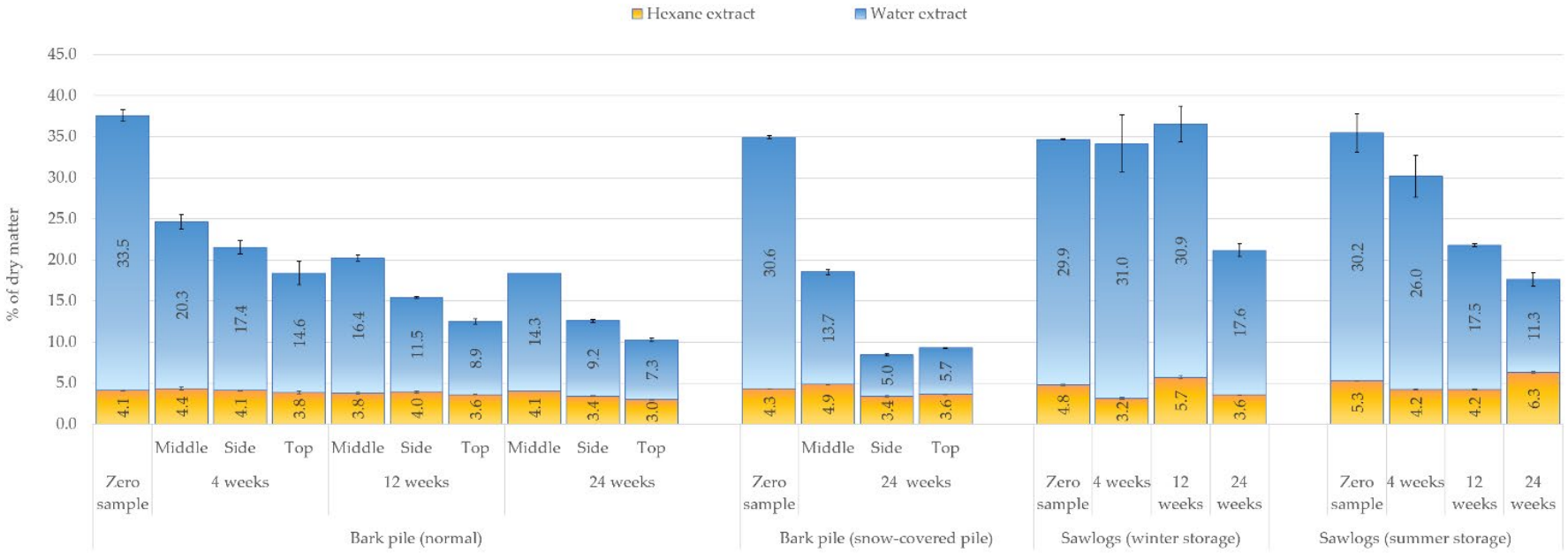


FIGURE 7. Comparison of the total dissolved solids in the bark samples stored in various ways: i) in piles during winter, a) uncovered and b) covered with snow, and ii) intact on sawlogs, a) during winter and b) during summer.

5.4 The limits of gas chromatographic identification

Identifying the individual chemical species extracted from the wood/bark samples in their extracted state from a mixture of other extracted compounds can prove challenging. Generally, chromatographic methods are best suited to separating and identifying different chemical species in the mixture. With gas chromatography, the primary tool used, only a small proportion of the produced extracts could be directly identified. While extractable, many compounds had too large a mass (being oligomeric or polymeric) to be admitted into the gas phase and transferred to the detector. Additionally, silylation, a standard procedure in gas chromatography, increasing compounds' volatility, did not make every compound volatile. Thus, from the TDS of any plant extract, only a portion could usually be identified with standard GC-FID/MS methods.

Figure 8 shows the relative amounts of identifiable and quantifiable extractives by GC-FID/MS in the water and *n*-hexane extracts from winter- and summer-stored sawlog bark samples. Regarding the *n*-hexane extracts, the total amount of identified free lipophilic extractives was approximately 51%, while the amount of esterified lipophilic extractives was slightly higher (approximately 57%). The amount of identifiable hydrophilic extractives was approximately 41%. Interestingly, while the amount of unidentified compounds remained almost constant during storage, a notable increase occurred in the amount of unidentified compounds in the water extracts during summer and winter. The difference was also visible: the water extracts from zero-samples and 4-week storage were light-brow to brown, while the samples from 12 to 24 weeks of storage were darker brown. Notably, this relative increase in unidentifiable compounds via GC-FID was concurrent with a dramatic drop in the total amount of extractives.

The relative increase in unidentified compounds could be explained in multiple ways: i) a possible increase in polymerization or photocatalytic reactions in certain compound groups, such as stilbenes; ii) an increase in the sample material of nanoscale (extractable) fine material ultimately included in the extracts, or, perhaps the most obvious explanation; iii) merely the more resilient, larger compound groups remaining since the total amount of small extractable compounds was lost more quickly.

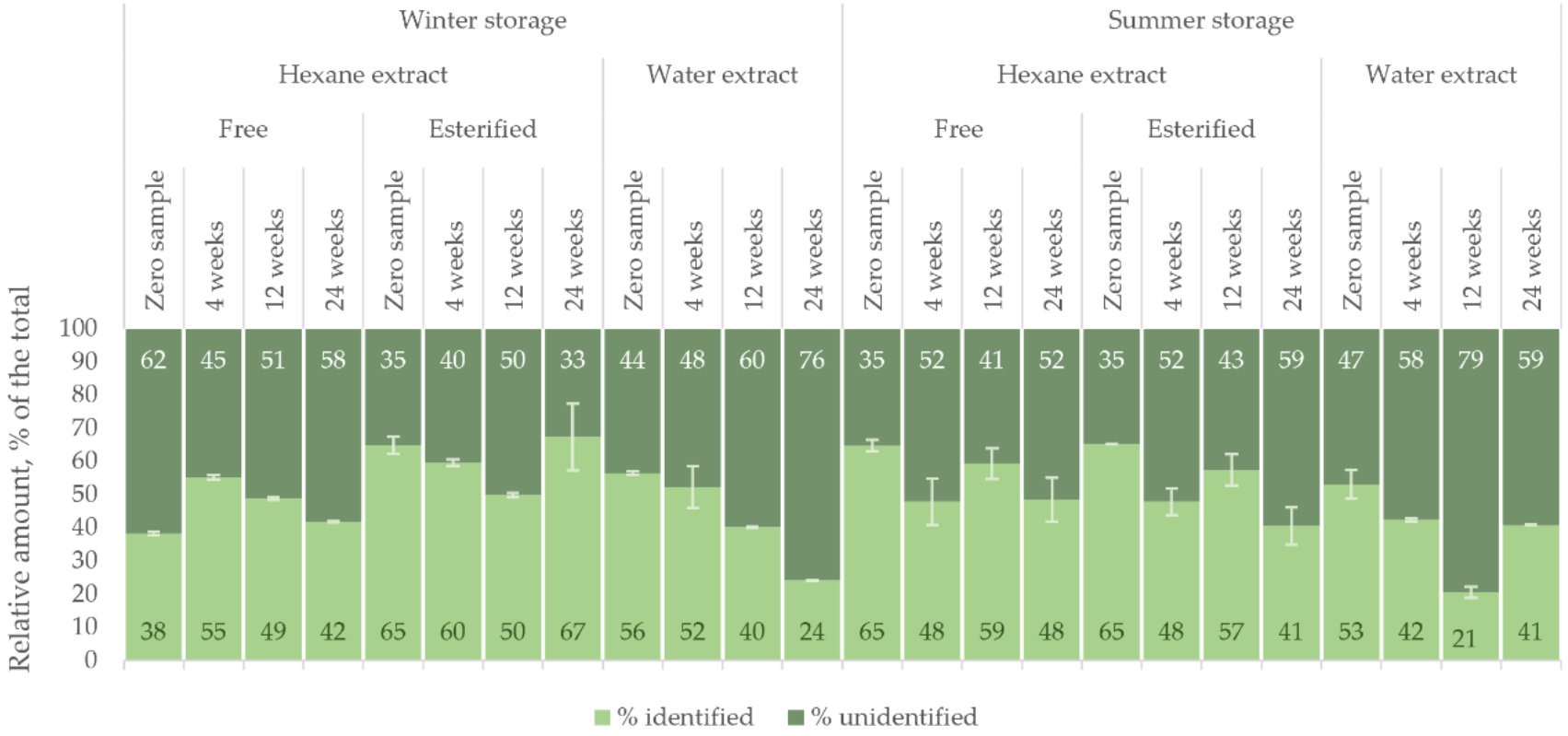


FIGURE 8. The relative amounts of identified and unidentified compounds in bark samples.

5.5 Storage setups versus extractive groups

5.5.1 Seasonal effects of storage

The seasonal effects of storage on *Picea abies* bark were evaluated by comparing two identical storage setups of sawlogs stored for 24 weeks. The results concerning lipophilic extractives showed that they underwent an increase in diterpenoids, steryl esters, sterols, and triglycerides during the summer. However, the lipophilic extractives remained resilient to degradation throughout the storage period during winter and summer.

Nevertheless, hydrophilic extractives were much more sensitive to seasonal changes, as observed in the initial amount of sugars and the rate at which the total amounts of individual extractive groups, such as sugars, stilbenes, and flavonoids, were depleted. UV light, heat, and microbial and fungal-related degradation were much greater during summertime, reflected in the faster loss of extractives.

A more detailed examination of the seasonal differences regarding extractive groups and individual extractives is given below while addressing hydrophilic and lipophilic extractives separately and considering the overall results from various storage setups.

5.5.2 Hydrophilic extractives

The effect of storage type on *Picea abies* bark could be evaluated by comparing pile and sawlog storage of fresh spruce bark (both during winter). Figure 9 presents a compilation of the hydrophilic extractive groups from the different storage setups. An overall view of the hydrophilic extractive groups revealed that in pile storage, the hydrophilic extractives were lost much faster at the beginning than in sawlog storage. However, the end result of the 24-week sawlog summer storage was close to that of pile storage. Nevertheless, winter-stored sawlogs showed a much slower decrease than other storage setups.

Comparing zero-samples of the setups confirms that the initial stages of each storage setup represented close chemical starting points for each bark sample. However, some differences were noted, especially in the seasonal effect of sawlog storage. For example, the initial amounts of free sugars and stilbenes in summer-stored sawlogs were 37% and 58% lower, respectively, than during winter storage of sawlogs, while the amount of organic acids was 79% higher.

After 4 weeks of storage, the hydrophilic extractive groups were dramatically reduced in the bark pile (approximately 48% based on the mean value of the three sampling points presented in Figure 9) compared to sawlog bark (during winter and summer storage). The covered bark pile results were unavailable for storage weeks 4 and 12.

In the winter-stored sawlogs, the total amount of hydrophilics was even slightly greater than in the zero-sample due to natural variation in the level of extractives in individual sawlogs. In the summer stored sawlogs, a 14% decrease

occurred in the total amount of hydrophilics during 4 weeks of storage. All the degrading chemical reactions were slowed considerably during winter. The decrease observed in hydrophilics during the first 4 weeks is mainly evident in the amount of free sugars, but in bark pile also in the amounts of stilbenes and flavonoids. Notably, in the bark pile, the amount of sugar alcohols was simultaneously increased by 64%, indicating the conversion of sugars into their respective alcohols.

After 12 weeks of storage, the reduction in the hydrophilic extractives in the bark pile was less than during the first 4 weeks. Nevertheless, the total amount of hydrophilic extractives was reduced in both the bark pile and the summer-stored sawlogs by approximately 30% from the level measured after 4 weeks of storage. However, the hydrophilic extractive level of the winter-stored sawlogs remained unaltered. This was an exceptional result, as it showed that, for practical purposes, the storage of sawlogs during winter could halt the degradation of extractives for at least 12 weeks given suitable circumstances. The results also suggested that in pile storage, more radical material loss occurred at the beginning stages of the storage during the first weeks, after which the degradation rate slowed considerably.

A major decrease in the extractive groups in the bark pile was observed in sugar alcohols and organic acids. In summer-stored sawlogs, all extractive groups except unidentified, distilbenes, and sesquistilbenes underwent a major decrease of 63–80%.

After 24 weeks of storage, the storage results for bark piles and summer-stored sawlogs were almost identical, although a few critical differences were observed. The amount of hydrophilic extractives in the uncovered bark pile had decreased by only 17% from the level measured after 12 weeks of storage. This reaffirmed that the rate of degradation of hydrophilic extractives is faster at the beginning stages of storage. Based on the results, sesquistilbenes significantly decreased during this storage stage, while free sugars, sugar alcohols, and organic acids increased slightly. The relative increase in sugars and their derivatives could be explained by glycosidic bonds in both the sesquistilbenes and other unidentified compounds undergoing degradation.

Furthermore, since the extraction of extractives occurred before the analysis of cellulose and hemicelluloses from the remaining material, it was expected that small molecular species stemming from cellulose and hemicelluloses degradation (and perhaps lignin to the same extent) would enter the extracts produced. Hence, mono- and disaccharides would also be released from hemicelluloses and cellulose on microbial degradation. The increase in organic acids would also naturally follow as a by-product of cellulosic degradation. For example, breaking cellulosic acetyl groups would increase the amount of extracted acetic acid.

The snow-covered bark pile generally showed noticeably similar results to the uncovered bark pile at 24 weeks. The most significant difference was that the amount of organic acids in the snow-covered bark pile was 46% lower, and the amount of sugars was 97% lower than in the uncovered bark pile. This difference

could be attributed to the snow covering of the covered bark pile, which had melted after 24 weeks and, thus, would have added cold-water extraction to the whole pile. In hindsight, it would have been interesting to take samples from the snow-covered pile while the covering remained intact to better evaluate its effectiveness in protecting against UV light and microbial activity. However, it now appears that it had practically no beneficial effect. One could instead argue that its result was detrimental because it probably caused the removal of hydrophilic extractives and mixed inorganic fine material to the raw material (from the snow covering).

In the winter-stored sawlogs, the most significant decrease (43%) in the total hydrophilic extractives was observed during this last storage stage from 12 to 24 weeks. Sugars, organic acids, stilbenes, sugar alcohols, and flavonoids decreased by 70–85% and sesquistilbenes and distilbenes by approximately 50% from the level during 12 weeks of storage. This degradation pattern resembled what occurred in pile storage after 4 weeks and summer sawlogs after 12 weeks. The ability of winter storage to protect some of the most volatile and easily lost compounds is evidenced by stilbenes still being identified in small amounts even after 24 weeks of storage.

In summer-stored sawlogs, a 36% decrease in hydrophilic compounds occurred from the 12-week level. Notably, the unidentified compounds decreased by 52% while the amounts of sugars, organic acids, sesquistilbenes, sugar alcohols, flavonoids, and other compounds increased. Especially remarkable was the 169% increase in organic acids. The observed increase in extractives is presumably linked to the breakdown of larger unidentified molecular species.

5.5.3 Lipophilic extractives

Figure 10 presents a compilation of the various lipophilic extractive groups from the various *Picea abies* bark storage setups. Overall, lipophilic extractives were more stable than hydrophilic extractives. The amounts of lipophilic compounds in bark piles decreased by only 8–14%. Resin and fatty acids were the most prominent groups throughout the storage periods. Triglycerides were more prominent at the beginning of the storage and decreased gradually, releasing more fatty acids. Interestingly, the amount of unidentified compounds appeared to increase throughout the storage, suggesting polymerization of fatty and resin acids.

Lipophilic extractive groups from the pile storage exhibited gradual and expected changes throughout the storage periods. However, with sawlog storage, the total amount of lipophilic compounds varied much more. This was expected since the extractives were gathered from the bark of individual sawlogs with natural variation in their extractive content depending on multiple variables regarding the tree (age, height, and width) but also the environment (exposure to UV light, levels of nutrients, soil type, and pollution). It is also known that the level of lipophilic compounds is affected by tree injuries and seasons (Ekman et al. 1979; Höll 1985; Krokene et al. 2008). For example, comparing the sawlogs

from winter and summer demonstrated that the level of triglycerides was 24%, diterpenoids 46%, sterols 63%, and steryl esters 74% higher in the summer-stored sawlogs. This was not a coincidence since similar results regarding the aforementioned four lipophilic extractive groups were also evident when comparing winter-stored bark pile samples to summer-stored sawlogs. Thus, while the data of sawlog lipophilic compounds were more challenging to interpret, some apparent seasonal differences and common trends could be observed, such as the gradual loss of fatty acids and triglycerides over time.

FIGURE 9. Compilation of the hydrophilic extractive groups from the various *Picea abies* bark storage setups as analyzed via GC-FID/MS. The percentage change between storage weeks is also indicated.

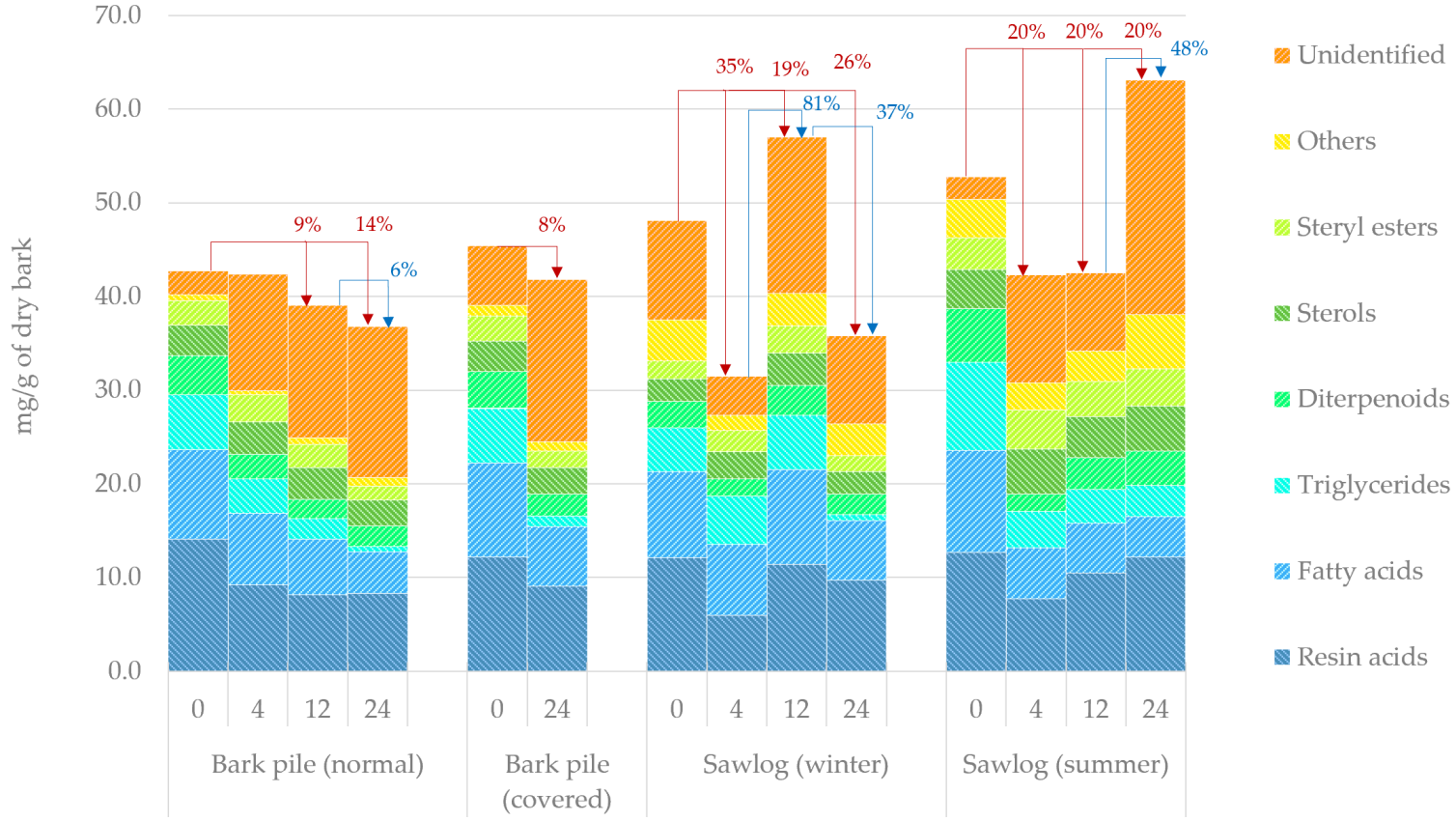


FIGURE 10. Compilation of the lipophilic extractive groups from the various *Picea abies* bark storage setups as analyzed via GC-FID/MS. The percentage change between storage weeks is also indicated.

5.6 Storage setups versus carbohydrate results

5.6.1 HPAEC results on holocellulose

The amount of holocellulose in the *Picea abies* bark samples was obtained via HPAEC. Figure 11 presents the results from sawlogs and bark pile storage setups at the initiation and end point of the storage. The relative amount of holocellulose decreased slightly if the results were based on the % of extractive-free bark. However, the total amount of holocellulose as a % of dry bark increased slightly during storage. Notably, hemicellulosic carbohydrates were also primarily included in these results. Glucose was by far the most abundant monosaccharide, while other monosaccharides (arabinose, xylose, mannose, and galactose) presented in approximately equal amounts.

The explanation for the apparent increase in the amount of holocellulose in dry bark is similar to that for the increase in proteins in needles in Figure 6. It concerns the relative increase after the extractives were consumed rather than the release/formation of new holocellulose (although some hemicellulosic species could be bound to lignin and released during storage). A similar relative increase was observed in lignin, in the amount of inorganic matter shown in Table 1, and in the amount of needle protein (Figure 6).

The largest changes occurred in the relative amounts of glucose and arabinose. In the bark pile, the relative amount of glucose increased during storage while the amount of arabinose decreased. Differences between sawlog samples were less consistent, but the amount of holocellulose appeared to decrease slightly during summer. This probably reflected seasonal variation in carbohydrate content. Many trees accumulate carbohydrates, such as hemicelluloses, during fall and use them throughout the cold winter period. Similar seasonal trends have been observed, with the starch content of *Picea abies* stem phloem and xylem peaking in October (Traversari et al. 2018).

5.6.2 Hemicelluloses

Figure 12 compiles the GC-FID results from acidic methanolysis from the studied bark samples. The results indicated a general increase in the amount of hemicelluloses in bark during storage. However, the increase was less prominent in the bark pile than in the sawlog samples, particularly during summer storage, where the amount of hemicellulose doubled. Again, it is notable that the relative increase in other bark constituents, such as cellulose, hemicelluloses, and lignin, was expected to occur due to the loss of extractives. However, the cellulose of bark during sawlog storage appeared to decrease faster than hemicelluloses. The amount of glucose in all storage setups and glucuronic acid during summer sawlog storage underwent the greatest relative increase. Increased microbial degradation of bark cellulose with warmer ambient temperatures might influence the increase in hemicellulose.

Interestingly, Čabalová et al. (2021), in their storage study of *Picea abies*, generally noted a decrease in hemicellulose content over time. Notably, however, their results were derived from a single 65-year-old tree and thus did not necessarily represent the wider view. Moreover, their extraction method would not have degraded the hemicelluloses in the sample and, probably included few hemicellulosic sugars in the extractive fraction. Hypothetically, if the amount of hemicellulosic sugars extracted by our hot-water extracts were added to the hemicellulose results presented in Figure 12, the results could show a general decrease in the hemicellulose fraction.

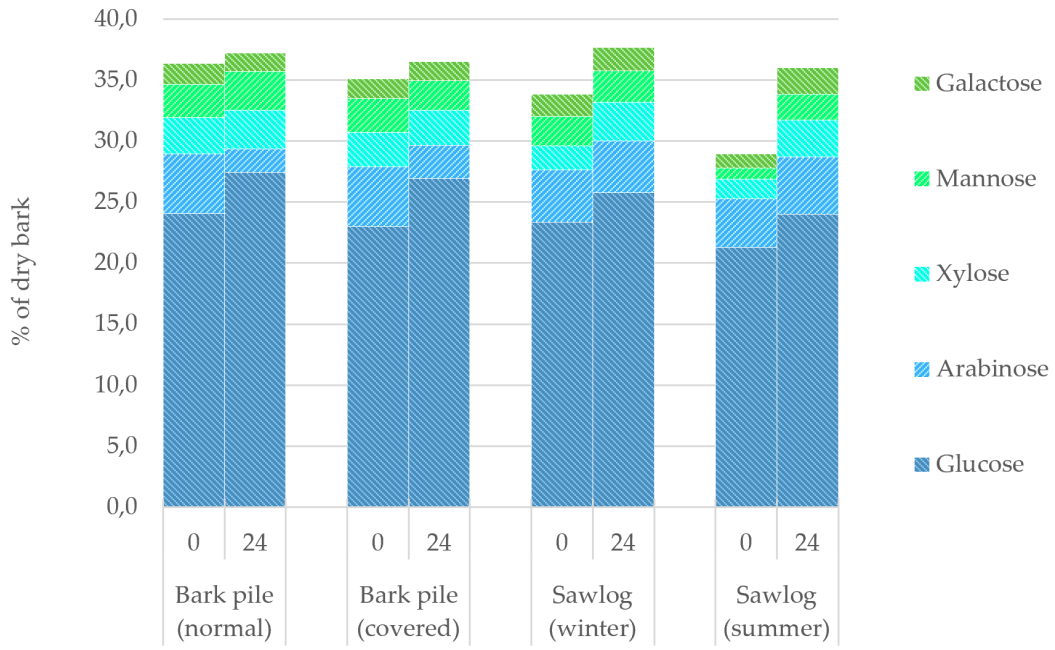


FIGURE 11 The amounts of holocellulosic monosaccharides in *Picea abies* bark samples detected via HPAEC chromatography.

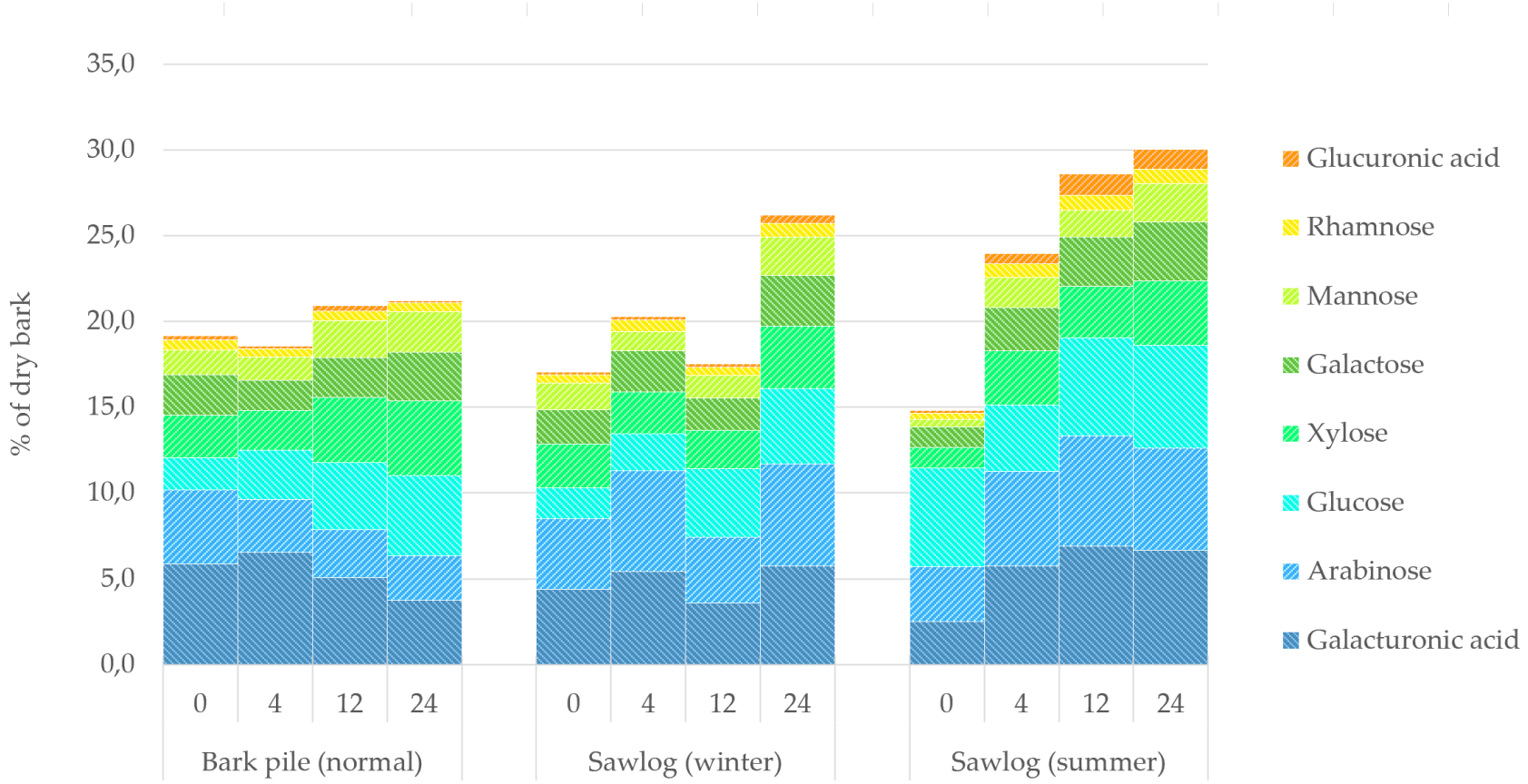


FIGURE 12. The amounts of hemicellulosic monosaccharides in *Picea abies* bark samples analyzed by GC-FID.

5.7 Storage setups versus individual extractives

5.7.1 Fatty acids

Figure 13 presents a compilation of the amount of determined fatty acids (mg/g of dry matter) from the various studied *Picea abies* assortments. The results from the bark samples also contained esterified fatty acids, while stump samples contained only those freely extractable via *n*-hexane. The presence of esterified fatty acids explained why the amount of bark fatty acids appeared much greater than that of stump fatty acids. However, since all samples originated from *Picea abies,* the order of significance of the individual fatty acids remained approximately the same in all samples. The most significant fatty acids were unsaturated linoleic acid (18:2), oleic acid (18:1), and *a*-linolenic acid (18:3). In bark pile samples, the amount of fatty acids generally decreased systematically. However, their concentration was more stable in winter-stored sawlogs and stump samples. The summer storage of sawlogs was particularly effective for fatty acid degradation.

It has been demonstrated that fatty acid degradation is limited during wet storage, indicating that oxidation reactions significantly impact their degradation (Ekman 2000). Hydrolysis reactions of triglycerides are also known to release more free fatty acids during storage. However, oxidation of fatty acids is common and can lead to the formation of radical species and volatile smelly aldehydes, such as pentanal or hexanal (Roffael 2016).

5.7.2 Resin acids

Figure 14 presents a compilation of the amounts of resin acids (mg/g of dry matter) determined from the various studied *Picea abies* assortments. The bark samples also contained esterified resin acids, while the stump samples contained only free resin acids. Together with fatty acids, they formed the most abundant lipophilic extractive groups in *Picea abies.*

In pile storage, the amount of resin acids decreased after 4 weeks of storage but then remained relatively stable. In stump samples, a slight decrease over time was also apparent. However, more fluctuation occurred in the amount of resin acids in sawlog bark. In most samples, dehydroabietic and isopimaric acids were the most abundant.

Resin and fatty acids undergo oxidation, degradation, and polymerization during storage (Roffael 2016). The observed results were consistent with the literature suggesting that during storage, the amount of abietadienoic resin acids with conjugated double bonds (such as levopimaric acid) decreases while the amount of dehydroabietic acids increases (Hemingway et al. 1971; Quinde and Paszner 1991). Loss of levopimaric acid could be attributed to radical oxidation reactions.

Previous studies regarding the seasonal variation of lipophilics in *Picea abies* samples have concluded that in *Picea abies* twigs, palustric and neoabietic acids

are more abundant during summer (Lorbeer and Zelman 1988). However, no such difference could be observed in bark samples. Instead, the bark samples of individual trees varied too much to determine any trends. Our results regarding lipophilic species mostly concurred with these observations.

5.7.3 Sterols and steryl esters

Figure 15 presents a simplified compilation of the major sterols (mg/g of dry matter) determined from the various studied *Picea abies*. Again, the results from bark samples also included esterified sterols, while the presented results from stump samples included only non-esterified sterols. Based on the results, sitosterol appeared to be the major sterol in *Picea abies* in all samples. However, a more prominent sterol might exist in an esterified form that could not be identified but only included in the amount of esterified sterols. Approximately half of the sterols appeared to exist naturally as steryl esters. Literature evidence indicates that steryl esters undergo slow hydrolysis during storage (Ekman 2000). Whether this occurred with our samples remained unclear.

Concerning degradation during storage, the amount of sterols was generally very stable. A slight decrease was apparent in the total amount of esters in bark samples from the bark piles observed at the end of the storage. Moreover, the amount of sitosterol gradually decreased in the uncovered bark pile. Similar conclusions were drawn concerning sterols in the winter-stored sawlogs. The concentration of sterols in the summer-stored samples was noticeably higher (2–3 mg/g of dry matter) than in the winter-stored samples. This result could be explained by seasonal variation in the lipophilic extractives in the bark. Stump samples had few free sterols with no indication of degradation during storage. The general distributions of sitosterol and campesterol were similar to those in bark samples.

5.7.4 Terpenoids

Terpenoids are substances found in plants, fungi, and animals and are responsible for the aromas and flavors of essential oils (Geron et al. 2000). They possess several beneficial properties, including anti-inflammatory, antiviral, and anticancer properties. Monoterpenoids, which are especially volatile, contribute to the scent of wood. The most common monoterpenoids in *Picea abies* are *a*-pinene, *β*-pinene, camphene, and limonene. Unfortunately, the GC-MS methods used to analyze our samples prevented the direct detection of monoterpenoids due to solvent delay. However, their maximum concentration in the studied lipophilic extracts could be determined from their inclusion in the unidentified compounds shown in Figure 10. A more detailed study of the behavior of individual *Picea abies* monoterpenoids during storage could be an exciting topic for further research.

According to Muilu-Mäkelä et al. (2021), the emission of monoterpenoids decreases during storage, and the rehydration and dehydration rates can affect the emission amount. Terpenoids are also prone to oxidation, hydroxylation,

epoxidation, and isomerization reactions, which can be facilitated by enzymes produced by fungi, such as laccases. For example, oxidation reactions were observed to affect limonene through various enzymatic oxygenation pathways (Wang et al. 2014). Microorganisms inhabiting plants, soil, and aquatic habitats are also known to use terpenoids as both carbon and energy sources (Marmulla and Harder 2014).

Figure 16 presents a compilation of the major diterpenoids (mg/g of dry matter) determined from the various studied *Picea abies* assortments. Diterpenoids were a significant lipophilic extractive group in the bark samples. As with lipophilic extractives, their amounts in the stump samples were generally approximately tenfold lower. Generally, the initial amount of diterpenoids in the bark pile- and the sawlog-stored samples was 4 mg/g of dry matter. The amounts of diterpenoids in the stored sawlogs during the summer were much higher, with noticeable increases in thunbergol and manool. As with sterols, this appeared to be related to the seasonal difference in bark samples. Alongside thunbergol, Δ-13-(*trans*) neoabienol was overall the most abundant diterpenoid, both comprising approximately 21% of the total amount of diterpenoids. In bark pile samples, thunbergol was quickly lost during storage and from the sawlogs stored during summer.

5.7.5 Triglycerides

Triglycerides, otherwise known as fats, are esters of fatty acids of various lengths that comprise a major lipophilic fraction of softwoods and hardwoods (Ekman 2000). They can be divided into saturated and unsaturated fatty acids. Generally, these compounds break down relatively quickly over time, releasing free fatty acids. Evidence of this is observed in our results in Figure 10. Research showed that the rate of hydrolysis is accelerated during wet storage. After 4 months of water storage, only 30% of the original triglycerides remain in spruce log sapwood (Ekman and Hafizoglu 1993). This degradation is probably due to the action of fungal lipase enzymes (Sharma et al. 2001).

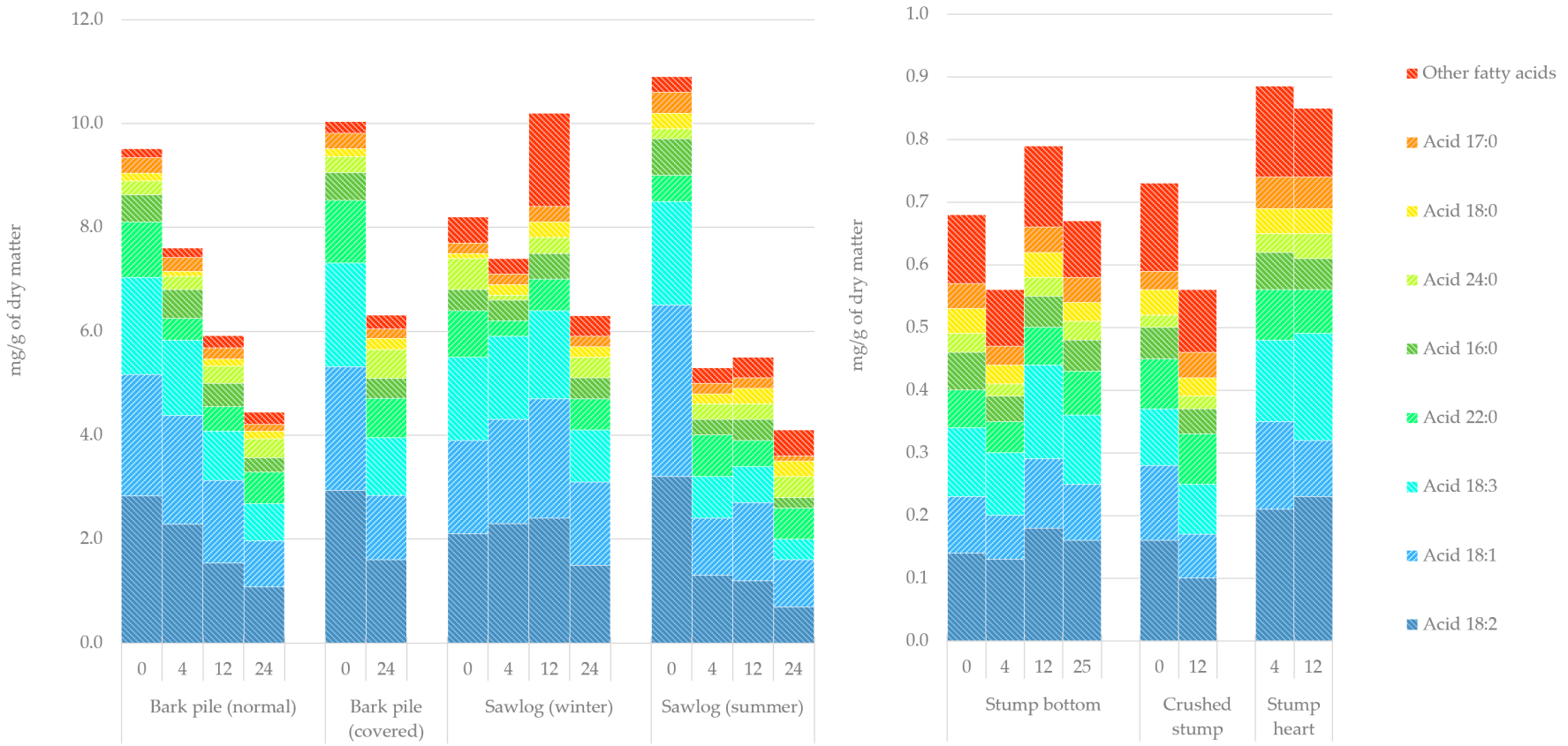


FIGURE 13. The compiled results for the main fatty acids found in the *Picea abies* samples. The bark and stump samples are presented on separate scales

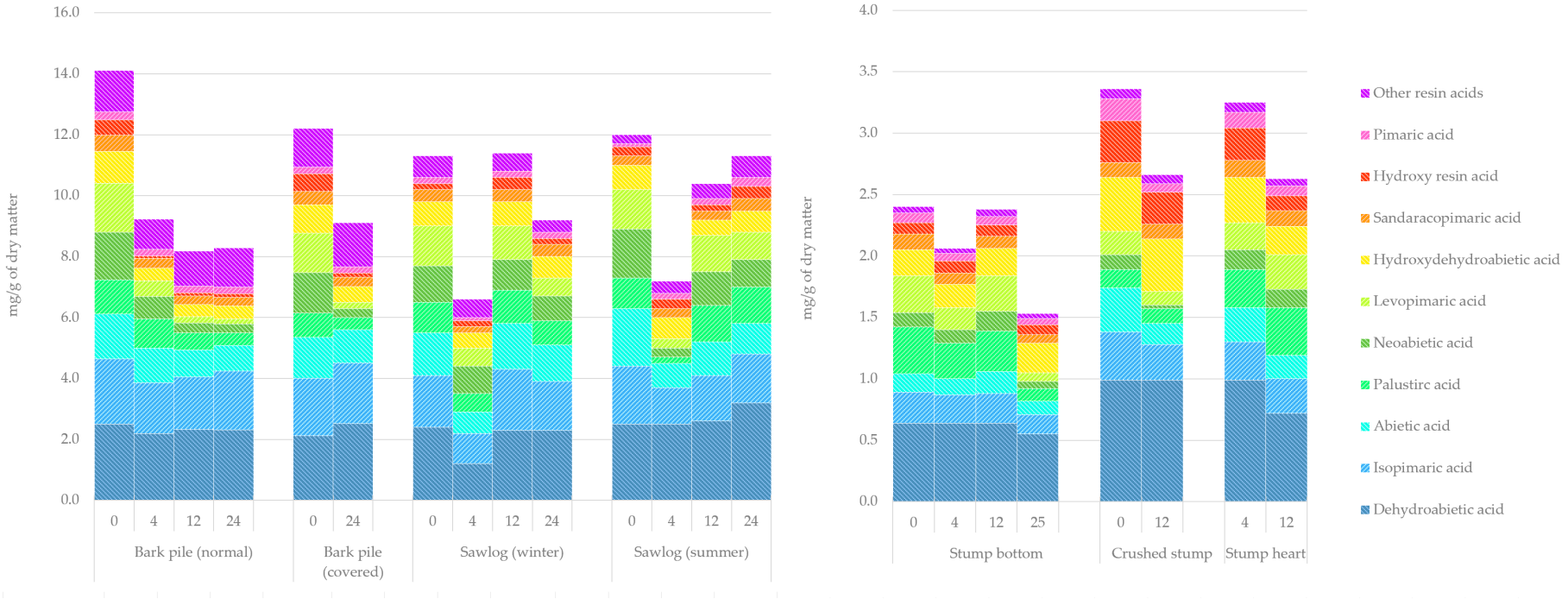


FIGURE 14. The compiled results of the main resin acids found in the *Picea abies* samples. The bark and stump samples are presented on separate scales.

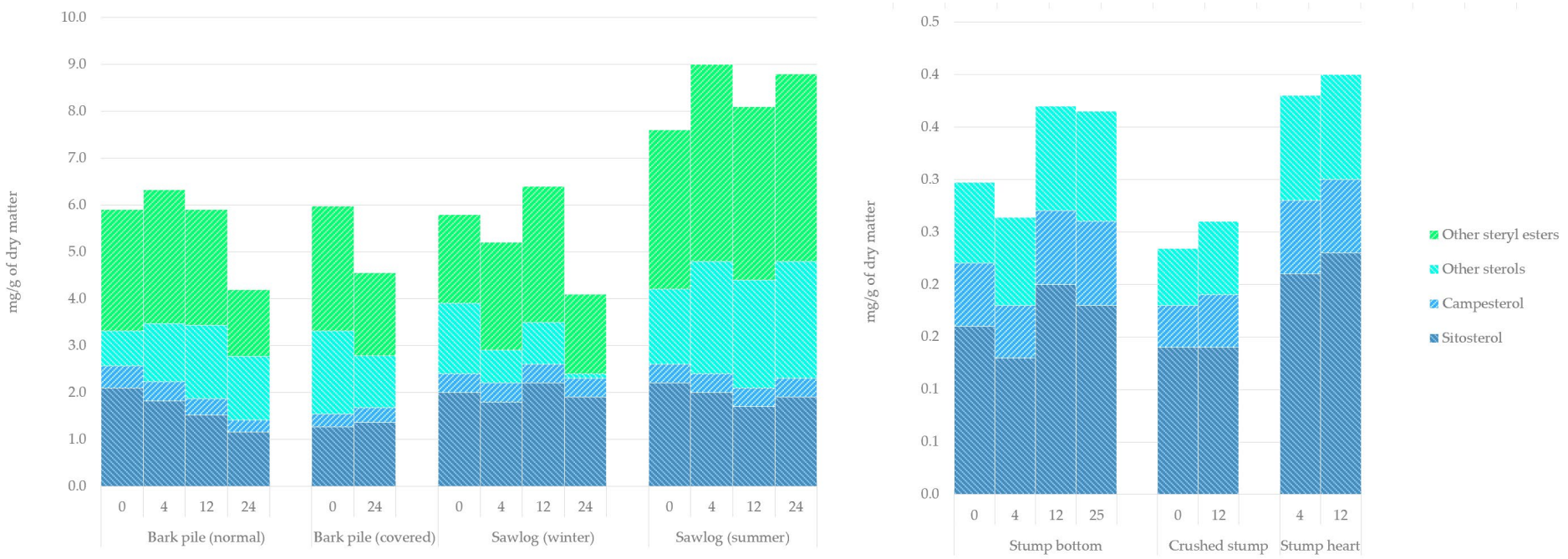


FIGURE 15. The amounts of sitosterol, campesterol, and other sterols as well as steryl esters presented from the various studied *Picea abies* assortments. The bark and stump samples are shown on separate scales.

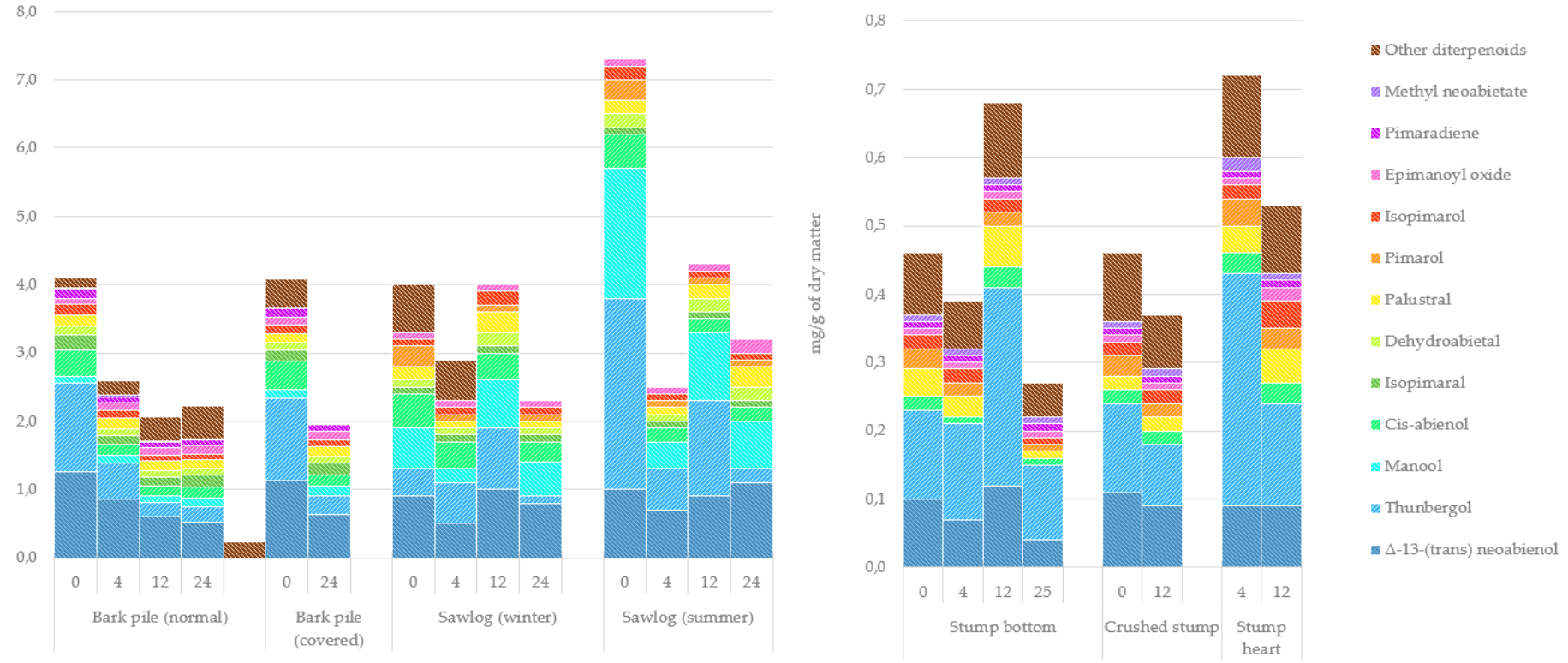


FIGURE 16. The amounts of diterpenoids from the various studied *Picea abies* assortments. The bark and stump samples are presented on separate scal

5.7.6 Sugars

Figure 17 presents a compilation of the various sugars identified in the hydrophilic *Picea abies* extracts. Different derivatives of glucose and sucrose comprised the vast majority of the identified sugars. The results presented represent the summation of all the various sugar derivatives. The large amounts of these species visible in the GC-FID spectra suggested that many extractives exist naturally as glycosides, and ionization in mass spectrometry may produce various glycosidic fragments. Moreover, it is known that each tautomeric form of silylated sugars with free carbonyl groups in GC-FID produces individual (sometimes overlapping) peaks, making the quantification and qualification of extractive mixtures challenging (Ruiz-Matute et al. 2011). Exploring other silylation methods may help solve the problem.

The initial amount of extracted sugars in the bark samples in piles and winter sawlogs was similar. The slightly higher amount of sugars in bark pile samples could indicate that the hemicellulosic sugars underwent more degradation and terminated in the water extracts in bark piles. However, the initial amount of sugars in summer sawlogs was less than half that in other bark samples. This indicated a significant seasonal effect of the warm summer on the amount of extracted sugars. It is known that during winter, many trees store their carbohydrates as energy reservoirs and protection against the cold, and to be used later during the growth period (Regier et al. 2010). *Picea abies* is also known for increased levels of sugars in trunk xylem tissue during cold periods (Wolfgang 1985). Among the summer-stored stump samples, the amount of sugars was generally very low. The highest amount of sugars was observed in the crushed stump samples, which included stump bark.

A notable difference was observed in the degradation rate in the bark pile and bark sawlog samples. In the sawlogs and stump samples, the level of sugars decreased gradually throughout the whole storage period. Conversely, in bark piles, a dramatic decrease of approximately 80% occurred during the first 4 weeks of storage, indicating either the presence of carbohydrate-consuming microbes or, as mentioned previously, the possibility of enzymatic conversion of these sugar constituents to monolignol building blocks forming new lignan (see Chapter 6.1.1). It was also notable that the increase in certain sugar species, such as galactose, during storage was only observed in pile storage, possibly indicating the degradation of the hemicellulose galactoglucomannan.

5.7.7 Sugar alcohols

Figure 18 presents the amounts of extracted sugar alcohols in the studied *Picea abies* samples. The initial sugar alcohol levels were almost identical in all bark zero-samples, with pinitol comprising more than half of all the sugar alcohols. After 4 weeks of storage, the amounts of sugar alcohols in the uncovered bark pile significantly increased, especially mannitol and inositol – probably due to enzymatic activity by microbes (Slatner 1998). A radical decrease in concentration followed this after 12 weeks of storage. In sawlogs, the amounts of sugar alcohols

decreased slightly during winter storage until week 12 and then decreased to very low levels at 24 weeks of storage. In summer-stored sawlogs, the decrease in sugar alcohol content was faster, reaching the lowest point after only 12 weeks of storage. In stumps, as with sugars, the highest concentrations occurred in the crushed stump samples due to bark material in the sample matrix.

5.7.8 Organic acids

Figure 19 presents a compilation of the organic acids identified among the hydrophilic *Picea abies* extracts. The most prominent organic acids in all samples were the gluconic, citric, and quinic acids. Pile storage had a stronger initial effect on the amounts of organic acids than sawlog storage. However, the acid levels in piles remained approximately the same after 4 weeks. In the sawlogs during winter, minimal degradation occurred until week 12, but after 24 weeks, the level of organic acids was lower than in the bark piles. Equally low levels of organic acids were reached during summer sawlog storage after 12 weeks. A considerable amount of organic acids, such as L-glutamic acid, was formed during the pile storage due to microbial degradation, which did not occur in other storage samples (Li 1965). In stump samples, citric acid was the most prominent, while gluconic acid was less represented than in bark samples. The amounts of organic acids decreased in all stump assortments, and the crushed stumps generally had the highest organic acid concentrations.

5.7.9 Flavonoids

Figure 20 presents a compilation of the flavonoids identified among the hydrophilic *Picea abies* bark extracts. Stump samples were not included in this figure because so few flavonoids were identified that their comparison would not be meaningful.

A similar profile was apparent for the bark zero-samples. Taxifolin glycoside, naringin, catechin, and taxifolin were the most prominent flavonoids in the samples, with ampelopsin appearing in some samples, especially during summer sawlog storage.

Generally, the degradation rate of flavonoids was fast, especially in pile storage. After 4 weeks, only a few flavonoids remained, while no apparent loss of flavonoids from sawlogs was observed during winter storage. During summer sawlog storage, flavonoids appeared to be degraded slightly faster than during winter sawlog storage.

5.7.10 Stilbenoids

Stilbenes are among the most intriguing extractives available from *Picea abies* due to their multiple applications in many fields, such as platform chemicals, pharmaceuticals, and antioxidants (Zahri et al. 2007). However, stilbenes are relatively sensitive to photodegradation under UV light. Figure 21 presents a compilation of the stilbenoids identified among the hydrophilic *Picea abies*

extracts. Stilbenes were rapidly lost in pile storage. After 4 weeks, the majority of them were lost. However, stilbene aglycones, such as rhapontigenin, piceatannol, and resveratrol, increased during pile storage. This was less evident in sawlog storage of bark. Approximately half of the available stilbenoids existed as distilbenes or sesquistilbenes in winter pile and sawlog storage zero-samples. The amounts of these larger stilbenoids were relatively stable in summer sawlog bark.

Among the individual stilbenoid glucosides in *Picea abies*, isorhapontin, astringin, and piceid were the most prominent. These were the only stilbenoids found in the stump samples. The amount of astringin was significantly lower during summer than during winter storage of sawlogs.

The degradation rate of stilbenoids was clearly lower in sawlog storage, especially during winter sawlog storage. Additionally, the amounts of distilbenoids and sesquistilbenes appeared more stable during storage than the individual stilbenoids.

5.7.11 Tannins

Condensed tannins (CTs), also known as proanthocyanidins, found in many plants are common antiviral and antibacterial polyphenols comprising interlinked flavan-3-ol units (Lacoste et al. 2015; Das et al. 2020). Proanthocyanidins are the primary cause of red coloring in autumn leaves. CTs also exist abundantly in the inner bark of *Picea abies*. Their extraction and use for their adhesive and foaming properties and the possibility of forming composites have recently received much attention. Other plants also have hydrolyzable tannins; however, they could not be found in *Picea abies* bark. CTs resist hydrolysis, while hydrolyzable tannins are easily hydrolyzed by enzymes, such as tannase, yielding ellagic or gallic acids (Bhat et al. 1998).

Based on the study by Jyske et al. (2020), the amount of CTs in *Picea abies* bark is more significant in winter-stored than in summer-stored sawlogs. UV results, being more unspecific and targeting other phenolics, show the amount of tannins to be as high as 100–200 mg/g of dry matter, while HPLC results show 20–50 mg/g of dry matter.

The amount of proanthocyanidins in the bark pile samples was evaluated by HPLC following thiolysis. The results revealed that the initial amount of proanthocyanidins (procyanidins and prodelphinidins) was 30–32 mg/g of dry matter, a result comparable with those mentioned above. However, after 4 weeks, the amount was only 6–15 mg/g – a more than 50% reduction from the original amount. After 24 weeks, only 10% of the initial amount remained. Rapid depolymerization (from degree of polymerization (DP) 8 to 3) of the tannin species appeared to occur during storage. Moreover, higher concentrations in the middle of the pile suggested that bark in the middle could be shielded from some degradation. Similar results were observed concerning many other extractives. Generally, it appeared that prodelphinidins resist environmental stress better than procyanidins.

Being highly hydrophilic, CTs could easily be leached out due to rainfall, air humidity, or condensates forming after the heating of the pile. The photodegradation of tannins from wood leachate via UV light has also been demonstrated, but this would not affect the inside of the pile (Petridis 2011). However, despite being more tolerant of fungi and bacteria than hydrolyzed tannins, CTs can also be consumed by microbes (Bhat et al. 1998). Another interesting known aspect is that in the presence of strong acids, CTs may also be converted to water-insoluble phlobaphenes (Roffael 2016). While acids are also lost during pile storage, it is also known that acid formation occurs during the pile storage of woody material. As reported by Fuller (1985) regarding the pile storage of wood chips, at 60–70 °C, the acetyl groups in glucose are cleaved to form acetic acid. A compilation of all these factors could probably explain the degradation of tannins in bark piles.

5.7.12 Lignans

Lignans are extractable compounds found in plants, comprising two or more phenylpropanoid molecules linked by covalent bonds (Bylund et al. 2005; Miura et al. 2007). They have been studied for potential health benefits, such as cancer prevention, inflammation reduction, antioxidant activity, and diabetes treatment. Only a minuscule amount of lignans was present in the *Picea abies* bark and needle samples. However, in the stump samples, lignans were the primary extractive group among the hydrophilic extractives. Thus, Figure 22 presents only a compilation of the results of the stump lignans. The results compare lignans detected in the stump water extracts (both with GC-FID and HPLC) and lignans from *n*-hexane extracts (GC-FID).

Notably, most lignans were generally detected via GC-FID. With the HPLC method used, reliable identification of the minor lignans was impossible. However, the identifiable lignans revealed strong similarities to the GC-FID results. Notably, sample preparation (e.g., silylation in GC-FID) and differences in the quantification methods used (internal standards in GC-FID and external in HPLC) would affect the result slightly. Lignans in *n*-hexane extract were a minor extractive group. Some very hydrophilic lignans, such as conidendric acid, were not extracted, while lipophilic lignan pinoresinol was present only in the *n*-hexane extract. HMR was the most abundant of all the studied extracts. Most of the available lignan-derived products from *Picea abies* are focused on HMR. Conidendric acid and todolactol were the next most abundant lignans.

Concerning degradation during storage, no clear trends and high variation were observed between individual stump samples. Lignans appeared well-protected; however, their amount was reduced after weeks of storage. Thus, if stumps were harvested to extract lignans, the results suggest this should be a viable option for up to 12 weeks of storage.

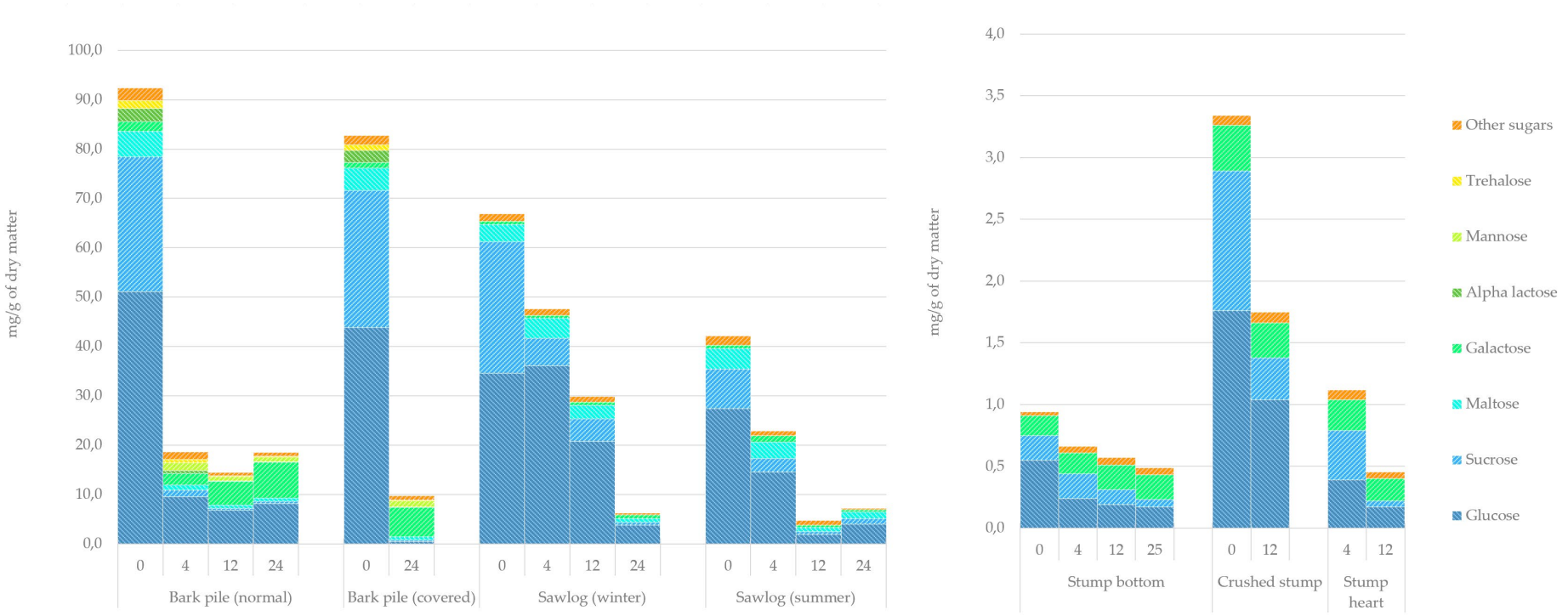


FIGURE 17. The amounts of extracted sugars in the *Picea abies* samples studied.

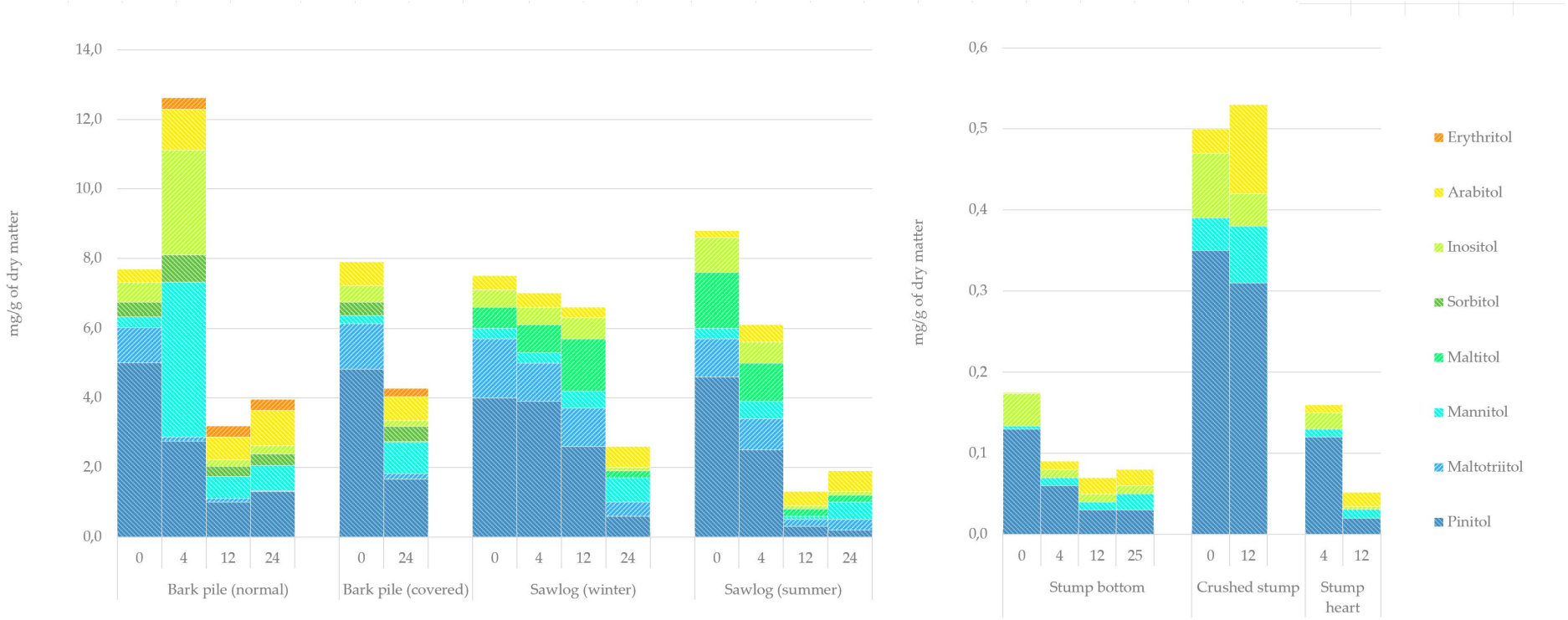


FIGURE 18. The amounts of extracted sugar alcohols in the *Picea abies* samples studied.

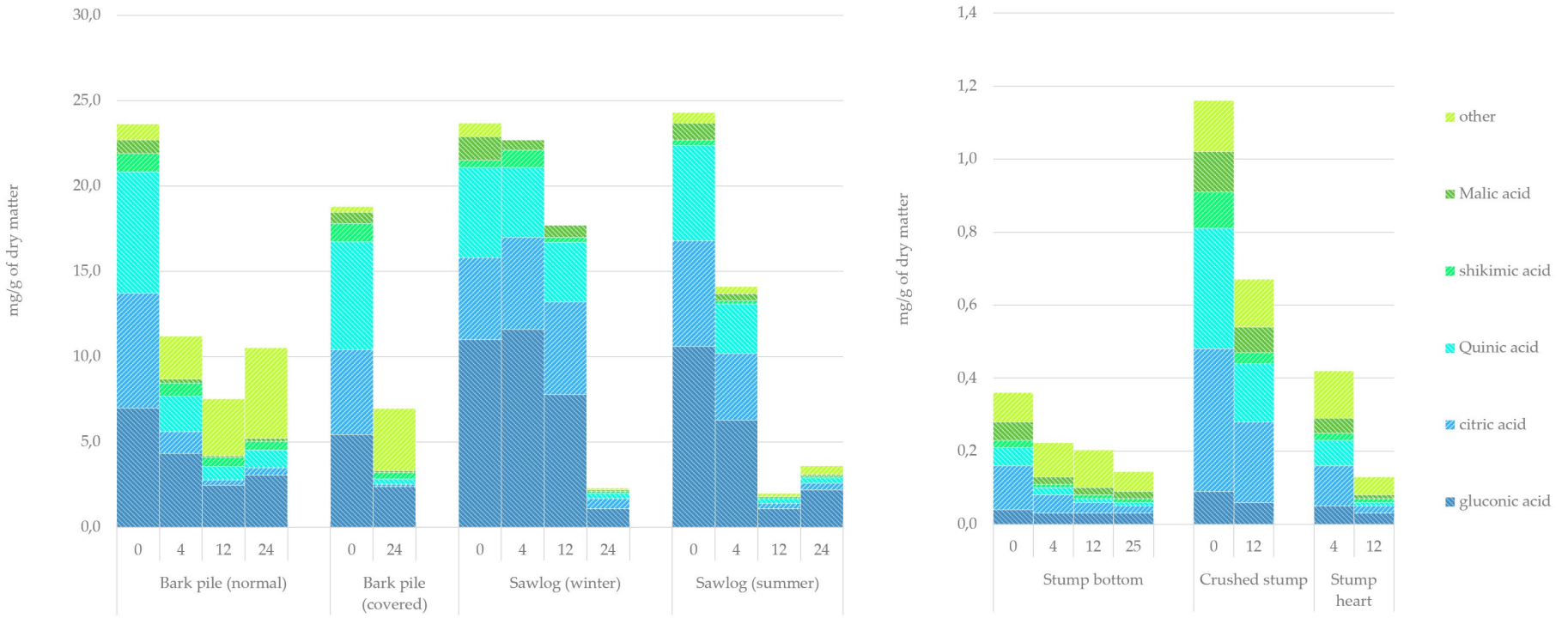


FIGURE 19. The amounts of extracted organic acids in the *Picea abies* samples studied.

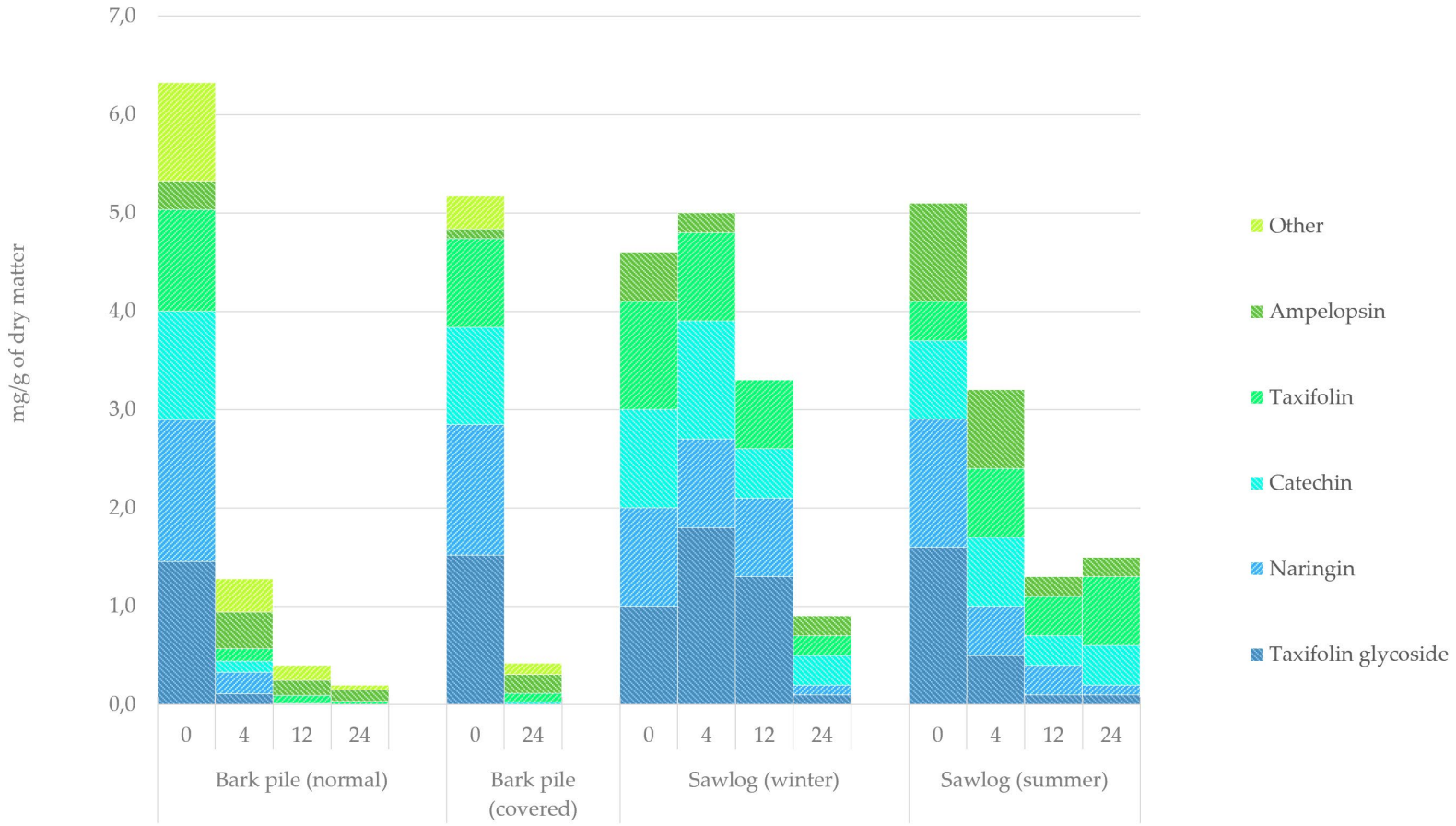


FIGURE 20. The amounts of extracted flavonoids in the *Picea abies* bark samples studied.

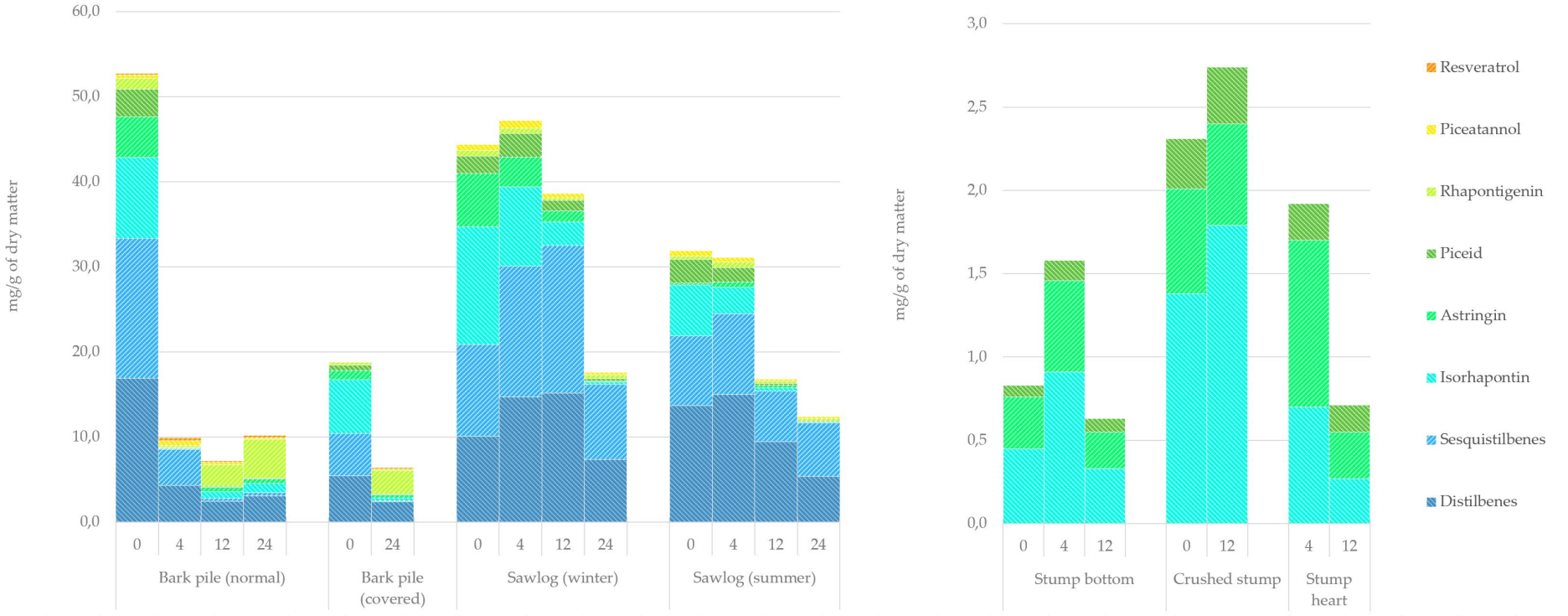


FIGURE 21. The amounts of extracted stilbenoids in the *Picea abies* samples studied.

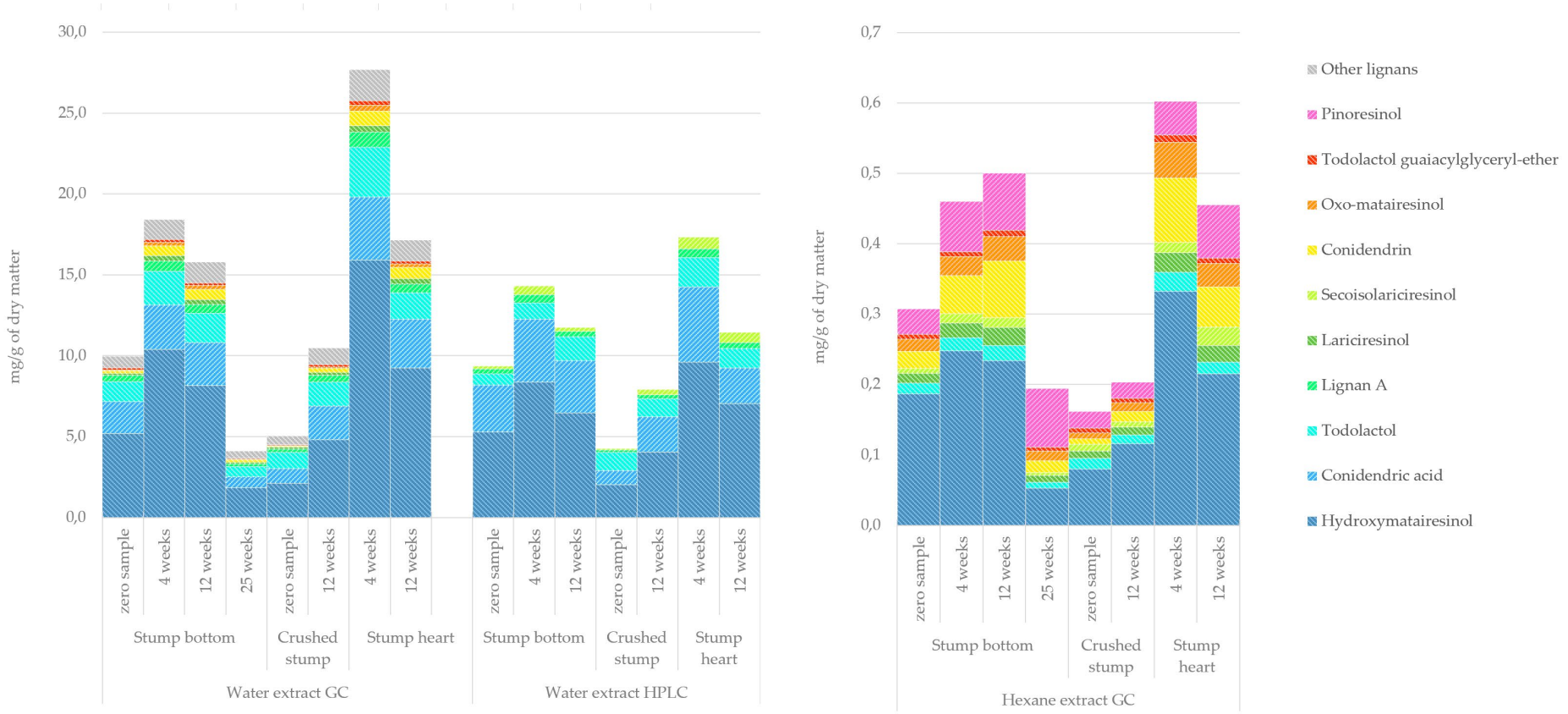


FIGURE 22. Compilations of the extracted lignans from the *Picea abies* stump samples.

6 CONCLUDING REMARKS

Abundant potential exists for using extractives, carbohydrates, and other derivatives from *Picea abies* industrial sidestreams, such as bark, needles, and stumps. Accessing the essential and widely applied hydrophilic extractives from bark, such as stilbenes and tannins, via simple extraction/purification methods appears the most viable of these options. However, stumps and harvesting residue can also be considered a good source of valuable chemicals for biorefineries.

The storage conditions and quality of these raw materials can significantly affect the preservation/loss of the valued compounds and should be considered carefully. A significant difference was demonstrated in the behavior of extractives (and carbohydrates) based on the chemical compositions of bark during sawlog and piled bark storage. The effect of seasonal variation was also examined regarding sawlog storage, and it was concluded that the observed degradation was more significant during summer. However, the results for stump storage suggested that the amount of lignans varies greatly between samples and that lignans are located more toward the pith of the stump. Generally, after 12 weeks, signs of significant alteration of the initial chemical composition were observed. In the bark pile, the degradation was substantial after only 4 weeks of storage, leading to the loss of almost half of its hydrophilic compounds. The results suggested that for valorization purposes, it is better to store biomass for as little time as possible, with as large a particle size as possible, as cold as possible, and, if possible, shielded from UV light and wetting. However, these guidelines, depend largely on the end goal set for the raw material – for example, whether it is to maximize the calorific value or the amount of extractable compounds.

The relative amounts of the non-extractive constituents (cellulose, hemicelluloses, lignin, and inorganics) as a percentage of dry matter in the studied samples increased, indicating that they were degraded more slowly. Interestingly, the relative increase in lignin appeared to be significantly linked to the rapid loss of extractable sugars, suggesting that enzymatic conversion of carbohydrates to monolignols occurs during storage. This relationship between

lost extractives and gained lignin should be investigated in more depth in the future.

The study of needle extractives was not the primary objective of this study. For future research, it could be interesting to determine how the relatively high concentrations of stilbenoids and flavonoids, among other compounds, respond to various storage conditions. This information would be helpful for those interested in investing in needle-derived applications. Additionally, a study of other possible storage options for *Picea abies* bark, such as using semipermeable coverings, could help optimize the storage conditions to achieve the fullest potential for the available material and the best possible practices for balancing preservation of quality and and cost-efficiency.

JOHTOPÄÄTÖKSET

Metsäkuusen (*Picea abies*) uuteainepitoisissa teollisissa sivuvirroissa, kuten kuoressa, neulasissa ja kannoissa on merkittävässä määrin hyödyntämätöntä potentiaalia liittyen hyödyllisten uuteaineiden, hiilihydraattien ja muiden kemiallisten yhdisteiden eristämiseen sekä puhdistamiseen. Erityisesti hydrofiilisten uuteaineiden, kuten stilbeenien ja tanniinien eristäminen metsäteollisuuden sivuvirroista, kuten puun kuoresta yksinkertaisten uuttoprosessien ja sopivien puhdistusmenetelmien avulla vaikuttaa varsin toteuttamiskelpoiselta vaihtoehdolta tulevaisuuden biojalostamoja silmällä pitäen. Samoin myös neulasia ja kantoja voidaan pitää arvokkaana biokemikaalien lähteenä.

Varastointiolosuhteet ja raaka-aineen laatu vaikuttavat suurelta osin kemiallisten yhdisteiden säilymiseen ja muutoksiin. Näin ollen kyseisten raaka-aineiden varastointiin tulisi myös kiinnittää erityistä huomiota. Tässä tutkimuksessa esitellyt metsäkuusen kuoresta saadut pölkkykoe- ja kuorikasa-varastoinnin tulokset osoittivat merkittäviä eroja uuteaineiden ja hiilihydraattien käyttäytymisessä erilaisissa varastointiolosuhteissa. Pölkkyvarastointi osoittautui kaikin puolin tehokkaammaksi keinoksi ylläpitää korkeaa uuteainepitoisuutta. Lisäksi tarkasteltiin myös vuodenaikavaihtelua pölkkyvarastoinnin yhteydessä ja todettiin, että kesällä havaittu hajoaminen oli huomattavasti talvella havaittua laajamittaisempaa. Kantovarastointi antoi toisaalta ymmärtää, että lignaanien määrä vaihtelee suuresti kantonäytteiden välillä ja että lignaanit ovat keskittyneet kannon keskiosaan. Yleisesti ottaen 12. varastointiviikon jälkeen havaittiin merkittäviä muutoksia kaikkien varastointinäytteiden alkuperäisissä kemiallisissa koostumuksissa. Kuorikasassa hajoamisreaktiot olivat jo neljän viikon jälkeen huomattavia, kun menetettiin noin puolet hydrofiilisista yhdisteistä.

Tulokset viittasivat siihen, että jalostustarkoitukseen on parempi varastoida raaka-ainetta mahdollisimman lyhyen ajan, niin suurella partikkelikoolla ja niin kylmässä kuin mahdollista ja suojattuna UV valolta sekä kastumiselta. Tämä yleisohje riippuu kuitenkin paljon raaka-aineen loppukäytöstä, kuten esimerkiksi siitä, pyritäänkö maksimoimaan raaka-aineen lämpöarvoa vai sen uuteainepitoisuutta.

Tutkittujen näytteiden uuteainevapaat jakeet (% kuiva-aineesta), kuten selluloosa, hemiselluloosat, ligniini ja tuhkapitoisuus, osoittivat yleisesti ottaen suhteellista pitoisuuden kasvua varastoinnin aikana, mikä viittasi niiden hajoavan uuteaineita hitaammin. Ligniinin suhteellinen kasvu vaikutti olevan yhteydessä sokereiden nopeaan vähenemiseen. Tämä saattoi viitata siihen, että varastoinnin aikana tapahtui entsymaattista hiilihydraattien konversiota monolignoleiksi. Suhdetta uuteaineiden vähenemisen ja ligniinin lisääntymisen välillä olisikin syytä tutkia tulevaisuudessa entistä tarkemmin.

Tämän tutkimuksen keskeisessä asemassa ei ollut neulasten uuteaineiden tutkiminen. Tulevaisuudessa olisi mielenkiintoista tutkia, millä tavalla vaihtelevat varastointiolosuhteet vaikuttavat muun muassa neulasten suhteellisen korkeisiin stilbeeni- ja flavonoidipitoisuuksiin, kuin myös muihin aromaattisiin yhdisteisiin. Tällaisesta informaatiosta olisi hyötyä erityisesti niille, joiden tavoittee-

na on kehittää neulasista johdettuja uuteaineperäisiä sovelluksia. Lisäksi toinen hyödyllinen tutkimus kuusen kuoren varastointiin liittyen olisi testata erilaisten osittaisesti läpäisevien suojakerrosten vaikutusta raaka-aineen hajoamisen kannalta, ja pyrkiä näin löytämään optimaaliset varastointiolosuhteet ja tasapaino käsittelymenetelmien hinnan sekä raaka-aineen laadun suhteen.

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ORIGINAL PAPERS

I

EFFECT OF SEASONAL STORAGE ON SINGLE-STEM BARK EXTRACTIVES OF NORWAY SPRUCE (*PICEA ABIES*)

by

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Article

Effect of Seasonal Storage on Single-Stem Bark Extractives of Norway Spruce (*Picea abies*)

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Abstract: Increasing the net value of forestry side-streams has both ecological as well as economic benefits for emerging biorefining industries. Spruce bark represents one of the nature's abundant sources of valuable extractives. In this study, the impact of storage on the quality and quantity of Norway spruce (*Picea abies*) extractives was examined as a function of storage time, environmental conditions and season (i.e., winter or summer). The bark from stored spruce saw logs was extracted with an accelerated solvent extractor (ASE) at 120 °C with hexane and water. The produced extracts were analysed qualitatively and quantitatively by gas chromatography with a flame ionisation detector (GC-FID) and high-performance liquid chromatography (HPLC) methods. The total amount of phenolics in the water extracts was evaluated by the Folin–Ciocalteu method, while the carbohydrate and lignin content of the extractive-free bark was estimated by acidic hydrolysis and acidic methanolysis. According to the results, storage season and temperature dramatically influenced both the chemical composition and degradation rate of bark extractives. After a storage period of 24 weeks, the winter-stored saw log bark retained 22% more hydrophilic extractives than the summer-stored bark. Lipophilic extractives, however, were 14% higher during the summer. Notably, the average amount of monomeric stilbenoids was 61% higher during the winter storage period. The initial total phenolic content in the water extracts was significantly higher during winter, but the degradation rate was about equal during winter and summer. The amount of cellulose in dry bark decreased from 17% to 11% and from 13% to 6% during winter and summer, respectively. By contrast, hemicelluloses increased from 17% to 26% and 15% to 30% during winter and summer, respectively. Overall, it was demonstrated that the seasonal factors of storage greatly affected the degradation rate of valuable spruce bark extractives, which should be considered in the planning stages of the raw materials procurement chain.

Keywords: spruce bark; biomass storage; degradation; extraction; wood extractives; hydrophilic; lipophilic; high-performance liquid chromatography (HPLC); gas chromatography (GC)

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1. Introduction

In boreal countries, such as Finland and Sweden, the annual consumption of woody biomass and generated by-products, such as bark and logging residues, is substantial. Norway spruce (*Picea abies*) bark is one of the major contributors in the class of secondary forest resources. The industrial use of spruce pulpwood and saw logs totalled 24.5 Mm^3 in Finland in 2019 (Luke statistics, 2021). In Finland, this equals 0.9–1.4 Mt/a (d.m.) of spruce bark generated at mills—a value calculated with an estimated average dry density of 380 kg/m^3 [1]. Despite the active promotion of bio-economical solutions, most of the forest industry's by-products are still being burned for heat and power.

The idea of converting these secondary resources into value-added products has genuine potential. Nevertheless, the prerequisite of the valorisation of forestry side-streams

is that the raw material is handled in a way that does not compromise its quality. The utilisation of valuable extractives and antioxidants from spruce bark is impossible if the raw material is already chemically barren by the time it reaches the end-user. The producers of a given raw material need to make prudent economic and logistical decisions regarding the raw material procurement chain so that the end-user will receive the raw material of the right quality. For this cause, in-depth knowledge and understanding of how the chemical and physical quality of the raw material is affected by different harvesting methods, means of transportation and storage conditions are required.

Softwood bark is known to be an abundant source of phenolic extractives. Norway spruce is rich in polyphenolic extractives and natural antioxidants, such as stilbenes and tannins [2,3]. According to Krogell et al. [3], dry bark spruce extractives (both lipophilic and hydrophilic) account for 38% by dry weight of inner bark and 22% of outer bark. In contrast, only 1.0–2.5% of spruce stemwood comprises extractives [4,5]. Thus, the harvesting of extractives from the bark is a promising prospect. However, bark is a complex mixture of natural polymers and unstable, volatile compounds, and the task of using them on an industrial scale is not a simple one. The production and emission of extractives as a response to various external influences is a constant activity carried out by living tree parenchyma cells, which continues even after tree felling. Moreover, as tree cells' access to nutrients is cut off, the production of new extractives eventually halts, and the remaining extractives' degradation is accelerated [6–9]. Therefore, it may be expected that the raw material losses begin at the harvesting site and continue throughout the procurement and supply chain until the final destination of the material is reached. The chemical composition of the extractives content of trees is likewise expected to change. To capitalise on a product utilising, for example, the stilbene glucosides of spruce bark (piceid, astringin and isorhapontin), understanding both the rate and nature of their degradation is of key importance.

The degradation mechanisms of woody biomass are well known [10], including living cell respiration, biological degradation, thermo-chemical oxidation reactions and moisture evaporation. Thermal degradation and auto-oxidation reactions are more influential in the pile storage of smaller particle-sized material, such as wood chips [11]. In the storage of wood logs, extractives fraction is mainly affected by the evaporation of volatile terpenoids [12,13], the hydrolysis of glycerides and waxes by plant enzymes and the activity of wood-colonising bacteria and fungi [9,14–16] through oxidative and polymerisation reactions [9], the photodegradation of phenolics [17–19] and the leaching of hydrophilic compounds by contact with water [20–23]. Thus, ambient conditions, such as the level of ultraviolet (UV) light, moisture, temperature and wind, are key variables for understanding and explaining the degradation rate of individual extractives compounds during storage. For example, moist storage conditions increase hydrolysis and leaching, while high temperatures and UV light increase photodegradation and polymerisation reactions [10].

Only a few older reports focus on conifer bark storage [11,24], although a recent interest in these studies can be observed [25–27]. However, most of these studies deal with only debarked bark stored in piles, whereas the storage of bark intact on saw logs is less researched [25]. The scope of many storage studies regarding the extractives content of bark or wood has been limited to looking at the amount of total dissolved solids (TDS) and larger extractives groups. In contrast, detailed studies of the degradation pattern of individual extractives are difficult to come across. Many storage studies are also limited in that, by having a single storage setup, they cannot take into consideration the seasonal effects of storage. Our experiment was conducted to fill this gap and provide easily accessible information useful in valorising bark for extractives-based products.

Our experiment aimed to study primarily the behaviour of extractives, carbohydrates and lignin content of saw log bark exposed to outdoor conditions as a function of season and storage time. The spruce bark samples were taken at the beginning of the experiment and after 4, 12 and 24 weeks. Duplicate experiments were conducted—one during the

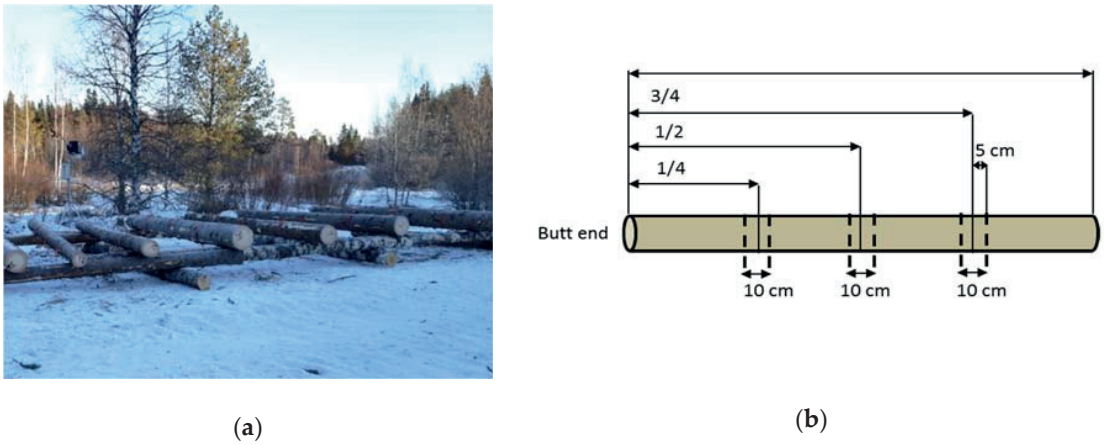
winter and one during the summer. Environmental conditions were gathered from the data provided by the Finnish Meteorological Institute [28].

It was concluded that the storage conditions significantly affected especially the content of hydrophilic extractives present in the bark, depending on the storage season. The hydrophilic extractives were found to be 22% more abundant in the bark stored during winter. The level of hydrophilic extractives remained high up to 12 weeks of storage during winter. The storing of bark intact on saw logs appeared to reduce the degradation of extractives and carbohydrates significantly compared to conventional pile storage of debarked bark. Based on the research, it seemed advisable that in the procurement chain of saw logs, if bark is to be utilised for its extractives content, to avoid unnecessary degradation, debarking should occur at the last possible moment.

2. Materials and Methods

2.1. Storage Setups and Sampling

The experimental setups for the Norway spruce bark winter and summer storage studies were constructed on 7 February 2017 and 29 May 2017, in Kälviä, Western Finland, respectively. Two identical experimental setups were established to study the seasonal effect of storage on the behaviour of extractives. Freshly felled trees were bucketed to saw logs and placed on a frame platform made from logs to keep them off the ground (Figure 1a) and prevent them from being buried under snow during winter or covered by vegetation during summer.



(**a**) (**b**)

Figure 1. (**a**) The setup for the saw log storage study. The image was taken at the beginning of the winter study. (**b**) Figure indicating the location of the three sample discs cut from each sample log.

For each of the trees used in the storage study, the tree's height, diameter at breast height and age of the tree were measured, as listed in Table 1. The sample discs for the calculation of tree age were cut from the remaining stumps. The sample logs were bucked to approximately 4.5 m long. The sampling frequency was (in addition to a zero-sample) after 4, 12 and 24 weeks. Because the logs' chemical composition changes from the butt end towards the top, three 10 cm discs were cut from each log (Figure 1b) to yield a more chemically uniform sample. Samples were taken from two saw logs during each sampling. The discs were then debarked manually.

2.2. The Average Temperature and Precipitation of the Sampling Location

The average temperatures and the amount of rain at the sampling location (Western Finland, Ostrobothnia region near Kokkola [63°54′44.03″ N, 23°25′17.0″ E]) were evaluated by utilising the 10 m × 10 m scale meteorological data provided by the Finnish Meteorological Institute [28]. Data used were the daily average temperatures (°C) and the daily rain (mm) amounts from 7 February 2017 to 13 November 2017. The data are presented in

Figure 2. There was an average difference of 5.7 °C during winter and summer storage; the bark in summer storage experienced a decrease in temperature starting from around 10 °C, rising to 15 °C after 4 weeks and ending at 0 °C at 24 weeks, while the bark in winter storage experienced a constant increase in temperature from around −5 °C and ending at 15 °C at 24 weeks. The average weekly amount of rain was 9.5 mm during the winter storage and 11.6 mm during the summer storage.

Table 1. Measurements taken from the saw logs used for the storage studies. Two log samples were taken at each sampling time.

	Storage Sample	Tree Age	Tree Height	D1.3m		Log Length	Log Diameter					
							Butt End		Middle		Top	
	weeks	years	dm	mm	mm	dm	mm	mm	mm	mm	mm	mm
Winter storage	0	119	225	362	345	47	310	319	290	306	261	270
	0	96	223	321	272	46	273	255	242	249	215	227
	4	97	210	301	299	47	260	258	234	238	216	219
	4	94	215	280	277	46	282	279	257	263	235	232
	12	73	206	260	263	46	234	226	204	207	191	185
	12	56	213	362	362	45	317	305	282	290	254	262
	24	96	224	323	341	46	264	269	238	244	207	210
	24	78	256	305	299	46	282	281	256	261	247	240
Summer storage	0	67	243	307	310	48	279	272	262	254	243	237
	0	70	245	362	357	44	310	311	300	291	292	289
	4	100	270	392	399	43	350	342	324	326	313	305
	4	95	255	360	364	49	303	310	281	284	254	256
	12	94	260	326	326	47	284	290	274	267	290	246
	12	58	225	304	300	48	256	239	225	219	206	199
	24	89	262	359	358	46	329	318	316	305	290	284
	24	93	252	285	278	48	271	260	246	242	226	224

2.3. Bark Initial Moisture Content

The average moisture contents in the bark samples at each sampling time are presented in Figure 2. The average moisture content for bark from the winter setup was 51.8% and from the summer setup 48.8%, indicating a slight decrease during the warm summer months. Also, there was an increase towards the end of the year (from September to December) as the weather cooled and the average amount of rain increased. This result concurs with Nurmi and Hillebrand [29] regarding the moisture content of harvest residues stored in uncovered piles. They found that the lowest moisture content was observed in winter-stored material at the end of the summer, and the moisture content increased if storage continued until the following winter.

2.4. Pretreatment and Basic Characterisation of Bark Samples

The discs' bark was removed manually and then ground into smaller particle sizes with a Jens Algol System woodchipper. The moisture content of the fresh bark samples was determined by a standard method [30]. The samples were dried at 105 °C in atmospheric air until a constant mass was achieved. The measurements were performed in duplicate. Evaluation of the relationship between log height and the bark's extractives content was outside of the scope of this study. Thus, the bark from the three discs was mixed to form a single sample representing the whole log. For chemical analysis, the samples from the two different logs from each sampling time were also mixed.

The bark was lyophilised (for at least three days) and ground with a Retsch SM 100 cutting laboratory mill (Retsch GmbH, Haan, Germany) equipped with a bottom sieve with trapezoidal holes (perforation size <1.0 mm) for chemical analysis. Samples were stored in a frozen state (<−20 °C). The dry matter content of each lyophilised bark sample was determined by drying 1 g of bark powder at 105 °C in an oven overnight in tared crucibles.

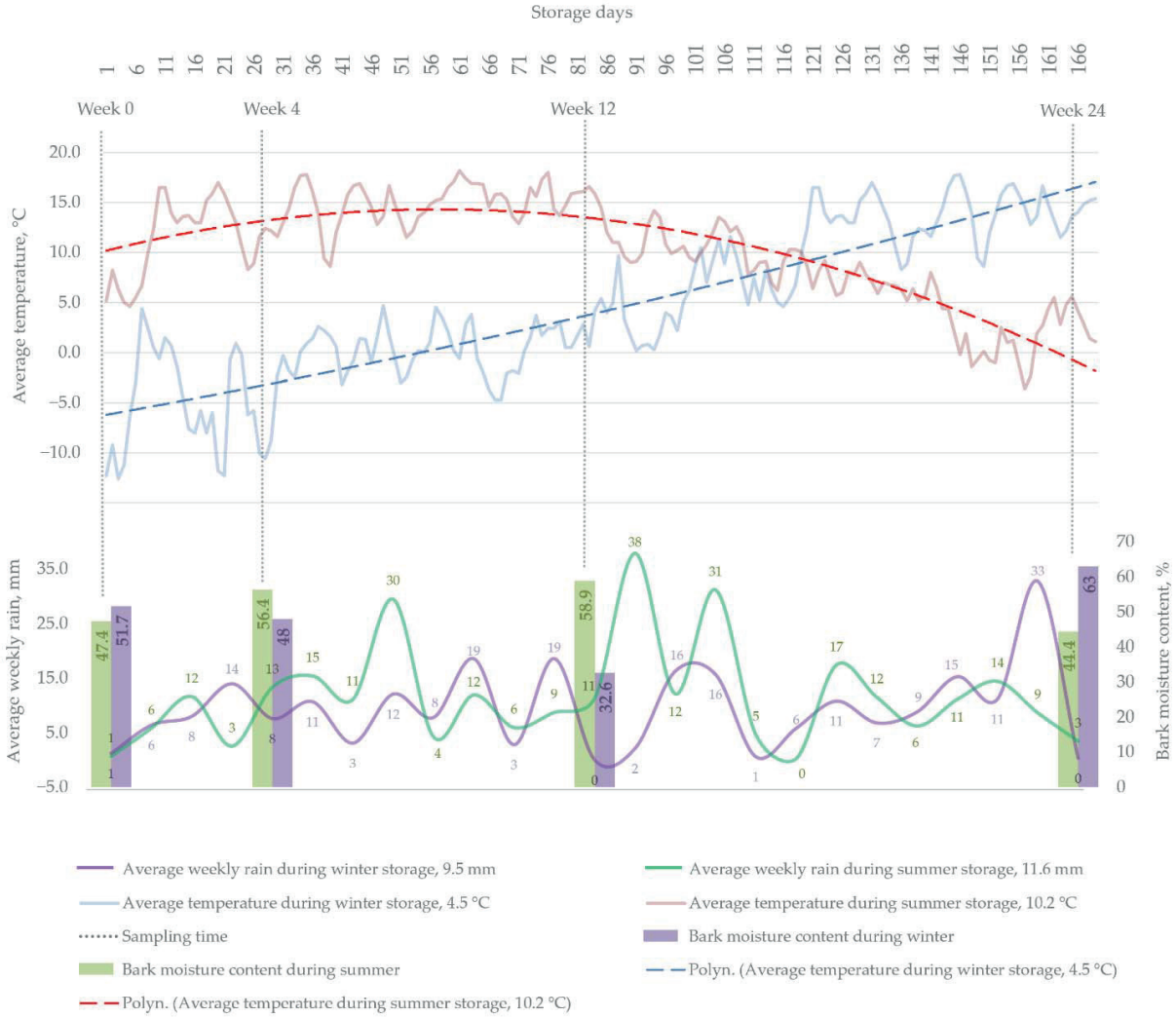


Figure 2. Average ambient temperatures and weekly amounts of rain during winter and summer storage, and the initial moisture content of bark samples at each sampling point. The sampling points (weeks 0, 4, 12 and 24) are indicated by vertical dotted lines.

2.5. Chemicals

The solvents used in the sample preparation of extractives were analytical grade acetone (BDH), high-performance liquid chromatography (HPLC)-grade hexane (VWR), methyl *tert*-butyl ether (MTBE, Lab-Scan), pyridine (BDH), 95% ethanol (EtOH, >94%, ETAX A, Altia Corporation), *n*-butanol (Merck), HPLC-grade methanol (MeOH, Merck), diethyl ether (≥99.8%, Sigma) and HPLC-grade acetonitrile (Fischer Chemicals). Bis(trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) used for silylation came from Regis Technologies.

The compounds used as internal standards in the gas chromatography (GC) analysis of extractives were heneicosanoic acid (99%, Sigma) and betulinol (≥98%, Sigma), cholesteryl margarate (≥97%, TCI America) and 1,3-dipalmitoyl-2-oleylglyserol (≥99%, Sigma). The standard used for the quantitative analysis of stilbenes with HPLC was *trans*-piceid (99%, PhytoLab). KOH (85%, Merck), NaOH (>98%, VWR), HCl (37%, VWR) and Na_2CO_3 (≥99.8, Sigma) and sulphuric acid (95–97%, Sigma) and bromocresol green (>95%, Sigma) were also used in the analysis.

2.6. Extraction of Bark Samples

The extractions of bark samples were done by a Dionex accelerated solvent extractor (ASE) 100 instrument using hexane and water as solvents for lipophilic and hydrophilic extractives, respectively. The extraction temperature was 120 °C, static extraction time 10 min, flush of the extraction cell 60%, nitrogen purge 70 s and the extraction pressure 1500 psi. For each extraction, 2 g of bark powder was loaded into an extraction cell (34 mL) plugged with a cellulose filter. Each sample was first extracted with hexane and then with ultra high-quality (UHQ) water. The extractive-free bark was consequently lyophilised and stored for carbohydrate analysis. The extractions were performed in duplicates for each sample.

2.7. Gravimetric Analysis of Total Dissolved Solids (TDS) and Preparation of Stock Solutions

Bark extracts were analysed gravimetrically to determine their TDS. The extractives collected in hexane were first evaporated to near dryness in a rotary evaporator; the extractives were subsequently transferred to a tared (Kimax®) test tube in acetone. Brief sonication of the mixture in an ultrasound bath assisted in the dissolution of lipophilic extractives. The acetone solution was evaporated under nitrogen flow to dryness and the gravimetric amount of total lipophilic extractives determined by weighing the dried extract until a constant mass was achieved. A stock solution of 100 mL was then prepared by dissolving the extractives in acetone.

The hydrophilic extract in water was first diluted to 100 mL with UHQ water and stored in a freezer as a stock solution; 10 mL of the stock solution was then frozen and lyophilised and the gravimetric amount of total hydrophilic extractives determined based on the mass of the lyophilised sample.

2.8. Analysis of Bark Extractives with Chromatographic Methods

2.8.1. Qualitative and Quantitative Analysis by Gas Chromatography (GC)

The methods used for the qualitative and quantitative analysis of spruce bark extractives groups and individual extractives (both free and esterified) were modified based on the methods reported by Örså and Holmbom (1994) [31].

For the preparation of a sample of bark extracts for qualitative or quantitative analysis, approximately 3 mg of extract was first dried (either under nitrogen flow or by lyophilisation). For quantitative analysis, internal standards were also added and the mixture dried under nitrogen flow. To analyse extractives groups using short-column gas chromatography with a flame ionisation detector (GC-FID), 100 µg of four internal standards, namely heneicosanic acid, betulinol, cholesteryl margarate and 1,3-dipalmitoyl-2-oleylglycerol, were added to the sample. For the quantitative analysis of individual compounds, only heneicosanic acid and betulinol were added. Both quantitative and qualitative samples were silylated before analysis by dissolving them in 500 µL of pyridine and 300 µL of the silylation reagent (BSTFA/TMCS, 99/1, v/v) and by keeping them capped in Kimax® test tubes in an oven at 70 °C for 1 h.

For the analysis of esterified lipophilic extractives, after the drying of the extract and internal standards, the sample was hydrolysed by adding 1 mL of KOH in 90% EtOH keeping the mixture in an oven at 70 °C for 3.5 h. After the hydrolysis, the sample was diluted with 1 mL of UHQ water, and the pH of the mixture was adjusted to 3.5 by the addition of 0.5 M sulphuric acid by using bromocresol green (2 drops) as an indicator solution. The hydrolysed compounds were extracted by adding 2 mL of diethyl ether to the sample mixture, mixing for 1 min, centrifuging the mixture for 5 min at 2500 rpm and separating the diethyl ether layer by pipetting. The extraction by diethyl ether was repeated two more times and the ether layers were combined. The diethyl ether was then dried under nitrogen flow (and lyophilisation if the sample was too moist), and the silylation of the sample was done as described previously.

The individual compounds were analysed quantitatively using an Agilent 6850 GC-FID equipment and qualitatively using an Hewlett Packard 5973 gas chromatograph–mass

spectrometry (GC-MS) equipment. The GC systems were equipped with an HP-5 column (30 m × 0.32 mm with 0.25 μm film), injecting the sample at 290 °C and detecting the compounds with a FID/MS detector at 300 °C. In the applied method, the starting temperature was 100 °C, where the sample was kept for 1.5 min, after which the temperature was first raised (6 °C/min) to 180 °C and then raised (4 °C/min) to 290 °C, where it was kept for 13 min. After this, it was finally raised (4 °C/min) to 300 °C, where it was kept for 20 min.

The extractives compound groups were analysed quantitatively with a short-column GC-FID equipped with an HP1/Simulated Distillation column (7.5 m × 0.53 mm with 0.15 μm film). The samples were injected on column at 90 °C, and the compounds were detected with FID at 320 °C. In the method used, the starting temperature was 90 °C, from which the temperature raised (12 °C/min) to 320 °C, and this temperature was kept for 10 min.

2.8.2. Qualitative and Quantitative Analysis by High-Performance Liquid Chromatography (HPLC)

For the qualitative analysis of bark samples with HPLC, 1 mg/mL dilution of bark water extract was prepared in MeOH/H_2O (50/50, *v*/*v*), and the sample was filtrated through a 0.2 μm polytetrafluoroethylene (PTFE) filter. The sample was then analysed with an Agilent 1290 LC (liquid chromatography) instrument equipped with a ZORBAX StableBond column (80Å C18, 2.1 mm × 100 mm, 1.8 μm, 1200 bar), a ZORBAX SB-C18 UHPLC guard column (2.1 mm, 1.8 μm), 1290 Infinity II Diode Array Detector and a 6460 triple quadrupole mass spectrometer (LC/DAD/QQQ). The LC columns were maintained at 30 °C. Two solvents were used for the mobile phase: (A) 0.1% formic acid in UHQ water and (B) 0.1% formic acid in acetonitrile. The mobile phase flow rate was 0.3 mL/min. The run method was modified based on the study by Gabaston et al. [32] and was as follows: 10% B (from 0–1.7 min), 10–20% B (from 1.7–3.4 min), 20–30% B (from 3.4–5.1 min), 30% B (from 8.5–11.9 min), 60–100% B (from 11.9–15.3 min), 100% B (from 15.3–17.0 min) and 100–10% B (from 17.0–17.3 min). Mass spectrometry analyses were performed in negative mode with a range of m/z 100–1200. The drying gas used was nitrogen at 10 L/min at 365 °C with a nebuliser pressure of 40 psi. The capillary voltage was 3100 V. Bruker Data Analysis 3.2 software was used for data processing. *Trans*-piceid was used as an external standard for quantifying the stilbenes and other phenolics separated and identified from the bark water extracts.

2.9. Carbohydrate Analysis

The extractives-free bark was analysed for its carbohydrate (cellulose and hemicelluloses) and acid-soluble and acid-insoluble lignin content using acid hydrolysis and acidic methanolysis. The cellulose and lignin contents were determined by acid hydrolysis [33] and the content of the hemicelluloses by acidic methanolysis.

2.9.1. Acid Hydrolysis

For acid hydrolysis, approximately 200 mg of the lyophilised extractives-free bark was weighed in a test tube. Each sample was prepared in duplicate. Then 4 mL of 72% cold sulphuric acid (stored in a fridge) was added to the sample. The test tube was maintained at 30 °C in a water bath for 1 h and was mixed with a glass rod every 5 min. The sample was then carefully transferred to a 250-mL autoclave bottle and was washed with 112 mL of UHQ water. The samples were then placed into an autoclave (MELAG Autoklav 23) at the pressure of 1 bar (~121 °C) for 1 h. The acid-insoluble lignin was separated from the mixture by filtering the sample with a tared borosilicate glass filter (Munktell MGA 413004) in a vacuum funnel. The amount of insoluble lignin was determined gravimetrically by drying the filter paper together with the solid residue in an oven at 105 °C to a constant mass. The filtrate was diluted to 500 mL with UHQ water and was consequently analysed with high-performance anion-exchange chromatography (HPAEC) for its monosaccharide content and with ultraviolet–visible (UV–Vis) spectrometry for its acid-soluble lignin content.

2.9.2. High-Performance Anion-Exchange Chromatography (HPAEC) Analysis of Monosaccharides

The monosaccharides formed during the acid hydrolysis were analysed from the 500 mL dilution using HPAEC. The standard solutions for HPAEC were prepared using a sulphuric acid concentration corresponding to the samples' background: cold 72% sulphuric acid (3 mL) was diluted in 500 mL with UHQ water. Fucose (500 ppm) was used as the internal standard. Three monosaccharide solutions were made for the preparation of standard solutions: (i) arabinose (400 ppm), galactose (200 ppm) and mannose (200 ppm); (ii) glucose (1000 ppm) and (iii) xylose (1000 ppm). The volumes for the five prepared standard solutions (STD1–STD5), as well as their monosaccharide concentrations, are shown in Table 2.

Table 2. Standard solutions for high-performance anion-exchange chromatography (HPAEC) measurements.

Mono-Saccharide (Standard)	STD1		STD2		STD3		STD4		STD5	
	V *	C **	V	C	V	C	V	C	V	C
Arabinose	1	4	3	12	6	24	10	40	15	60
Galactose	1	2	3	6	6	12	10	20	15	30
Mannose	1	2	3	6	6	12	10	20	15	30
Glucose	5	50	10	100	15	150	20	200	25	250
Xylose	2	20	4	40	6	60	8	80	10	100

* Volume (mL) of used solution in 100 mL volumetric flask. ** Solution concentration (ppm).

The bark samples (500 mL, UHQ dilution) from acid hydrolysis were analysed with HPAEC (Dionex) using 1 M sodium acetate, 0.5 M sodium acetate + 0.1 M NaOH and 0.3 M NaOH solutions as eluents. The analytes were separated in CarboPac PA1 + Guard PA1 columns and were detected using an ED50 detector using carbohydrate pulsing. Post-column elute was pumped by an IC25 isocratic pump. Samples for HPAEC analyses were prepared by pipetting 2 mL of internal standard solution into a 20 mL volumetric flask and filling the flask with the diluted sample (500 mL) from acid hydrolysis. This solution (1.0–1.5 mL) was transferred into an HPLC vial by filtrating it through a syringe filter (Phenex RC 0.2 μm).

2.9.3. Ultraviolet–Visible (UV–Vis) Measurement of Acid-Soluble Lignin

The amount of acid-soluble lignin was determined from the 500 mL dilution following acid hydrolysis by UV–Vis spectrometry at 205 nm according to TAPPI UM 250 standard [34] using an extinction coefficient for softwood of 120 L/(gcm) [35]. The analysis was done with a Perkin Elmer Lambda 35 UV–Vis spectrometer.

2.9.4. Acidic Methanolysis

The amount of hemicelluloses in the spruce bark samples was determined from the extractive-free lyophilised bark by acidic methanolysis modified from the method by Bertaud et al. [36]. An internal standard solution was prepared by dissolving 10 mg of sorbitol into 100 mL of MeOH. For an external standard solution, 10 mg of pure arabinose, galactose, glucose, xylose, mannose, galacturonic acid and glucuronic acid was dissolved in 100 mL of UHQ water. Methanolysis reagent was prepared by cooling 100 mL of MeOH in an ice bath and carefully adding and mixing 16 mL of acetyl chloride to the cold MeOH. The reagent was stored at −20 °C.

For methanolysis, 2–3 mg of the extractive-free bark sample was weighed in a pear-shaped flask. For a standard sample, 1 mL of a standard solution containing all the monosaccharides was added to another pear-shaped flask, and the solvent was evaporated by a rotary evaporator. The methanolysis reagent (2 mL) was then added to both the sample flasks and standard flasks. The sample flasks were capped with screwed caps, sonicated in an ultrasound bath and kept in an oven at 100 °C for 3 h. The flasks were cooled before opening them to reduce their pressure. Pyridine (80 μL) and 1 mL of the

internal standard were added to the samples, and the solvent was evaporated by a rotary evaporator accompanied by a water bath (35–45 °C). Pyridine (80 µL) and 250 µL of silylation reagent were added to the dried samples. The samples were sonicated in an ultrasound bath and kept in a shaker at room temperature for 40 min. The samples were then filtrated with glass wool and placed in vials for GC analysis.

The samples were analysed with an Agilent gas chromatograph using an HP-5 column (30 m × 0.32 mm with 0.25 µm film), injecting at 260 °C and detecting with a FID at 290 °C. The method used was as follows. The starting temperature was 100 °C, where the sample was kept for 2 min. The temperature was raised (4 °C/min) to 220 °C, where it was maintained for 2 min and was finally raised (15 °C/min) to 300 °C and kept there for 2 min.

2.10. Total Phenolic Content (TPC)

The bark samples' total phenolic content (TPC) was evaluated from the hot-water extracts of bark with the Folin–Ciocalteu method. The Folin–Ciocalteu method was used as described by Singleton and Rossi [37]. Hot-water extracts of bark were diluted, and 0.5 mL of the solutions was pipetted into a Kimax® test tube. The 0.2 N Folin–Ciocalteu reagent (2.5 mL) and 2.0 mL of sodium carbonate solution (75 g/L) were added. The samples were vortexed and placed in a hot-water bath at 50 °C for 5 min. After cooling (10 min), the absorbance was measured at 760 nm with Perkin Elmer Lambda 35 UV–Vis spectrometer.

2.11. Statistical Analysis of Bark Extractives

To analyse the statistical differences in the bark's chemical constituents between storage times (i.e., storage time in weeks since the onset of the storage experiment) and seasons (winter vs. summer), a two-way analysis of variance (ANOVA) was used with the assumptions of the normal distribution (assessed by histograms) and the homogeneity of variance. The dependent variables for the analyses were the quantitative amounts of the different extractives groups analysed by GC-FID/HPLC and spectrophotometric methods (UV–Vis). Because of the limited data set of only eight observations per response variable, the assumptions of normality and variance homogeneity were initially not met. To solve the problem, data transformations that maximised normality and minimised variance between sample groups were utilised.

The utilised transformations for the extractives groups were as follows. Logarithmic transformation (Ln) was used to normalise the distribution of all extractives groups except for triglycerides, alcohols, sugars and unidentified hydrophilic extractives. With triglycerides and unidentified hydrophilic extractives, cosine transformation was used to normalise the distribution, whereas sine transformation was used for alcohols, and the absolute value of cosine transformation was used for sugars.

3. Results and Discussion

3.1. Overall Change in Chemical Composition

In summary of the results, the overall change in the chemical composition of winter- and summer-stored bark is presented in Figure 3. According to the results, the proportion of extractives in bark gradually decreased while the extractive-free fraction increased. The hot-water extract was the most substantial fraction at the beginning of the storage period, both during winter and summer. In contrast, at the end of the storage period, hemicelluloses and lignin comprised the largest fractions during winter and summer, respectively. It should be noted that since no new hemicellulose or lignin is expected to be formed during storage, their observed increase was relative to the loss of extractives. The amount of cellulose and lignin was not determined for samples of weeks 4 and 12, and thus, were included in the group of unidentified compounds of bark. By comparing the zero-samples to 24-week samples, it seems that, in general, the relative proportion of unidentified compounds increases slightly during storage.

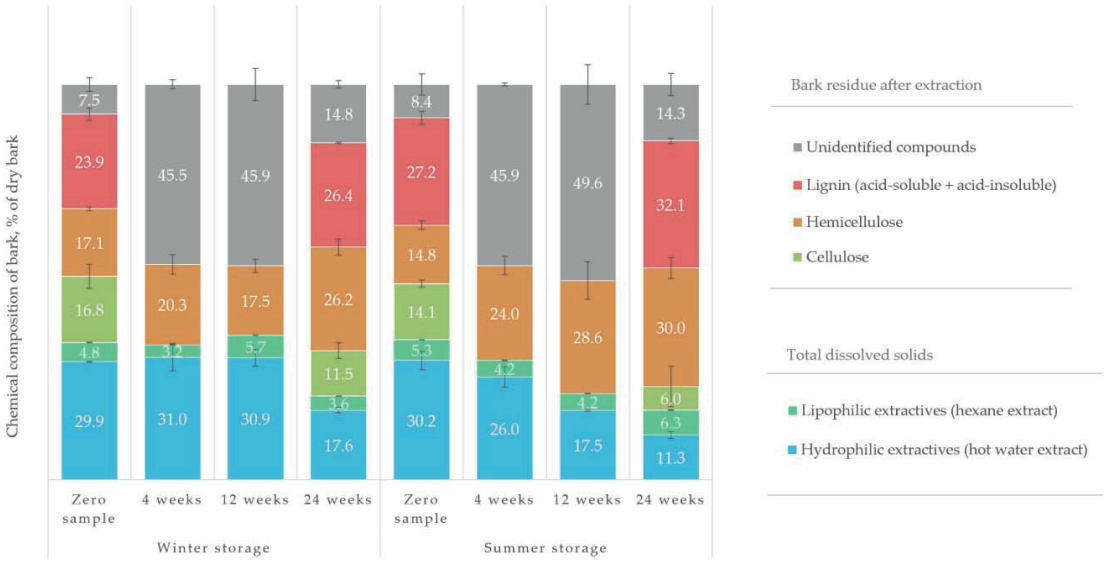


Figure 3. Summary of the overall chemical change in bark samples during the winter and summer storage. Total dissolved solids (both hydrophilic and lipophilic), carbohydrate content (cellulose and hemicelluloses), the amount of lignin and unidentified compounds are shown. The amount of cellulose and lignin was not determined for samples of weeks 4 and 12.

3.2. Total Dissolved Solids (TDS)

The TDS of the hexane and water extracts of spruce bark from both the winter and summer saw log storage experiments are shown in Figure 3. The TDS of the water extracts were much higher than those of the hexane extracts, indicating that hydrophilic extractives were more abundant in spruce bark in comparison to the lipophilic extractives.

3.2.1. TDS of Hydrophilic Extractives

The initial TDS of hydrophilic extractives in the bark zero-samples during both the winter and summer experimental setups were about equal. Overall, the hydrophilic extractives degraded much faster than the lipophilic extractives. However, the degradation rate in the hydrophilic extractives was more pronounced during the summer, when the samples were exposed to higher temperatures, higher levels of UV light and the increased activity of insects, bacteria and fungi. The TDS of hydrophilic extractives decrease consistently during summer storage, but during winter, the total amount of hydrophilic extractives remained unaffected up to 12 weeks of storage. The TDS of the saw log bark at 12 weeks of winter storage was notably high in both the water and the hexane extracts. This result should be interpreted in light of the fact that all bark for any given sample came from just two separate logs.

The natural variation in extractives content between any two trees is affected by several factors, the impact of which is not always clear. Such factors include the age of the tree, weather conditions, soil nutrients, pollution and the level of exposure to UV radiation. In the case of the bark sample at 12 weeks of winter storage, both logs contributing to the sample happened to be from trees over 30 years younger than the average age of the sample trees. The exact age of the sample trees could not be taken into account during the construction phase of the experimental setups since the ages were determined later at the laboratory. The trees were instead chosen based on a similar outward appearance (height and diameter), yet a significant difference was observed in the trees' age. This result indicated that the trees have grown at markedly different speeds, probably affected by different sunlight exposure, which in turn affected the extractives fractions. It should be noted that some disparity between trees is always expected regardless of the criteria used to choose the raw material.

This study's scope is limited because of the relatively small sample size (bark from only two logs per sample). Thus, it cannot provide a reliable relationship between all the sample variables (such as tree age and TDS). Such an inquiry would require tens of biological replicates per sampling, which was excessive for this study. This study's scope was rather to provide a directive overview of the chemical degradation occurring in the spruce bark extractives during winter and summer storage, which would benefit industrial parties interested in the valorisation of woody biomass. Also outside of the scope of this study was the detailed study of the extractives' differences in the bark from different heights of individual logs. It is well known that the concentration of extractives in bark decreases as one moves from stump to crown. However, industrial biorefining of bark would necessarily be holistic and not targeted at bark from only a specific area of the log. Thus, a mean value for the saw log bark extractives content was aimed at by combining the bark from the three sample discs cut from the logs (according to Figure 1b) and by combining the bark of the two individual saw logs of each sampling.

3.2.2. TDS of Lipophilic Extractives

The total amount of lipophilic extractives in the bark remained relatively stable throughout the storage period of 24 weeks, both during winter and summer. However, this result does not mean that the lipophilic extractives remained chemically unaltered during the storage period. The variation in the amount of TDS of lipophilic extractives was 3.2–5.7% of dry matter during winter storage and 4.2–6.3% dry matter during summer storage, indicating that lipophilic extractives were more abundant in bark during the summer. Similar TDS levels (4.0–5.0%) for lipophilic extractives from spruce bark have been reported previously [38] utilising the Soxhlet extraction method.

3.3. Qualitative and Quantitative Analysis of Bark Extracts

3.3.1. Qualitative Analysis by Gas Chromatography-Mass Spectrometry (GC-MS)

The most prominent lipophilic and hydrophilic extractives identified by GC-MS from the spruce bark hexane and water extracts are listed in Tables 3 and 4, respectively. The extractives are listed according to their significance among all the analysed samples. The main categories of lipophilic extractives were resin and fatty acids, diterpenoids, sterols and fatty alcohols (i.e., waxes). The main categories of hydrophilic extractives were sugars, organic acids, stilbenes, alcohols and flavonoids. The category defined in the table as others comprised the extractives, which (although visible in the GC chromatograms) could not be identified. Identifying these compounds was impossible either because their amount was too low, the peaks overlapped, a lack of a meaningful library match or the MS method's solvent delay caused the peaks to be undetected. The reference chromatograms from the long-column GC-MS/FID analysis of the hexane extracts of both free and esterified extractives are given in Appendix A and Figures A1 and A2, respectively, while the chromatogram of the short-column GC-FID for the hexane extract is shown in Figure A3. The long- and short-column reference chromatograms for the water extract's hydrophilic compounds can be seen in Figures A4 and A5, respectively. The compounds listed in Tables 3 and 4 are linked to the chromatogram peaks with specific symbols to help with their interpretation.

As can be seen from Table 3, resin and fatty acids together accounted for more than half of the identified lipophilic compounds, with diterpenoids, sterols and fatty alcohols being the minor compounds. The most important resin acids found from the spruce bark samples were dehydroabietic, isopimaric, abietic, levopimaric, neoabietic and palustric acids, representing 77% of the total amount of resin acids. The most prominent fatty acids were 18:1, 18:2, 18:3, 22:0, 16:0, 20:0 and 24:0, representing 88% of the total amount of fatty acids. It should be noted that many triglycerides and steryl esters had fatty acids bound to them, which were released upon hydrolysis with KOH (see Section 2.8.1).

Table 3. Primary spruce bark lipophilic extractives identified and quantified by gas chromatography-mass spectrometry (GC-MS).

Lipophilic Extractives Groups		**Amount, % of Total Lipophilic Extractives**		
Resin acids		44		
Fatty acids		21		
Diterpenoids		14		
Other		10		
Sterols		8		
Fatty alcohols		3		
Resin acids	Compound	Amount, % of total resin acids	Retention time, min	Symbol
	Dehydroabietic acid	23	30.91	a
	Isopimaric acid	16	29.91	b
	Abietic acid	10	31.44	c
	Levopimaric acid	10	30.73	d
	Neoabietic acid	9	33.19	e
	Palustirc acid	9	30.32	f
	Hydroxydehydroabietic acid 1	3	34.60	g
	Sandaracopimaric acid	3	29.62	h
	Hydroxydehydroabietic acid 2	3	34.01	i
	4-Hydroxycinnamic acid	2	21.89	j
	Imbricatolic acid	2	33.00	k
	Pimaric acid	2	29.30	l
	Unidentified hydroxy resin acid	2	35.53	m
	Ferulic acid	1	25.04	n
	2,4-Dihydroxybutanoic acid	1	11.23	o
Fatty acids	Compound	Amount, % of total fatty acids	Retention time, min	Symbol
	Acid 18:1	24	27.43	p
	Acid 18:2	23	27.32	q
	Acid 18:3	16	26.93	r
	Acid 22:0	9	35.31	s
	Acid 16:0	6	23.93	t
	Acid 20:0	5	31.70	u
	Acid 24:0	5	38.71	v
	Acid 18:0	4	27.89	w
	Acid 17:0	3	25.37	x
	Acid 25:0	2	35.10	y
	Acid 15:0	1	7.66	z
	Docosanedioic acid	0.5	42.55	A
Diterpenoids	Compound	Amount, % of total diterpenoids	Retention time, min	Symbol
	Δ^{13}-(*trans*-)neoabienol	26	26.34	B
	Manool	18	25.95	C
	Thunbergol	17	25.76	D
	cis-Abienol	9	26.94	E
	Palustral	5	28.01	F
	Dehydroabietal	4	28.63	G
	Cubebene	4	8.67	H
	Isopimarol	3	28.18	I
	Epimanoyl oxide	3	23.61	J
	Pimarol	3	31.00	K
	Isopimaral	2	27.46	L
	Cadinene	1	9.21	M
Sterols	Compound	Amount, % of total sterols	Retention time, min	Symbol
	Sitosterol	74	48.02	N
	Campesterol	16	46.05	O
	Diglycerol	6	10.54	P
	24-Methylenecycloartanone	3	55.75	Q
	Acid 18:2 monoglyceride	1	10.22	R
Fatty alcohols	Compound	Amount, % of total fatty alcohols	Retention time, min	Symbol
	Alcohol 22:0	37	33.72	S
	Alcohol 24:0	28	37.18	T
	Alcohol 18:0	17	26.16	U
	Alcohol 15:0	17	32.51	V

Table 4. The primary spruce bark hydrophilic extractives identified via gas chromatography-mass spectrometry (GC-MS).

Hydrophilic Extractives Groups		**Amount, % of Total Hydrophilic Extractives**		
Sugars		37		
Organic acids		25		
Others		12		
Stilbenes		11		
Alcohols		10		
Flavonoids		4		
Sugars	Compound	Amount, % of total sugars	Retention time, min	Symbol
	Glucose	59	19.31, 19.95, 21.45, 21.66, 23.30	a, b, c, d, e
	Sucrose	20	36.44, 45.43, 52.05	f, g, h
	Maltose	9	34.64, 38.06, 38.61, 40.46	i, j, k, l
	Galactose	2	15.76, 21.94, 44.69	m, n, o
	Cellobiose	1	44.94, 47.65, 50.76	p, q, r
	Trehalose	1	37.45	s
	Lactulose	1	44.50	t
	α-Lactose	1	44.30	u
Organic acids	Compound	Amount, % of total organic acids	Retention time, min	Symbol
	Gluconic acid	47	19.66, 23.77	v, w
	Aconitic acid	24	19.82	x
	Quinic acid	20	20.80	y
	Malic acid	4	13.20	z
	Shikimic acid	2	19.45	A
	Docosanedioic acid	1	42.55	B
	3,4-Dihydroxymandelic acid	1	41.14	C
Stilbenes	Compound	Amount, % of total stilbenes	Retention time, min	Symbol
	Isorhapontin	54	66.45	D, E
	Astringin	18	65.80	F
	Piceid	17	61.25	G
	cis-Piceatannol	6	39.56	H
	Rhapontigenin	5	39.35	I
Alcohols	Compound	Amount, % of total alcohols	Retention time, min	Symbol
	Pinitol	31	19.94	J
	Salicin	14	41.87	K
	Guaiacyl glycerol	13	16.42, 40.33	L, M
	Maltotriitol	11	39.00, 43.34	N, O
	Maltitol	10	44.00	P
	Inositol	6	25.40	Q
	Arabitol	6	17.87	R
	Mannitol	5	22.26	S
	Dihydroconiferin	4	40.78	T
Flavonoids	Compound	Amount, % of total flavonoids	Retention time, min	Symbol
	Taxifolin glycoside	26	42.39	U
	Taxifolin	21	41.51	V
	Catechin	21	40.07	W
	Naringin	20	45.79, 48.80	X, Y
	Ampelopsin	12	42.17	Z

Detailed qualification of the lipophilic compounds visible only in the short-column spectrum, that is, steryl esters and triglycerides, was limited because this measurement was done on equipment without a mass detector and only with FID. The general group identification of triglycerides and steryl esters, as seen in Figure A3, was, however, possible due to previous studies that used the same method for the group analysis of wood extractives [31,39].

The results in Table 4 demonstrate that the most significant part (37%) of the hydrophilic water extract in all the samples was accounted for by various soluble sugars, followed by organic acids (25%). Stilbenes and alcohols made up 11% and 10% of the identified extractives, respectively, while the amount of flavonoids present was only 4%. Even with short-column GC, a relatively large part of the water extract was left uniden-

tified. The unidentified hydrophilic compounds contained such large oligomeric and polymeric molecules that they could not be analysed in the gas phase even after silylation. Such compounds would presumably have contained procyanidins, oligomeric phenolic compounds (e.g., stilbenes) and sugars as well as lignin. Based on a recent study's analytical pyrolysis results, there also appeared to be lignin fragments present in hot-water extracts of spruce [40]. However, as shown in Figure A5, the short-column GC did reveal two additional compound groups, which, based on a previous study [41], could be identified as dilignans and sesquilignans. However, the lack of monomeric lignans in the studied bark samples, combined with the fact that HPLC analysis of water extracts confirmed the presence of distilbenes, suggested that these compounds should more likely be counted as distilbenes and sesquistilbenes. Nevertheless, the lack of a mass detector in the short-column GC prevented the final confirmation of this presumption.

3.3.2. Quantitative Analysis by Gas Chromatography with a Flame Ionisation Detector (GC-FID)

The changes in the amount of lipophilic extractives in the bark samples during winter and summer storage are shown in Figure 4. The average amount of identified lipophilic extractives during winter and summer was around 43 mg/g and 50 mg/g of dry matter. In comparison, approximately 20 mg/g and 23 mg/g of dry matter were left unidentified using chromatographic methods during winter and summer, respectively. Based on the total amount of extractives, no clear trends in the extractives decay could be observed. Instead, the changes that were seen could be understood to reflect the natural variation in the amount of lipophilic compounds between any saw logs of similar outward appearance.

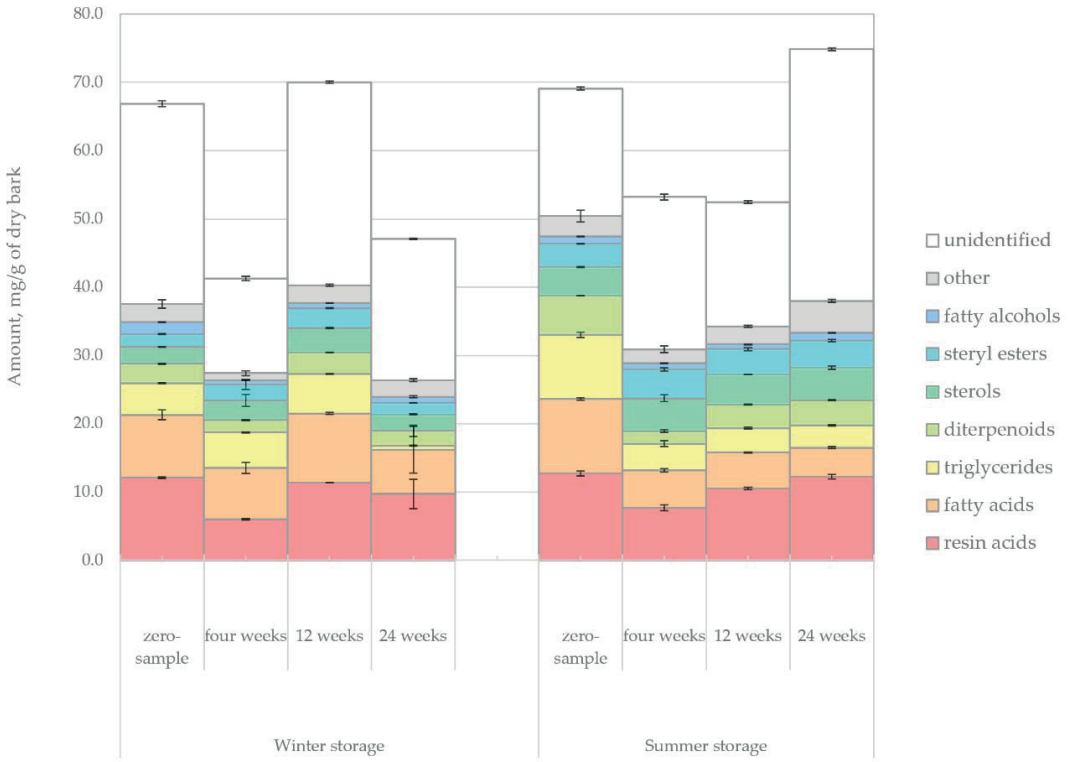


Figure 4. Changes in the amount of lipophilic compounds (mg/g of dry matter) in saw log bark during summer and winter storage periods. Results presented here are a combination of the quantified long-column gas chromatography with a flame ionisation detector (GC-FID) individual free and esterified compounds and the short-column GC-FID extractives groups (triglycerides and steryl esters).

A more in-depth view of the different compound groups revealed clear trends, such as the gradual decrease of triglycerides because of hydrolysis, a well-known reaction during wood storage [9,15,42]. While the summer-stored bark lost triglycerides faster at the beginning of the storage period, from week 12 onwards (as the temperatures decreased from 15 °C to 0 °C), hydrolysis reactions were also reduced. In the winter-stored bark, triglycerides' hydrolysis was limited for the first 12 weeks of storage, after which, as the temperatures rose to 20 °C, the triglycerides were lost rapidly. A similar pattern of degradation could be seen in the total amount of fatty acids. In the summer-stored bark, compared to the winter-stored bark, there was a 44% increase of diterpenoids, 19% increase of resin acids, 4% increase of sterols, 51% increase of other lipophilic compounds, 73% increase of steryl esters and 24% increase of triglycerides during storage. The higher lipophilic content during the summer storage agreed with a previous study [43], where it was shown that higher temperatures increased the hydrolysis of esterified compounds.

The changes in the amount of hydrophilic compounds during the winter and summer storage periods are shown in Figure 5. Unlike in the case of lipophilic compounds, clear trends were observed for hydrophilic extractives. The total amount of extractives decreased 41% during winter storage and 62% during summer storage. This difference could easily be understood due to the seasonal effects, namely, elevated microbial activity, increased rate of hydrolysis and increased UV radiation during the warm summer months. During summer, the decrease of extractives was immediately noted after 4 weeks of storage, but the extractives content remained high for 12 weeks during winter storage.

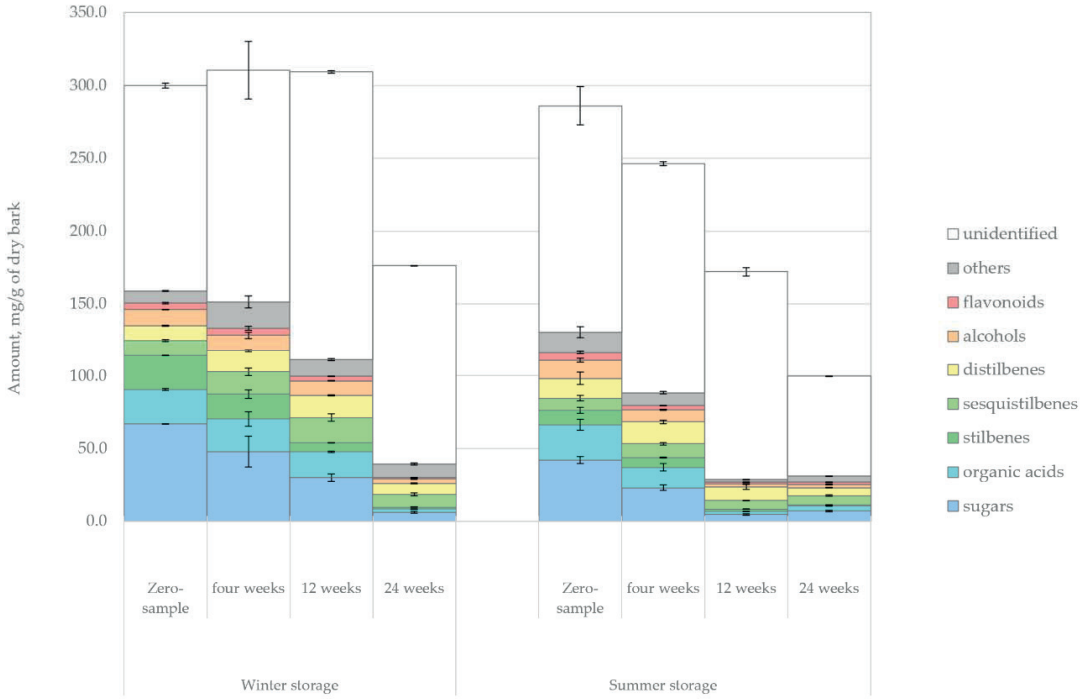


Figure 5. Changes in the amount of hydrophilic compounds (mg/g of dry matter) in saw log bark during summer and winter storage periods. The identification of the extractives group sesquistilbenes is conjectural. It is also possible that this group should be identified as sesquilignans.

It should be noted that the degradation rate of hydrophilic extractives reported here is not as significant as in previous studies that focus on the storage of chipped wood, bark or logging residue in large piles. Ekman, as cited by Sjöström [42], has reported even 50% degradation of hydrophilic extractives in 50 days. A faster loss of hydrophilic compounds in pile-stored material, compared to the storage of saw logs, could be expected because,

in pile storage, various factors increase the likelihood of material losses, such as smaller particle size, pile compactness and self-heating caused by microbial activity [10,11,44].

A more in-depth look at the individual compounds' changes during storage based on extractives groups is given in Figures 6–10 for lipophilic extractives and in Figures 11–15 for hydrophilic extractives. The exact amounts of the different compounds presented at each figure as well as their standard deviations are presented as Supplementary Files (link to Supplementary Files can be found at the end of the article).

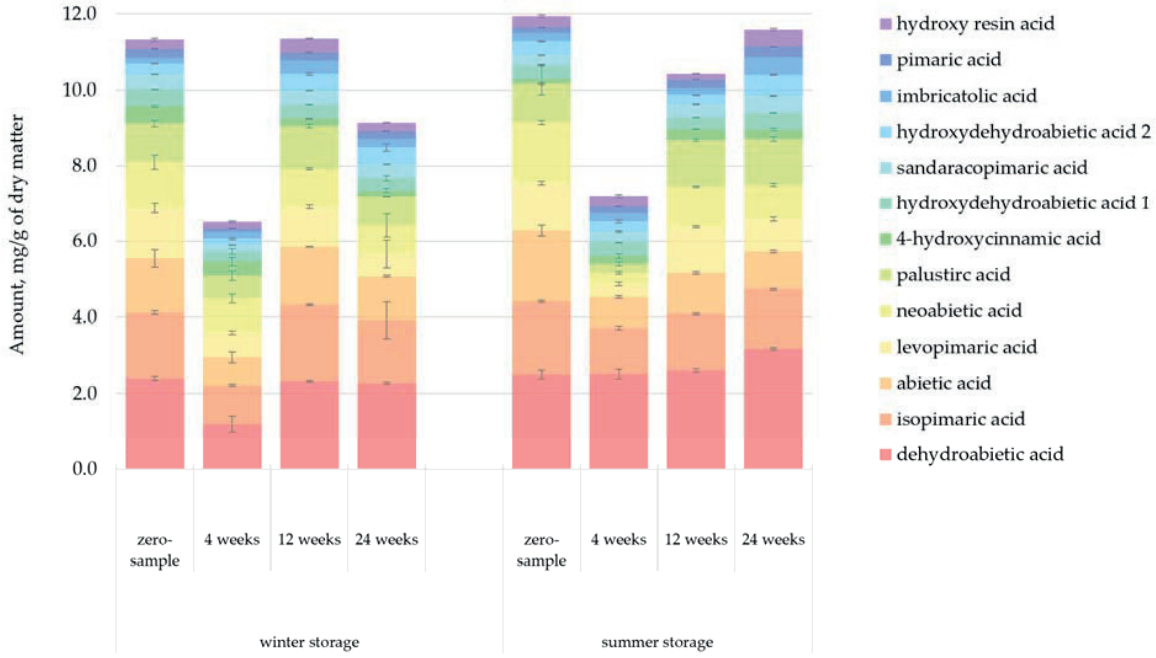


Figure 6. Changes in resin acids during summer and winter storage periods of saw log bark.

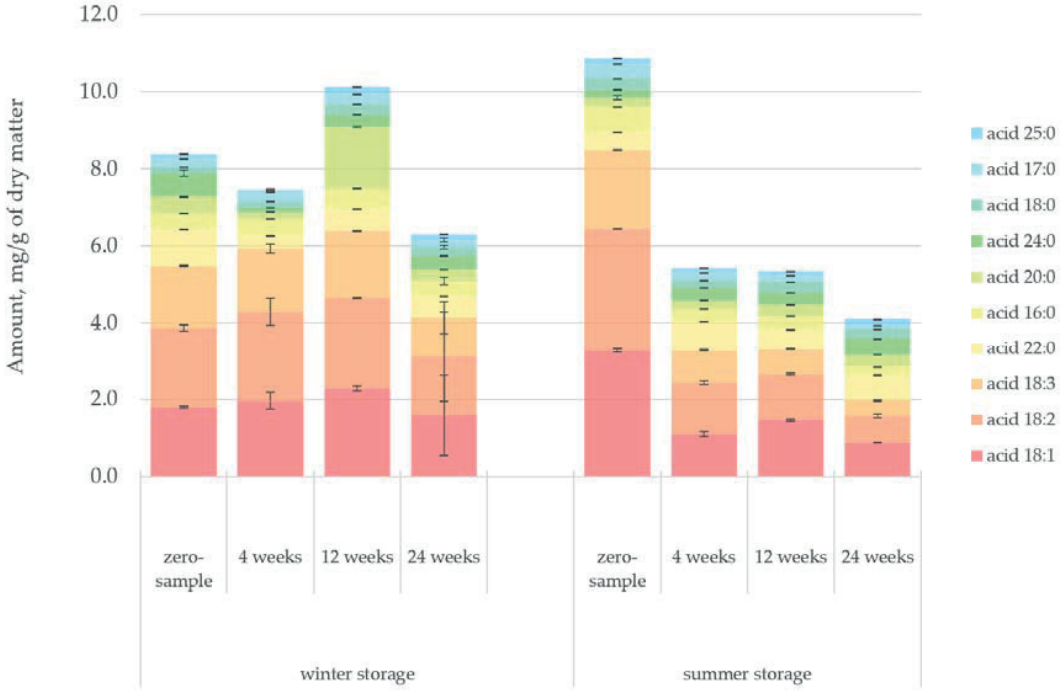


Figure 7. Changes in fatty acids during summer and winter storage periods of saw log bark.

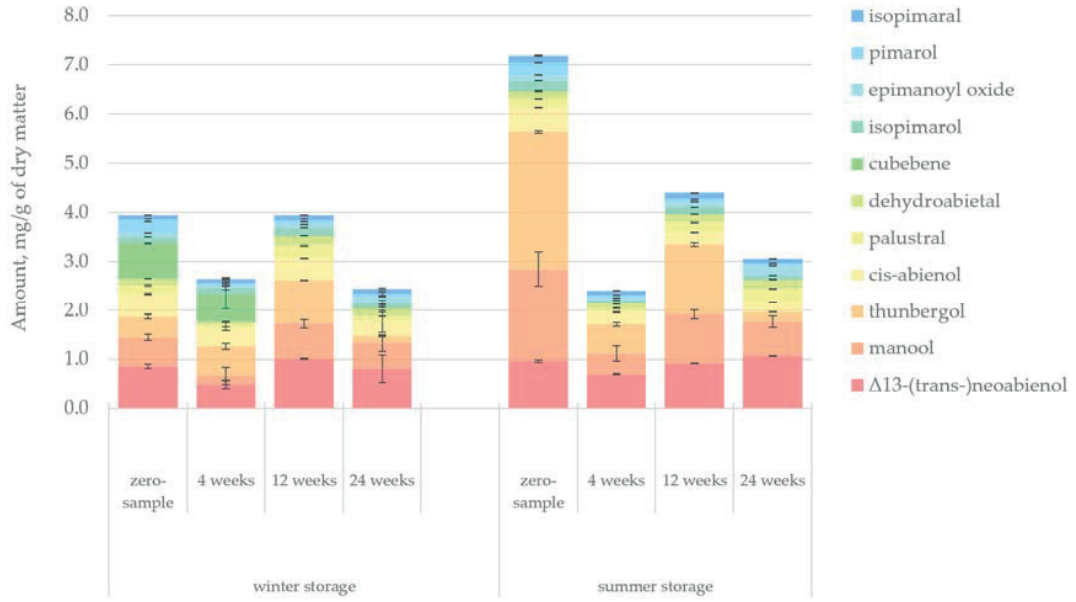


Figure 8. The changes in diterpenoids during summer and winter storage periods of saw log bark.

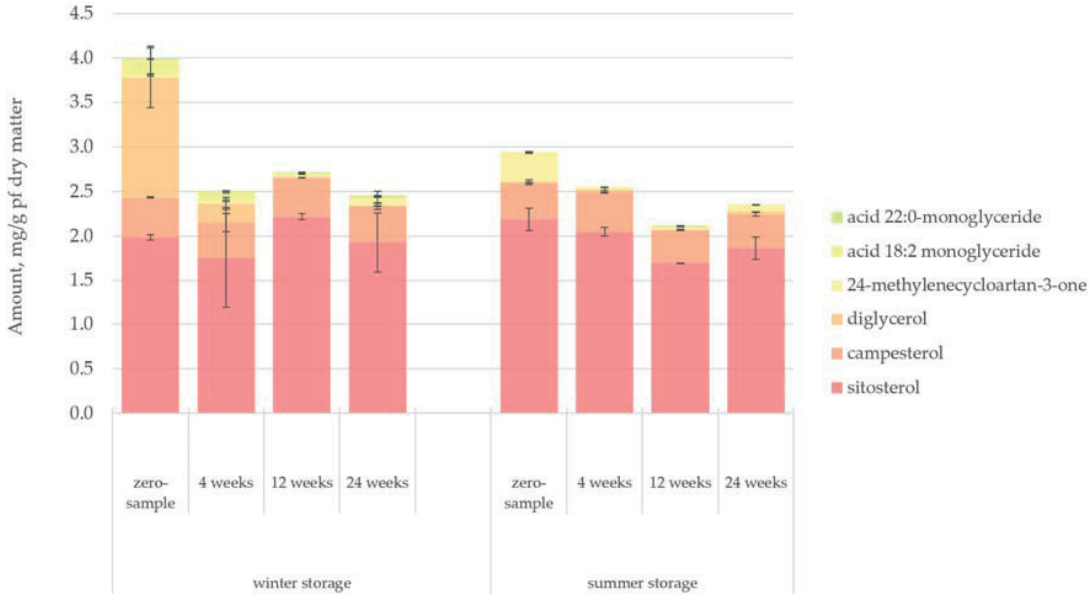


Figure 9. Changes in sterols during summer and winter storage periods of saw log bark.

Figure 6 presents the quantified amount of resin acids in the lipophilic bark extracts. There appeared to be a general correlation between the total amount of resin acids and the total amount of lipophilic extractives. The total amount of resin acids remained relatively constant throughout the storage periods. Previous studies have demonstrated that resin acids can be resistant to microbial degradation [16]. Samples from week 4 had a notably lower amount of resin acids compared to other samples. However, this result should be interpreted as a sign of the natural variance in the amount of lipophilic compounds between trees, not as a general principle.

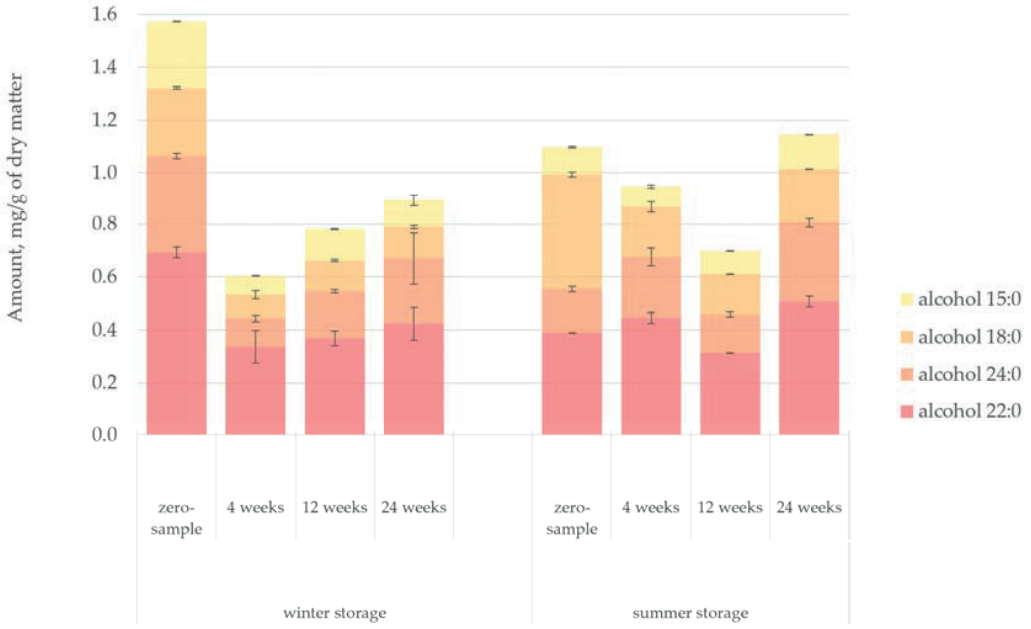


Figure 10. Changes in fatty alcohols during summer and winter storage periods of saw log bark.

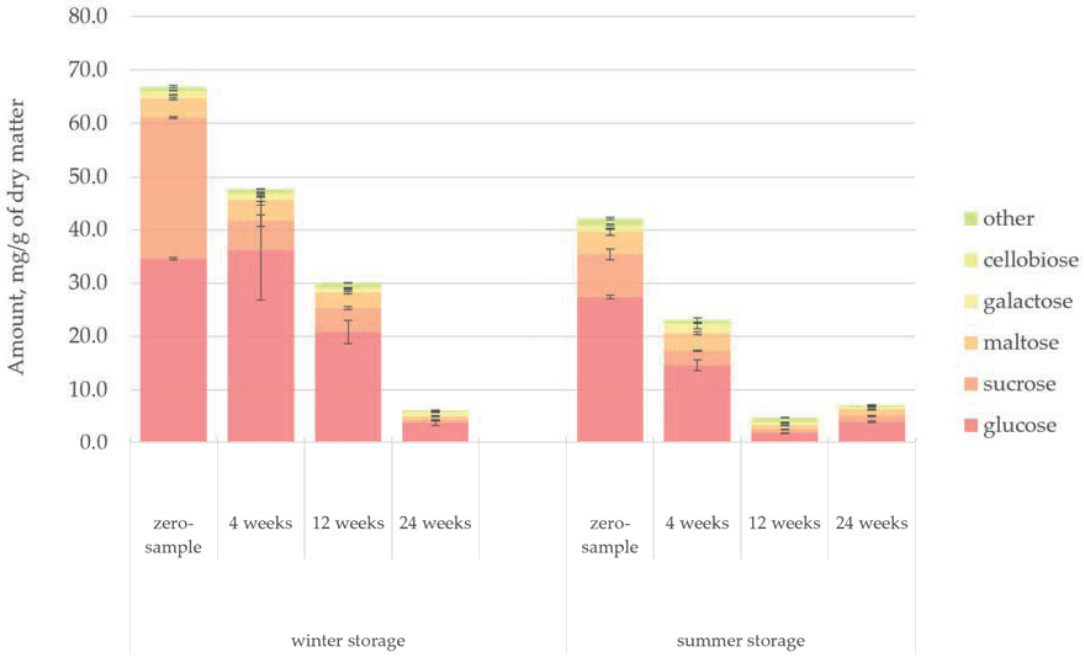


Figure 11. Changes in sugars during summer and winter storage periods of saw log bark.

Figure 7 presents the quantified amount of fatty acids in the lipophilic bark extracts. The rate of fatty acid degradation followed a quite predictable path in both storage studies. The initial amount of fatty acids was higher at the beginning of the summer storage period. However, fatty acids also decreased faster during summer, primarily due to increased hydrolysis, oxidation and reactions of conjugated double bonds. The sample from the winter storage period at week 12 appeared to be an anomaly, having a generally high concentration of fatty acids but also a much higher amount of acid 20:0 compared to other samples. The difference might be explained by the fact that, as seen in Table 1, the sample logs from the winter storage period at week 12 were around 30 years younger than the

average age of the trees used in the study. The same observation was true for the zero-sample saw logs from the summer storage period, which had an equally high concentration of fatty acids. This result suggested that a tree's age influences the amount of fatty acids in spruce bark. For this study, having a significant variance in tree age was an interesting factor to note but not an objective that was pursued. For meaningful inferences from tree age to extractives content to be made, the number of samples would have needed to be significantly higher. As a general principle, older trees have more resin in the heartwood. Moreover, while this behaviour could also be expected of bark, the authors of this paper are not aware of any studies that carefully demonstrate the relationship between tree age and the total extractives content of Norway spruce bark.

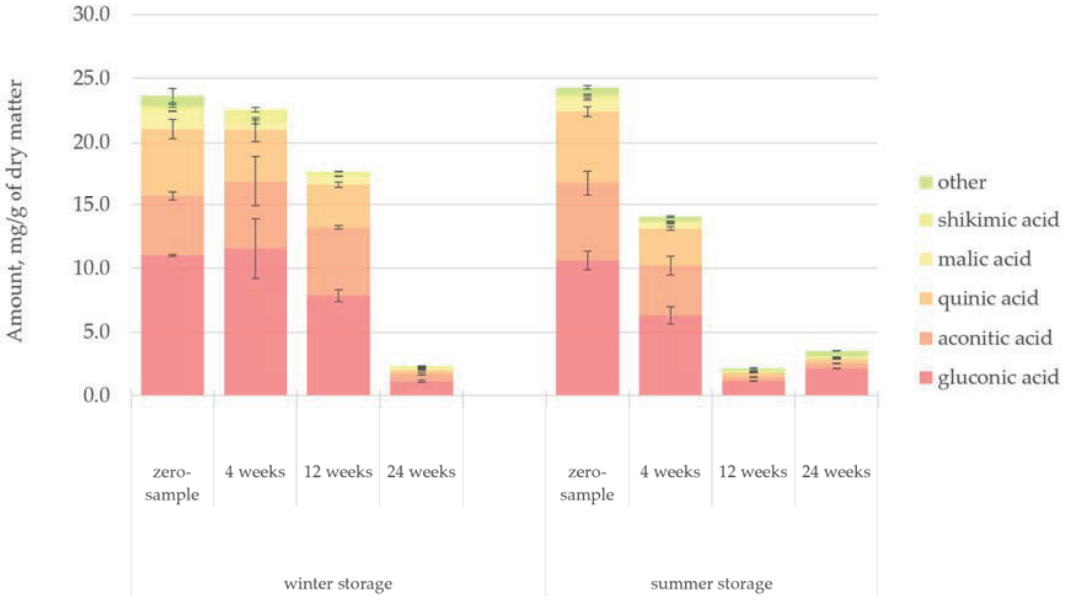


Figure 12. Changes in organic acids during summer and winter storage periods of saw log bark.

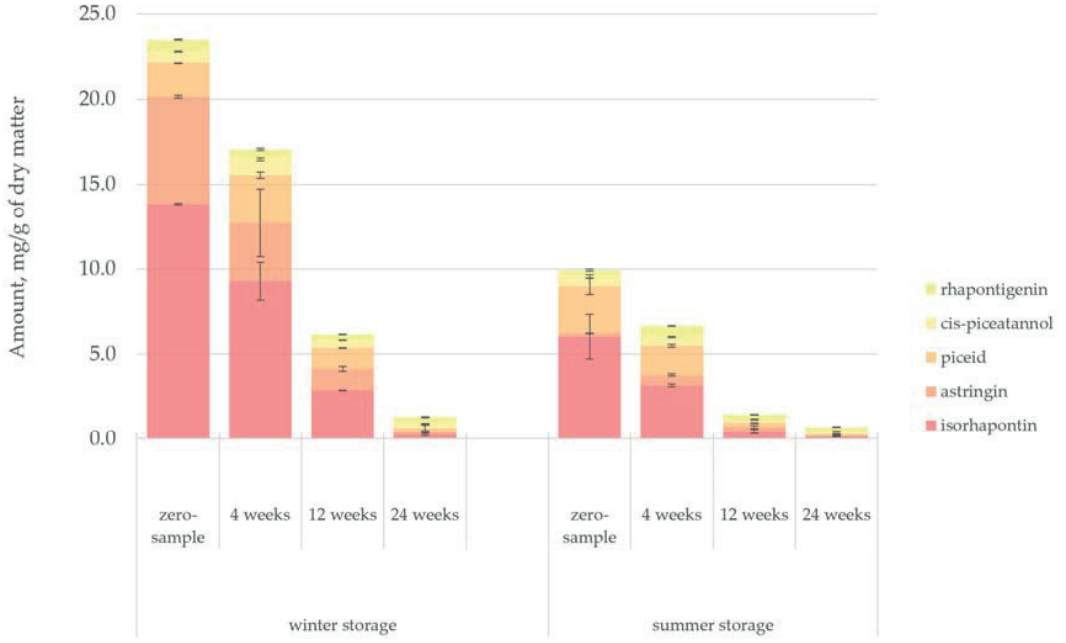


Figure 13. Changes in stilbenes during summer and winter storage periods of saw log bark.

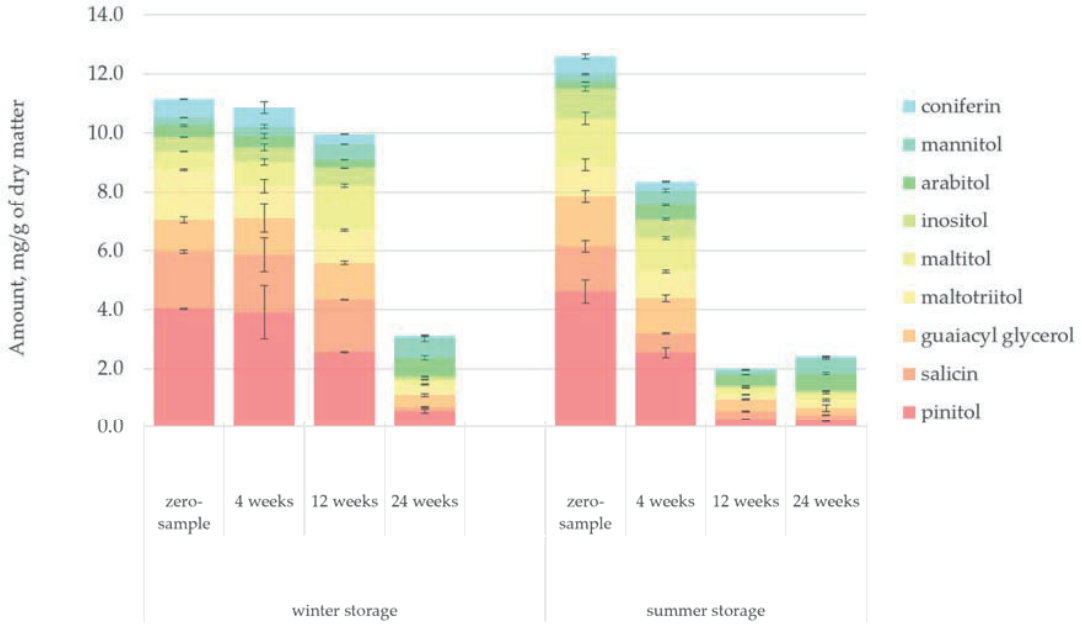


Figure 14. Changes in alcohols during summer and winter storage periods of saw log bark.

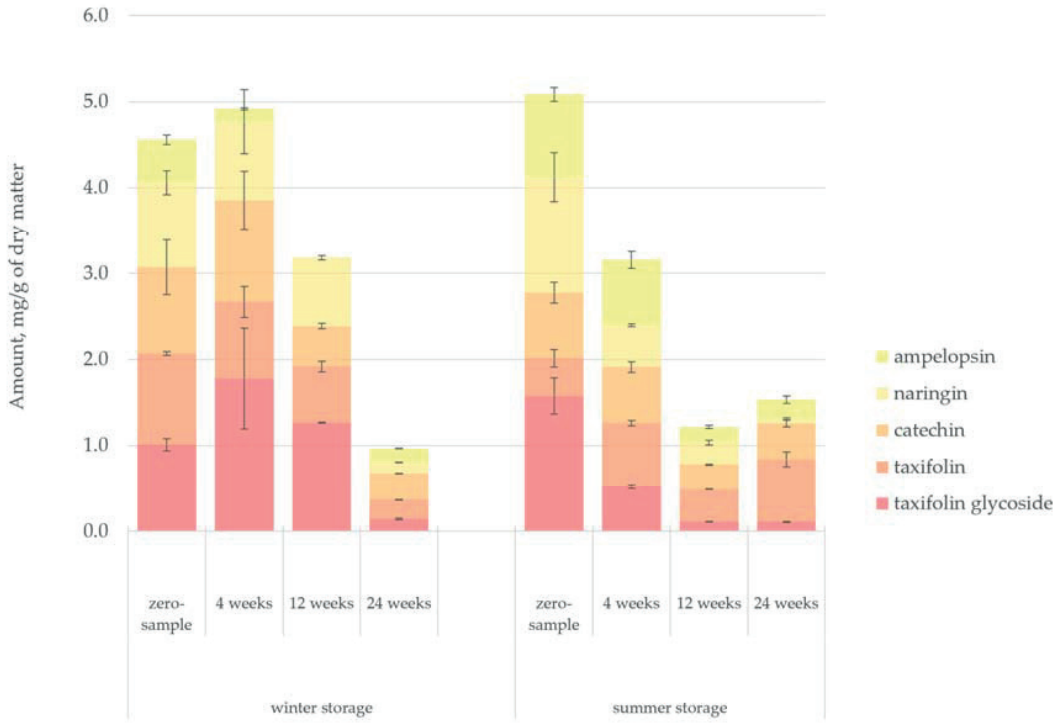


Figure 15. Changes in flavonoids during summer and winter storage periods of saw log bark.

Figure 8 presents the quantified amount of diterpenoids in the lipophilic bark extracts. The total amount of diterpenoids was higher during the summer storage period, especially the amount of manool and thunbergol. Nevertheless, the amount of cubebene appeared to be higher during the winter storage period. As noted before regarding resin acids, samples at week 4 had particularly low concentrations. Despite this variation, the total amount of diterpenoids slowly decreased as the storage period lengthened.

Figure 9 presents the quantified amount of sterols in the lipophilic bark extracts.

Sitosterol was the most significant and abundant sterol in spruce bark, while campesterol was overall the second abundant, remaining relatively constant in all samples. Diglycerol appeared with a high concentration on the zero-samples of the winter-stored bark. However, its amount was quickly reduced as the storage period continued and the ambient temperature rose. During the summer storage period, diglycerol could not be detected even at the zero-sample.

Figure 10 presents the quantified amount of fatty alcohols in the lipophilic bark extracts. The degradation pattern of fatty alcohols in the bark samples paralleled the degradation pattern of sterols, more or less. Particularly high concentration of fatty alcohols (especially alcohol 22:0) was observed at the beginning of the winter storage period. On the other hand, the zero-sample from the summer storage period had a notably high concentration of alcohol 18:0. Although the total amount of fatty alcohols remained stable, after the initial decrease in the amount of fatty alcohols, there appeared to be a slight increase until the end of the storage period. This increase could be explained by the gradual conversion of fatty acids or triglycerides into fatty alcohols.

Figure 11 presents the quantified amount of sugars in the hydrophilic bark extracts. The results indicated that the concentration of simple sugars in the extracts decreased systematically and significantly. Similar results have been reported in storage studies of wood [45]. Glucose and sucrose alone made up most of the detected sugars. A clear difference could be noticed in the initial sugar concentration between the winter- and summer-stored bark. The winter-stored bark had a 37% higher sugar concentration initially. Saccharides are especially prone to degradation since they could easily be leached out by rain and provide an excellent nutrient source for micro-organisms. The enhanced activity of micro-organisms during warm seasons also explains the more significant reduction of saccharides observed during the summer storage period.

Figure 12 presents the quantified amount of organic acids in the hydrophilic bark extracts. Gluconic, aconitic and quinic acids accounted for most of the organic acids present in the hot-water extract of spruce bark. Gluconic acid was found to be the most abundant organic acid, probably related to the high glucose level in the bark samples. It has been shown that gluconic acid may be obtained by the oxidation of glucose catalysed by the glucose oxidase enzyme [46]. The degradation pattern of organic acids followed that of the sugars, although the initial amount of organic acids in bark during storage was not affected by season. The degradation of organic acids was slower than that of sugars, but again a clear difference in degradation rate was observed between the winter and summer storage. The winter-stored bark retained 76% of the original amount of organic acids until 12 weeks of storage, while there was a 42% decrease in the concentration of the organic acids during the first 4 weeks of the summer storage period.

Figure 13 presents the quantified amount of stilbene glucosides (by long column GC-FID) in the hydrophilic bark extracts. Stilbenes from conifer bark have been the focus of many previous studies, giving them promise as platform chemicals and antifungal agents [2,25,32,47–49]. The three primary stilbene glucosides in spruce bark, isorhapontin, astringin and piceid, along with the stilbene aglycones *cis*-piceatannol and rhapontigenin, were identified. The total amount of stilbenes was 23.5 mg/g of dry bark in the winter storage zero-sample and 9.9 mg/g of dry bark in the summer storage zero-sample. Gabaston et al. [32] reported a similar concentration (18.5 mg/g of bark) in a sample stored for 1 month in the dark. On the other hand, much higher stilbene concentrations have been reported on the root bark of Norway spruce [48].

The degradation of the stilbene glucosides and aglycones in the bark samples followed a similar pathway as seen for the sugars. The evident influence of the season was seen in that the zero-sample of the summer-stored bark contained 58% fewer stilbenoids than the zero-sample of the winter-stored bark. However, the degradation rate of stilbenoids was approximately the same during both winter and summer.

Stilbenes are known for being sensitive to UV light and may easily undergo photocatalysed *cis/trans* isomerisation and rearrangement reactions to form phenanthrene

structures [17,18,50]. Because of exposure to an increased amount of UV light and higher temperatures during the summer storage period, the loss of stilbenes would be expected to be amplified. Lesions exposing the inner bark (e.g., those caused during harvesting) would be expected to increase stilbenes' degradation because they are primarily located on the inner bark [49]. Increased microbiological activity during summer also explains the reduction of stilbenes because, although stilbenoids are antimicrobial by nature, they are not inert to microbial or enzymatic degradation. The increased UV light appeared to have a particularly significant effect on the level of the stilbenoid astringin. Astringin had a substantially lower concentration during the summer, but during winter, it was the second most abundant stilbenoid after isorhapontin.

Figure 14 presents the quantified amount of alcohols in the hydrophilic bark extracts. The degradation pattern of alcohols in the saw log bark during storage closely resembled that of organic acids. Most of the alcohols found in the hot-water extract, including pinitol, maltotriitol, maltitol, inositol, arabitol and mannitol, were sugar alcohols. It was evident that temperature dramatically affected the reduction of alcohols. During the winter storage period, until week 12 (i.e., when the ambient temperature according to Figure 2 was approximately 5 °C), the amount of alcohols remained relatively high. However, during the summer storage period, the amount of alcohols decreased by 34% immediately after 4 weeks of storage. The slight increase in the level of alcohols during the summer storage period from week 12 to 24 (a similar pattern as was seen for organic acids, sugars and flavonoids) could be explained by the natural variance between the saw logs (e.g., the age of the trees). However, the decrease in the summer storage ambient temperature from week 12 to 24 (from 13 °C to 0 °C according to Figure 2) could also explain the results.

Figure 15 presents the quantified amount of simple flavonoids in the hydrophilic bark extracts. Among the identified flavonoids, a remarkably similar degradation pattern to alcohols was observed. However, flavonoids appeared to be more sensitive than alcohols to ambient temperature increase towards the end of the winter storage period. The amount of identified flavonoids was quite insignificant compared to other extractives groups, although it should be noted that the compound group designated as 'others' (in Figure 5) most likely included several unidentified flavonoid derivatives. The identification of individual flavonoids from bark extracts was especially challenging because of the myriad of compounds that were eluted at the same time during the GC-MS analysis (Figure A4). All of the identified flavonoids eluted approximately at the retention time of 40–50 min, containing many overlapping peaks.

3.3.3. Quantitative and Qualitative Analysis by HPLC

The used HPLC method was targeted specifically for the identification and quantification of stilbenes from the bark extract. A tentative identification and quantification of several stilbene monomers and dimers were achieved based on the compounds' mass fragmentation. The qualitative and quantitative results are presented in Table 5, and possible structures for some of the compounds are suggested in Appendix B Figure A6. Similar molecular structures have also been suggested for stilbene dimers in earlier studies [32,47,51]. The qualification and quantification of stilbenes were done for the zero-sample and the sample of the winter-stored saw logs stored for 24 weeks. The quantification was undertaken via external standard *trans*-piceid. A standard calibration curve with concentrations ranging from 1 μg/mL to 40 μg/mL was prepared with a good linearity and correlation coefficient R^2 with the value of 0.9991.

Table 5. The qualitative and quantitative results of the high-performance liquid chromatography (HPLC) analysis of bark hot-water extract.

Retention Time (min)	[M-H] *m*/*z*	Main Fragments (*m*/*z*)	Tentative Identification	Amount (mg/g of Dry Matter)		Change (%)
				Zero-Sample	After 24 Weeks	
7.6	405	811, 473, 243	astringin	8.8	1.5	−82.5
9.7	389	809, 567, 435, 299, 227	*trans*-piceid	3.7	1.7	−52.6
10.5	419	839, 465, 257	*trans*-isorhapontin	14.7	2.3	−84.6
Total stilbenoid monomers				27.1	5.5	−79.6
11.5	823	707, 665, 299	isorhapontin dimer 1	2.5	1.3	−49.6
12.3	809	845, 575, 541, 299, 187	astringin dimer 1	1.1	0.6	−45.5
12.5	809	823, 555, 163	astringin dimer 2	2.1	0.8	−60.7
13.3	823	859, 555, 299	isorhapontin dimer 2	3.1	4.8	58.4
14.6	837	569, 299	isorhapontin dimer 3	1.9	1.9	0.1
Total stilbenoid dimers				10.8	9.5	−11.7
Total stilbenoids (monomers and dimers)				37.9	15.0	−60.3

The three stilbene glucosides, *trans*-isorhapontin, astringin and *trans*-piceid, were the most prominent of the identified stilbenoids. Small amounts of several dimeric stilbenoid species were also recognised, at least three of which were assumedly based on dimeric isorhapontin and two on dimeric astringin species. These results also facilitated identifying the compounds in Figure A5 with the retention time range of 16–18 min as dimeric stilbenoids. In the zero-sample, *trans*-isorhapontin was the most abundant of the identified monomeric stilbenoids (14.7 mg/g of dry matter), astringin came second (8.8 mg/g of dry matter) and *trans*-piceid third (3.7 mg/g of dry matter). These results agreed with those obtained by GC-MS, although the HPLC samples' reported amount was slightly higher. This difference could be explained by the fact that in comparison to GC, the HPLC method is more sensitive, the sample preparation for analysis is more straightforward and the delay between extraction and identification of the compounds is shorter. Hence, less degradation and error due to sample treatment occurred.

Among the identified stilbenoids, dimeric stilbenoids were the least abundant. The dimeric species with the main fragment of *m*/*z* 823 were identified as isorhapontin dimers 1 and 2, which were the most abundant in the zero-sample at 2.5 mg/g and 3.1 mg/g of dry matter, respectively. Other dominant dimeric species were those with the main fragment *m*/*z* 809, identified as astringin-based stilbene dimers, and one with the main fragment *m*/*z* 837, identified as isorhapontin dimer 3.

If the dimeric species were accurately identified, it would suggest that *trans*-piceid does not form similar dimeric species as do astringin and *trans*-isorhapontin. This would be understandable since, in the proposed structures for the dimeric species, the ether bonds connecting the monomeric species are formed via the two phenolic hydroxyl/methoxy groups of astringin and isorhapontin (at ring B; see Appendix B Figure A6). Because *trans*-piceid only has one phenolic hydroxyl group in ring B, it would only form single ether bonds, which are not as strong.

As shown in Table 5, there was approximately a 60% decrease in the total amount of the identified stilbenoids. The degradation of stilbenoid monomers and that of dimers was not, however, equal. The monomers decreased by almost 80%, while the dimers decreased by only around 10%. It should be noted that some of the dimeric species were more abundant at the end of the storage period. The amount of isorhapontin dimer 2 increased by almost 60%. This result may indicate that polymerisation reactions play an essential role in the degradation of stilbenoids. However, evaluating the mechanisms of the polymerisation reactions (whether they be enzymatic in nature or something else) was not within the scope of this study.

When looking at both dimeric and monomeric species, it appears that overall astringin-related stilbenoids corresponded with the most significant losses during storage. This observation was also confirmed by the GC results (Figure 13), especially during the sum-

mer storage period, where even the initial amount of astringin in the zero-sample was vanishingly small and reached undetectable levels by week 24 of the storage period. The loss of astringin might be explained by the fact that, of the stilbenoid monomers, astringin has the highest amount of the free phenolic hydroxyl groups, which is known to increase reactivity and antioxidant activity.

3.4. Change in Carbohydrate and Lignin Content

The results from the carbohydrate and lignin analysis of the bark samples are shown in Table 6. The carbohydrate content was measured from the extractives-free bark at the beginning and the end of the storage period. For each set of extractives-free bark, two samples were taken for carbohydrate analysis. Since the extractions were duplicated, the carbohydrate results for each week were presented as the mean values from four measurements. The cellulose content presented in Table 6 was calculated as the difference between the monosaccharide content (from acid hydrolysis) and the hemicellulose content (from acidic methanolysis). The compounds designated as 'others' were the solid leftovers unexplained by the methods used and were calculated by subtracting the total carbohydrate and lignin content from the samples' dry mass. This group presumably contained inorganic compounds.

Table 6. Change in chemical composition of extractives-free spruce bark during storage (% of dry matter).

	Storage Time (Weeks)	Cellulose	Hemicelluloses	Acid-Insoluble Lignin	Acid-Soluble Lignin	Others
Winter storage	0	25.8 ± 5.4	26.1 ± 2.4	35.1 ± 3.4	1.6 ± 0.03	11.5 ± 4.6
	24	14.6 ± 0.4	33.2 ± 3.9	32.2 ± 1.0	1.3 ± 0.05	18.8 ± 1.1
Summer storage	0	22.0 ± 3.1	22.9. ± 2.4 *	40.8 ± 2.5	1.3 ± 0.1	13.0 ± 0.8
	24	7.3 ± 3.1	36.4 ± 3.3	37.8 ± 0.6	1.2 ± 0.03	17.3 ± 3.7

* A result based on a duplicate sample, while other hemicellulose results were based on four samples.

According to Table 6, at the beginning of the winter storage period, 51.9% of the extractives-free bark was made up of carbohydrates (i.e., hemicelluloses and cellulose), and 44.9% at the beginning of summer storage. On the other hand, the total lignin amount was 36.7% at the beginning of the winter storage period and 42.1% at the beginning of the summer storage period. At the end of the 24-week storage periods, the total carbohydrate content was 47.7% and 43.7% during winter and summer, respectively. At the end of the 24-week storage period, the total lignin content was 33.5% and 39.0% during winter and summer, respectively. The total carbohydrate and lignin content remained relatively stable, ensuring the bark's value, for example, for bioenergy production. These results might be somewhat misleading in that they only deal with the remaining bark on the saw logs and do not consider the total material losses. The total mass losses could not be evaluated by direct observation as the bark needed to remain intact on the saw logs and could not be removed until the sampling.

Table 6 shows that the carbohydrate and lignin content changes in the bark samples followed similar trends both during the summer and winter storage periods. At the beginning of the storage periods, the hemicellulose content was 0.4% and 2.4% higher than the cellulose content during winter and summer, respectively. The amount of hemicellulose increased by 7.1% and 13.5% during the winter and summer storage periods, respectively. The cellulose content decreased by 11.2% and 13.2% during the winter and summer storage periods, respectively. Hence, at the end of the 24-week storage periods, the hemicellulose content was 18.7% and 29.1% higher than the cellulose content during winter and summer, respectively.

At the beginning of the storage periods, the amount of acid-insoluble lignin was 33.5% and 39.5% higher than the amount of acid-soluble lignin during winter and summer, respectively. The amount of acid-insoluble lignin decreased by 2.9% and 3.0% during winter and summer, respectively. The amount of acid-soluble lignin decreased by only

0.3% and 0.1% during winter and summer, respectively. The amount of other compounds increased by 7.3% and 2.8% during winter and summer, respectively.

The results indicated that the content of cellulose, hemicelluloses and acid-soluble lignin in the bark was lower during the summer storage period, while the amount of acid-insoluble lignin was higher during the summer storage period. The observed decrease of the cellulose content during the storage periods could be explained by the ceasing of the active natural protective measurements (for example, synthesis of protective compounds) of trees after their felling (due to lack of nutrients) against microbial and enzymatic activity as well as their exposure to UV light and weather. The lower initial level and higher degradation rate of cellulose during the summer storage period could be primarily explained by the increased amount of UV light and higher temperatures, which in turn would have enabled more microbial and enzymatic activities.

3.4.1. Changes in Monosaccharide Content

The HPAEC results shown in Figure 16 indicate changes in the content of bark monosaccharides in the acid hydrolysate. The results show that the glucose units were the most prevalent in the samples throughout the storage periods, making up 30–36% of the extractives-free bark, while the arabinose, mannose, xylose and galactose units accounted for 5–7%, 1–4%, 3–4% and 2–3% of dry matter, respectively. The released monosaccharide units were 5.5% more abundant during the winter storage period as opposed to the summer storage period. The storage had the most significant effect on the loss of glucose units. It should be noted that, in their native state, most monosaccharide units presented here exist either as oligomers and polymers or as glycosylated units bonded to lignin and other polyphenolics.

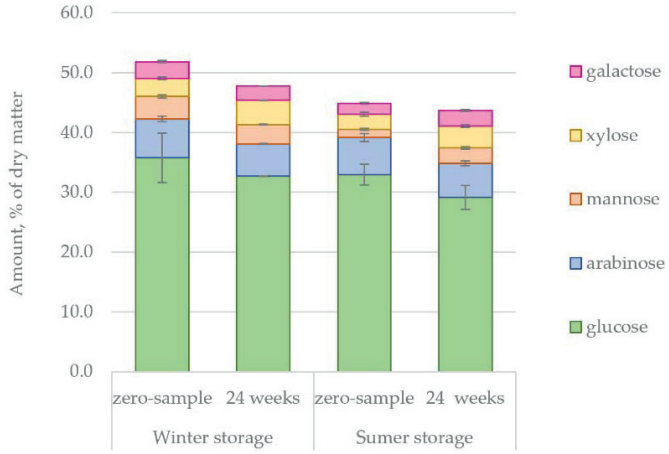


Figure 16. The high-performance anion-exchange chromatography (HPAEC) results of the changes in monosaccharide content during winter and summer storage.

3.4.2. Change in Hemicellulose Content

The changes in the hemicellulose content of extractives-free spruce bark during storage were determined by acidic methanolysis. The analysis results presented in Figure 17 indicated similar trends in winter- and summer-stored bark. The relative proportion of the hemicellulose content in extractives-free bark increased gradually during the storage periods. Arabinose and galacturonic acid units were the most abundant units during the winter storage period, corresponding to 6–9% and 6–8% of dry matter, respectively. Arabinose, galacturonic acid (the main component of pectin) and glucose were the most abundant units during the summer storage period, totalling 5–8%, 4–9% and 6–9% of dry matter, respectively. The most noteworthy differences were detected in the amounts of glucose and glucuronic acid. During the summer storage period, the amount of glucose in hemicelluloses increased by 39%, and the amount of glucuronic acid by 62%. This could

be seen as the result of cellulose's degradation as the microbial activity would increase towards the end of the storage period. However, it should be noted that the total amount of glucose in the extractives-free bark decreased during the storage periods (Figure 16) as well as in the water extracts (Figure 5). Correlation with the ambient temperature, increasing towards the end of the winter storage period and at the beginning of the summer storage period, can also be observed (Figure 2).

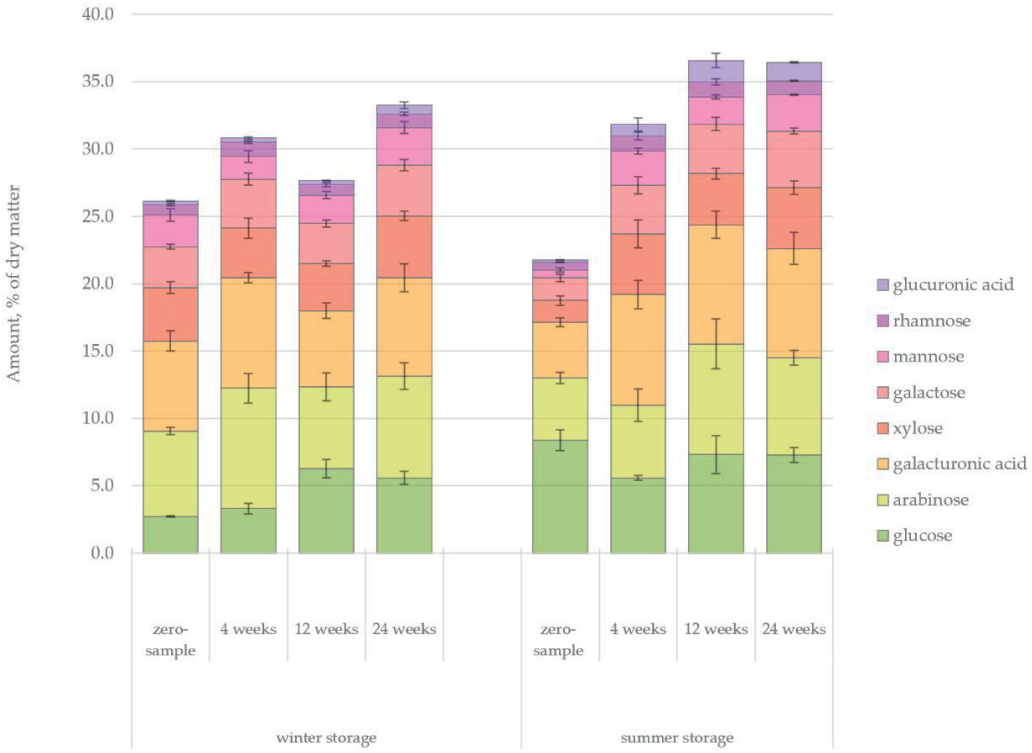


Figure 17. Changes observed in monosaccharide units of hemicelluloses during the winter and summer storage periods of spruce bark.

3.5. Evaluation of Total Phenolic Content (TPC)

The results indicating the total phenolic content (TPC) of the hot-water extracts, as evaluated with the Folin–Ciocalteu method, are shown in Figure 18. The TPC values are indicated in the figure by a blue dotted line. An overview of the results suggested that the level of TPC was 29% lower during the summer storage period, although the rate of degradation was quite similar in both experiments. The TPC value would presumably also contain the oligomeric and polymeric phenolic compounds. Thus, if the TPC results are compared with the amounts of phenolic compounds from the GC analyses (the columns in Figure 18), an estimate of polyphenols can be obtained. As can be seen, the estimated amount of polyphenols explained approximately 48% of the compounds left unidentified by GC. A large amount of the polyphenolic compounds would most likely contain polyphenols, such as lignin and condensed tannins. A more in-depth HPLC evaluation of the procyanidin content in the inner and outer bark of the same bark material has recently been published [25]. Those HPLC results appeared to be approximately 50% lower than the amounts of polyphenols estimated in Figure 18. This difference in results may be attributed to the presence of lignin and other reducing agents, which also affect the UV reading in the Folin–Ciocalteu method. The unidentified non-phenolic compounds, on the other hand, could contain, for example, polysaccharides.

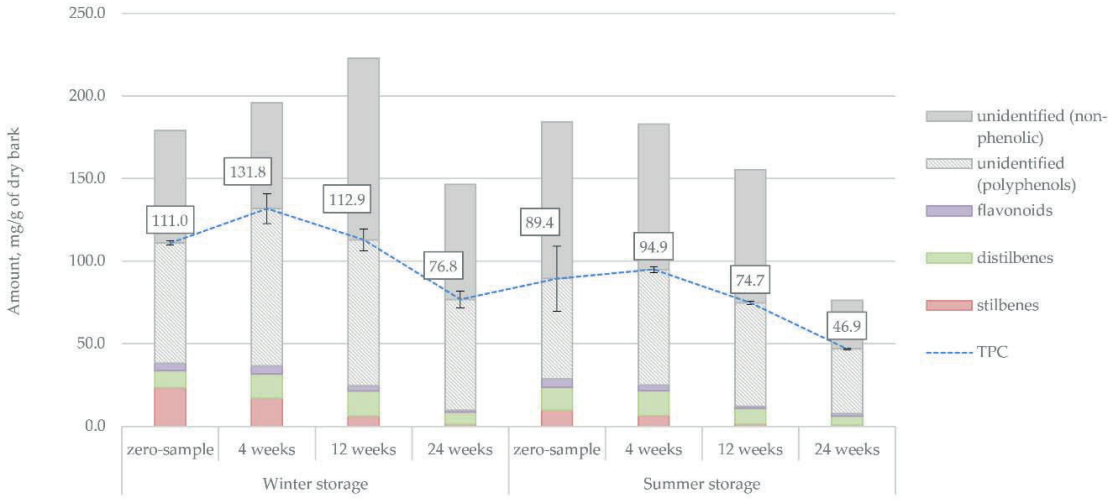


Figure 18. The total phenolic content (TPC) of the water extracts. The values of stilbenes, distilbenes, flavonoids and unidentified compounds from the GC analyses, as seen in Figure 5, are shown for reference.

3.6. Results of the Statistical Analysis

The *p*-values for the independent variables (season and storage time) are presented in Table 7. The results indicated that season has a statistically significant effect on the amount of triglycerides, steryl esters, sterols, stilbenes, alcohols, sugars, unidentified hydrophilic extractives and total phenols. Likewise, according to results, storage time has a statistically significant effect on the amount of resin acids, stilbenes, sugars, unidentified hydrophilic extractives and total phenols. The statistically significant results agree with what can be deduced from the quantification results. However, concerning some extractives groups, for example, flavonoids, the actual effect of storage time seems to be greater than what the statistical results can show.

Table 7. The statistical differences (*p*-values) between storage time and season with regard to the quantitative amounts of extractive groups. Results showing statistical significance are bolded.

Dependent Variable	***p*-Values for Independent Variables**	
Lipophilic extractives (gas chromatography with a flame ionisation detector (GC-FID))	Season	Storage time
Resin acids	0.247	**0.037**
Fatty acids	0.177	0.235
Triglycerides	**0.007**	0.460
Diterpenoids	0.143	0.137
Sterols	**0.017**	0.545
Steryl esters	**0.020**	0.458
Fatty alcohols	0.883	0.274
Other	0.130	0.124
Unidentified	0.930	0.752
Hydrophilic extractives (GC-FID)	Season	Storage time
Sugars	**0.018**	**0.011**
Organic acids	0.408	0.190
Stilbenes	**0.012**	**0.004**
Sesquistilbenes	**0.058**	0.434
Distilbenes	0.554	0.128
Alcohols	**0.020**	0.313
Flavonoids	0.553	0.138
Other	0.240	0.517
Unidentified	**0.002**	**0.028**
Total phenols (ultraviolet–visible (UV–Vis) spectrometry)	**0.009**	**0.016**

Here only the main effect of storage concerning extractives groups was evaluated. To meaningfully evaluate the mixed effect of season and storage time statistically and consider the factors of tree age and sampling point and their effect on the behaviour of extractives would have required many more individual samples. This would be an interesting study for the future. However, for our purposes, to aid in the industrial-scale utilisation of bark, a more generalised look at extractives' behaviour during storage was feasible.

4. Conclusions

Seasonal variation is shown to affect the extractives content (predominantly hydrophilic compounds) of spruce bark obtained from stored saw logs. The compounds, which were the most sensitive to UV light and hydrolysis, degraded the fastest. For example, stilbenoid monomers were much more abundant during the winter storage period than during the summer storage period. An increase in ambient temperature also enhanced the microbial activity during summer, leading to higher extractives losses. The most apparent decrease of extractable compounds can be seen for hot water-extractable saccharides. The degradation patterns, especially for lipophilic extractives detected between the sample logs, were not always as straightforward and uniform as could be expected. Tree age, soil fertility and damage caused to bark (for example, during harvesting) could also significantly affect the extractives content and composition and explain some of the discrepancies observed between samples.

This study provided a comprehensive overview of how the extractives groups of spruce bark (both lipophilic and hydrophilic) behave while in storage. It has been demonstrated that high levels of extractives were retained in the bark of stored saw logs, especially during winter. This information could be particularly useful for parties interested in developing biorefinery concepts by utilising complex chemical biomass, such as bark, which would benefit from having a plan for the procurement and storage of the raw material. The development of high-value extractives-based products from conifer bark remains a potential prospect and an innovative undertaking. However, a great deal of time and energy could be saved if appropriate care is first taken to ensure the proper storage of raw material, which would maintain the quality of the extractives.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12060736/s1, Table S1: Values for Figure 4. Extractives groups in spruce bark hexane extract (amount, mg/g of dry bark), Table S2: Values for Figure 5. Extractives groups in spruce bark water extract (amount, mg/g of dry bark), Table S3: Values for Figure 6. Resin acids in spruce bark hexane extract (amount, mg/g of dry bark), Table S4: Values for Figure 7. Fatty acids in spruce bark hexane extract (amount, mg/g of dry bark), Table S5: Values for Figure 8. Diterpenoids in spruce bark hexane extract (amount, mg/g of dry bark), Table S6: Values for Figure 9. Sterols in spruce bark hexane extract (amount, mg/g of dry bark), Table S7: Values for Figure 10. Fatty alcohols in spruce bark hexane extract (amount, mg/g of dry bark), Table S8: Values for Figure 11. Sugars in spruce bark water extract (amount, mg/g of dry bark), Table S9: Values for Figure 12. Organic acids in spruce bark water extract (amount, mg/g of dry bark), Table S10: Values for Figure 13. Stilbenes in spruce bark water extract (amount, mg/g of dry bark), Table S11: Values for Figure 14. Alcohols in spruce bark water extract (amount, mg/g of dry bark), Table S12: Values for Figure 15. Flavonoids in spruce bark water extract (amount, mg/g of dry bark), Table S13: Values for Figure 16. Monosaccharides in extractives-free bark (amount, % of dry matter), Table S14: Values for Figure 17. Hemicelluloses in extractives-free bark (amount, % of dry matter), Table S15: Values for Figure 18. Total phenolic content (TPC) of water extracts.

Author Contributions: J.N.: funding acquisition, the conceptualisation of the study and writing (review and editing); H.E.B.: conceptualisation of the study, writing (review and editing) and supervision of experimental work; E.S.H.: writing (original draft preparation), chemical analysis, analysis method development, data analysis and interpretation and visualisation; O.L.: writing (review and editing) and conceptualisation of the study; R.A.: writing (review and editing) and supervision. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A. Chromatograms

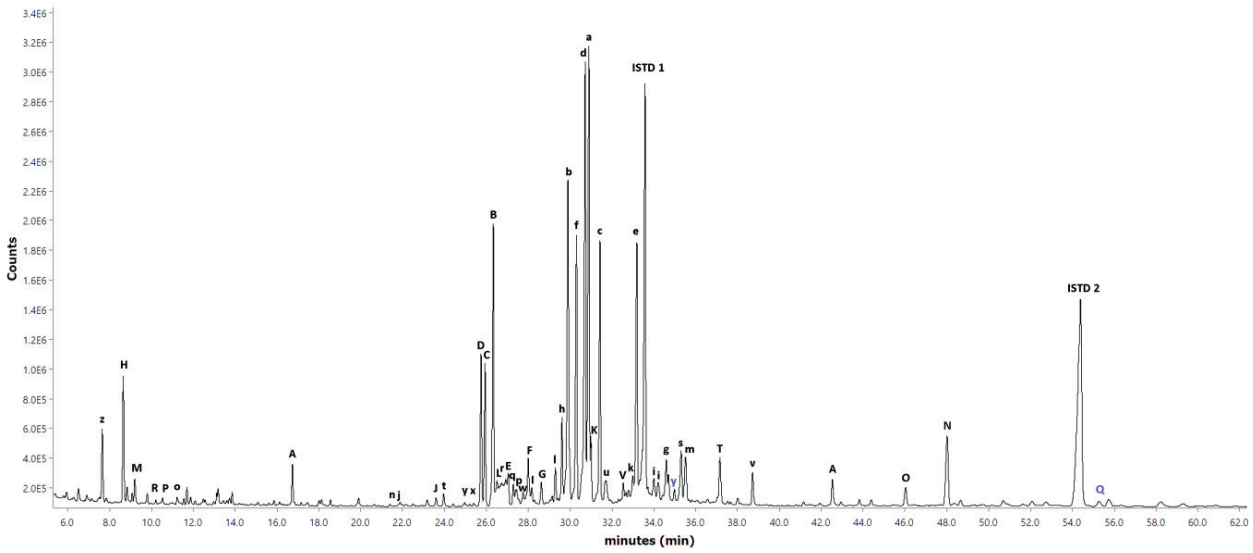

Figure A1. Reference gas chromatography with flame ionisation detection or mass spectrometry (GC-FID/MS) chromatogram for hexane extract of spruce bark. For alphabetical peak symbols, see Table 3.

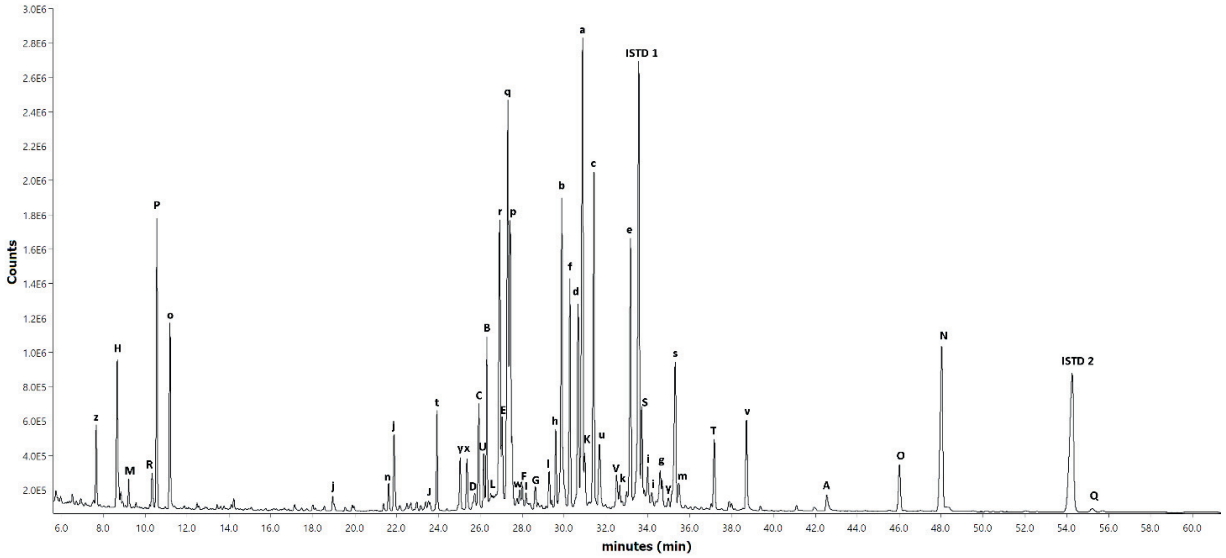

Figure A2. Reference gas chromatography with flame ionisation detection or mass spectrometry (GC-FID/MS) chromatogram for hexane extract of spruce bark (esterified compounds). For alphabetical peak symbols, see Table 3.

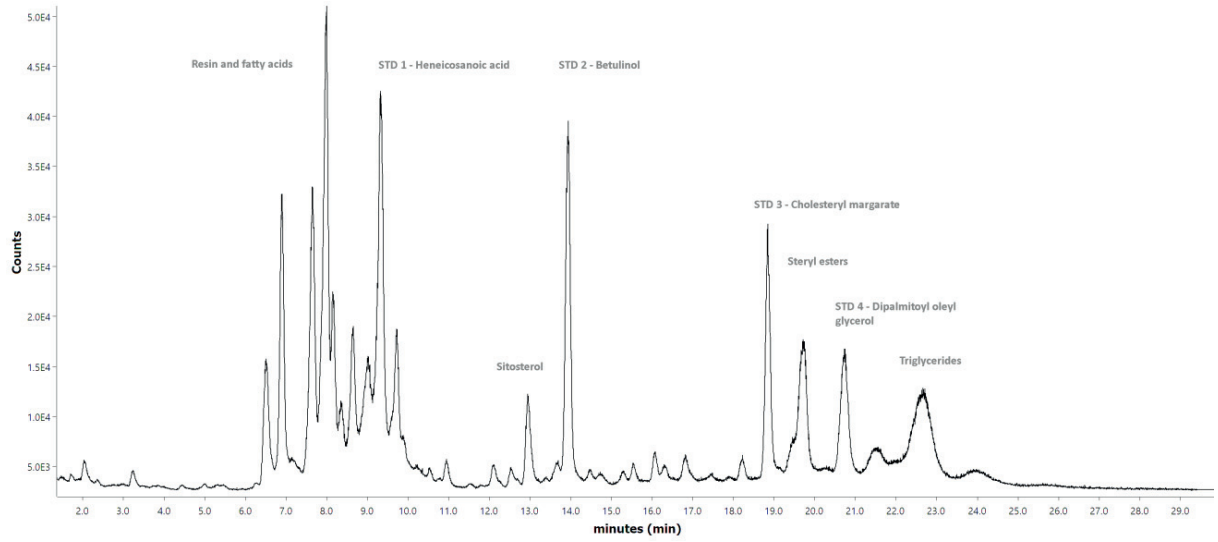

Figure A3. Reference gas chromatography with flame ionisation detection (GC-FID; short-column) chromatogram for hexane extract of spruce bark.

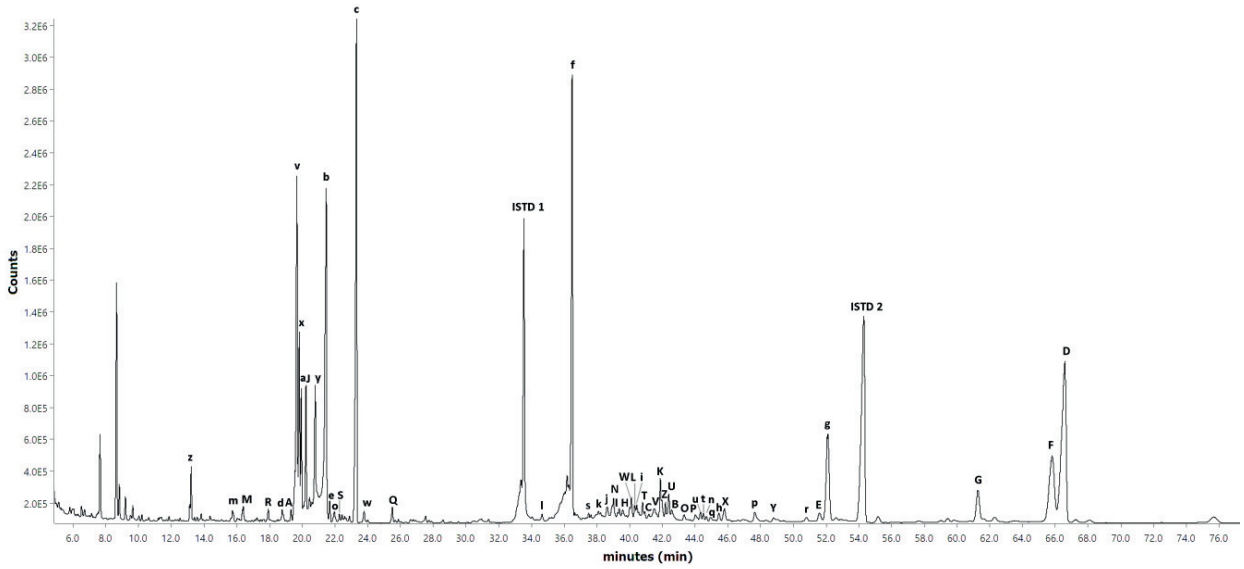

Figure A4. Reference gas chromatography with flame ionisation detection or mass spectrometry (GC-FID/MS) chromatogram for spruce bark water extract. For alphabetical peak symbols, see Table 4.

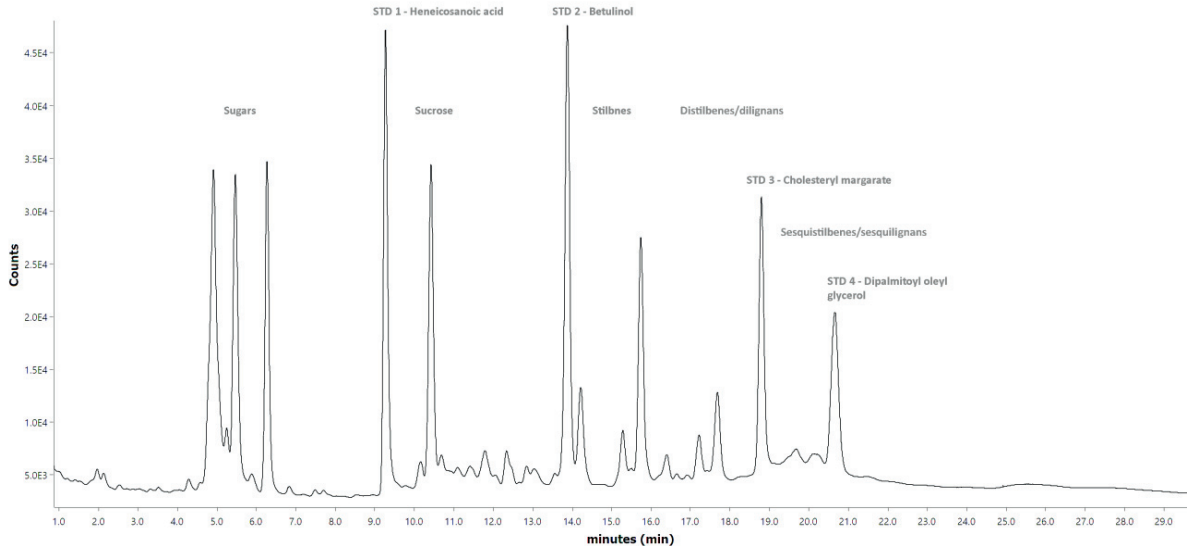

Figure A5. Reference gas chromatography with flame ionisation detector (GC-FID; short-column) chromatogram for spruce bark water extract.

Appendix B. Suggested Stilbenoid Structures

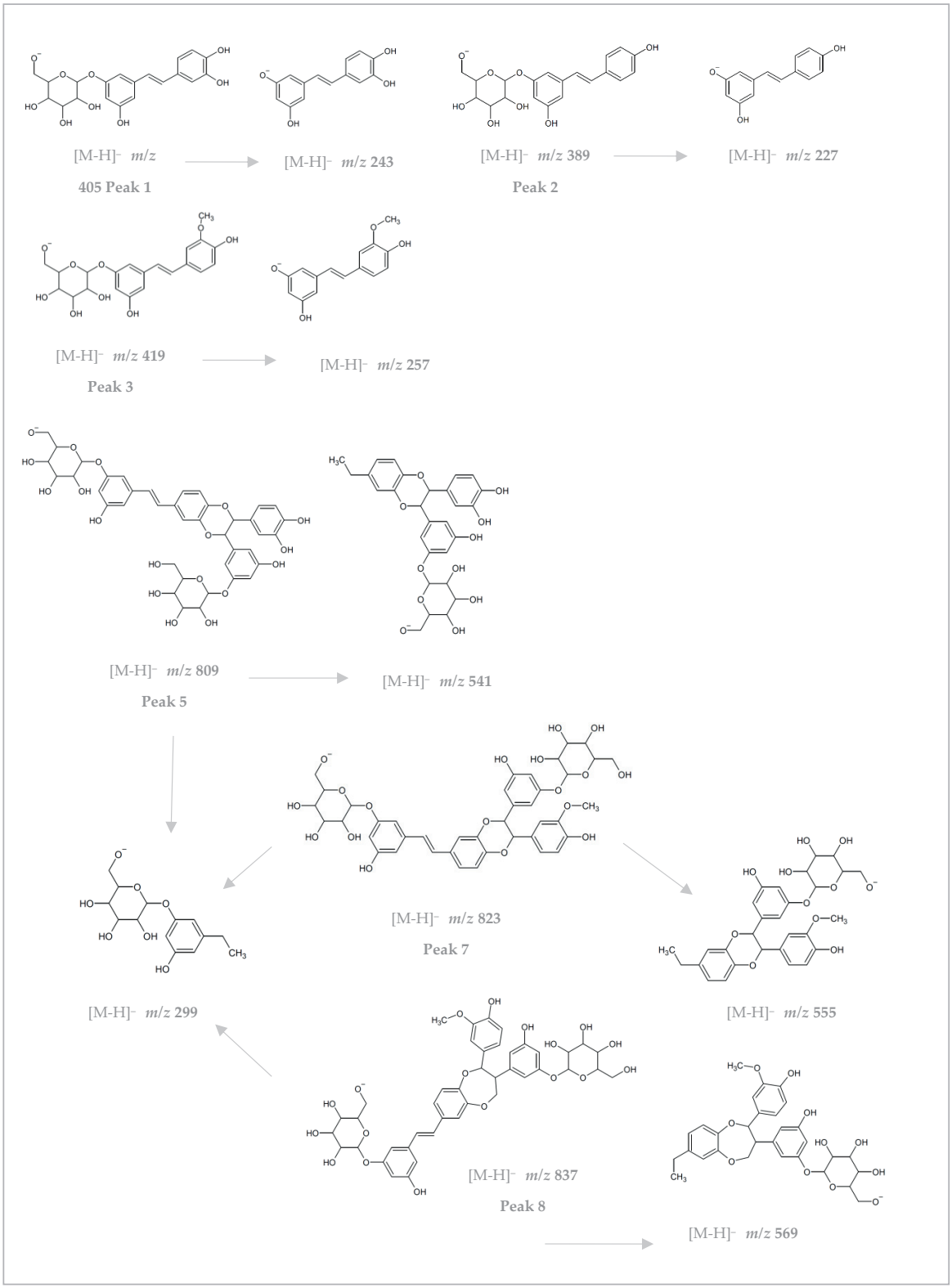

Figure A6. Suggested structures and fractionation of some detected compounds from the high-performance liquid chromatography (HPLC) analysis of bark hot-water extract.

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Supporting material – Tabulated values of Figures 4–18.

Table S1 Values for Figure 4. Extractives groups in spruce bark hexane extract (amount, mg/g of dry bark)

Compounds	Winter storage				Summer storage			
	zero-sample	4 weeks	12 weeks	24 weeks	zero-sample	4 weeks	12 weeks	24 weeks
Unidentified	29.3 ± 0.5	13.9 ± 0.3	29.7 ± 0.2	20.7 ± 0.1	18.7 ± 0.2	22.3 ± 0.4	18.2 ± 0.2	36.8 ± 0.2
Resin Acids	12.1 ± 0.1	6.0 ± 0.1	11.4 ± 0.04	9.7 ± 2.2	12.7 ± 0.4	7.7 ± 0.4	10.5 ± 0.2	12.2 ± 0.3
Fatty Acids	9.2 ± 0.7	7.5 ± 0.8	10.1 ± 0.1	6.4 ± 3.4	10.9 ± 0.2	5.5 ± 0.3	5.3 ± 0.1	4.3 ± 0.1
Triglycerides	4.7 ± 0.1	5.2 ± 0.06	5.8 ± 0.003	0.6 ± 0.1	9.4 ± 0.4	3.9 ± 0.5	3.6 ± 0.1	3.3 ± 0.1
Steryl Esters	1.9 ± 0.1	2.3 ± 0.7	2.9 ± 0.1	1.7 ± 0.1	3.4 ± 0.1	4.2 ± 0.3	3.7 ± 0.2	4.0 ± 0.2
Sterols	2.4 ± 0.04	2.9 ± 0.9	3.5 ± 0.1	2.4 ± 0.1	4.2 ± 0.1	4.8 ± 0.5	4.4 ± 0.03	4.8 ± 0.2
Fatty Alcohols	1.7 ± 0.02	0.6 ± 0.06	0.8 ± 0.04	0.9 ± 0.1	1.1 ± 0.02	0.9 ± 0.1	0.7 ± 0.01	1.1 ± 0.04
Diterpenoids	2.8 ± 0.1	1.8 ± 0.06	3.2 ± 0.01	2.2 ± 0.8	5.7 ± 0.04	1.8 ± 0.2	3.4 ± 0.1	3.7 ± 0.06
Other	2.7 ± 0.6	1.0 ± 0.4	2.6 ± 0.2	2.5 ± 0.2	3.0 ± 0.9	2.0 ± 0.5	2.6 ± 0.1	4.7 ± 0.2

Table S2 Values for Figure 5. Extractives groups in spruce bark water extract (amount, mg/g of dry bark)

Compounds	Winter Storage				Summer Storage			
	zero-sample	4 weeks	12 weeks	24 weeks	zero-sample	4 weeks	12 weeks	24 weeks
Unidentified	140.9 ± 1.6	159.2 ± 19.7	198.3 ± 0.8	136.8 ± 0.04	155.6 ± 13.1	158.0 ± 1.3	143.4 ± 2.9	68.7 ± 0.1
Sugars	66.9 ± 0.03	47.8 ± 10.6	30.0 ± 2.6	6.1 ± 0.7	42.1 ± 2.5	23.1 ± 1.9	4.8 ± 0.6	7.1 ± 0.5
Stilbenes	23.5 ± 0.03	17.1 ± 2.9	6.1 ± 0.1	1.3 ± 0.3	9.9 ± 2.0	6.7 ± 0.2	1.4 ± 0.2	0.7 ± 0.08
Organic Acids	22.4 ± 0.7	21.9 ± 5.0	17.1 ± 0.6	2.2 ± 0.2	40.0 ± 3.8	26.7 ± 2.6	6.2 ± 0.1	16.7 ± 0.4
Alcohols	11.1 ± 0.2	10.9 ± 2.3	10.0 ± 0.2	3.1 ± 0.4	12.6 ± 1.4	8.3 ± 0.4	2.0 ± 0.1	2.4 ± 0.3
Sesquistilbenes	10.8 ± 0.7	15.4 ± 2.5	17.3 ± 2.6	8.8 ± 0.9	8.2 ± 1.9	9.5 ± 0.9	5.9 ± 0.3	6.3 ± 0.6
Distilbenes	10.1 ± 0.5	14.7 ± 0.9	15.2 ± 0.4	7.4 ± 0.3	13.7 ± 4.4	15.0 ± 1.1	9.5 ± 1.9	5.4 ± 0.2
Flavonoids	4.6 ± 0.5	4.9 ± 1.4	3.2 ± 0.1	1.0 ± 0.01	5.1 ± 0.8	3.2 ± 0.003	1.2 ± 0.03	1.5 ± 0.2
Others	8.4 ± 0.3	18.0 ± 4.1	11.4 ± 0.7	9.4 ± 0.6	14.5 ± 3.7	8.6 ± 0.9	1.7 ± 0.1	4.0 ± 0.2

Table S3 Values for Figure 6. Resin acids in spruce bark hexane extract (amount, mg/g of dry bark)

Compounds	Winter Storage				Summer Storage			
	zero-sample	4 weeks	12 weeks	24 weeks	zero-sample	4 weeks	12 weeks	24 weeks
Hydroxy Resin Acid	0.2 ± 0.04	0.2 ± 0.02	0.4 ± 0.02	0.2 ± 0.001	0.3 ± 0.03	0.3 ± 0.04	0.2 ± 0.01	0.4 ± 0.04
Pimaric Acid	0.2 ± 0.1	0.1 ± 0.004	0.2 ± 0.001	0.2 ± 0.01	0.1 ± 0.003	0.2 ± 0.01	0.2 ± 0.004	0.3 ± 0.01
Imbricatolic Acid	0.2 ± 0.01	0.2 ± 0.003	0.4 ± 0.02	0.2 ± 0.01	0.2 ± 0.001	0.2 ± 0.01	0.2 ± 0.01	0.5 ± 0.01
Hydroxydehydroabietic Acid 2	0.3 ± 0.01	0.2 ± 0.03	0.4 ± 0.03	0.5 ± 0.1	0.4 ± 0.01	0.3 ± 0.04	0.2 ± 0.001	0.5 ± 0.02
Sandaracopimaric Acid	0.4 ± 0.01	0.2 ± 0.002	0.4 ± 0.0001	0.4 ± 0.01	0.3 ± 0.003	0.3 ± 0.02	0.3 ± 0.01	0.4 ± 0.01
Hydroxydehydroabietic Acid 1	0.5 ± 0.004	0.3 ± 0.1	0.4 ± 0.001	0.2 ± 0.1	0.4 ± 0.01	0.4 ± 0.001	0.3 ± 0.003	0.2 ± 0.01

4-Hydroxycinnamic Acid	0.5 ± 0.03	0.4 ± 0.1	0.2 ± 0.02	0.2 ± 0.1	0.1 ± 0.4	0.2 ± 0.1	0.3 ± 0.01	0.2 ± 0.03
Palustric Acid	1.0 ± 0.1	0.6 ± 0.1	1.1 ± 0.1	0.8 ± 0.01	1.0 ± 0.02	0.2 ± 0.1	1.2 ± 0.03	1.2 ± 0.1
Neoabietic Acid	1.2 ± 0.2	0.9 ± 0.1	1.0 ± 0.03	0.8 ± 0.3	1.6 ± 0.1	0.3 ± 0.03	1.1 ± 0.02	0.9 ± 0.1
Levopimaric Acid	1.3 ± 0.1	0.6 ± 0.1	1.1 ± 0.04	0.6 ± 0.4	1.3 ± 0.1	0.3 ± 0.04	1.2 ± 0.03	0.9 ± 0.1
Abietic Acid	1.4 ± 0.2	0.7 ± 0.2	1.5 ± 0.01	1.2 ± 0.02	1.9 ± 0.2	0.8 ± 0.03	1.1 ± 0.04	1.0 ± 0.04
Isopimaric Acid	1.7 ± 0.1	1.0 ± 0.03	2.0 ± 0.02	1.6 ± 0.5	1.9 ± 0.03	1.2 ± 0.1	1.5 ± 0.03	1.6 ± 0.03
Dehydroabietic Acid	2.4 ± 0.1	1.2 ± 0.2	2.3 ± 0.02	2.3 ± 0.03	2.5 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	3.2 ± 0.04

Table S4 Values for Figure 7. Fatty acids in spruce bark hexane extract (amount, mg/g of dry bark)

Compounds	**Winter Storage**				**Summer Storage**			
	zero-sample	4 weeks	12 weeks	24 weeks	zero-sample	4 weeks	12 weeks	24 weeks
Acid 25:0	0.1 ± 0.01	0.1 ± 0.02	0.2 ± 0.02	0.1 ± 0.01	0.1 ± 0.001	0.1 ± 0.01	0.1 ± 0.001	0.2 ± 0.003
Acid 17:0	0.2 ± 0.00003	0.2 ± 0.001	0.3 ± 0.002	0.2 ± 0.1	0.4 ± 0.003	0.2 ± 0.01	0.2 ± 0.001	0.1 ± 0.001
Acid 18:0	0.1 ± 0.01	0.2 ± 0.01	0.3 ± 0.002	0.2 ± 0.1	0.3 ± 0.001	0.2 ± 0.01	0.3 ± 0.004	0.3 ± 0.002
Acid 24:0	0.6 ± 0.1	0.1 ± 0.01	0.3 ± 0.01	0.4 ± 0.02	0.2 ± 0.02	0.3 ± 0.01	0.3 ± 0.003	0.4 ± 0.003
Acid 20:0	0.4 ± 0.01	0.2 ± 0.01	1.6 ± 0.02	0.3 ± 0.01	0.2 ± 0.1	0.2 ± 0.002	0.3 ± 0.001	0.3 ± 0.003
Acid 16:0	0.4 ± 0.001	0.4 ± 0.003	0.5 ± 0.001	0.4 ± 0.1	0.7 ± 0.002	0.3 ± 0.01	0.4 ± 0.003	0.2 ± 0.004
Acid 22:0	0.9 ± 0.01	0.3 ± 0.002	0.6 ± 0.01	0.6 ± 0.01	0.5 ± 0.01	0.8 ± 0.01	0.5 ± 0.002	0.6 ± 0.01
Acid 18:3	1.6 ± 0.02	1.6 ± 0.1	1.7 ± 0.02	1.0 ± 0.4	2.0 ± 0.01	0.8 ± 0.02	0.7 ± 0.01	0.4 ± 0.02
Acid 18:2	2.1 ± 0.1	2.3 ± 0.4	2.4 ± 0.02	1.5 ± 1.2	3.2 ± 0.01	1.3 ± 0.04	1.2 ± 0.02	0.7 ± 0.1
Acid 18:1	1.8 ± 0.03	2.0 ± 0.2	2.3 ± 0.1	1.6 ± 1.0	3.3 ± 0.1	1.1 ± 0.1	1.5 ± 0.03	0.9 ± 0.004

Table S5 Values for Figure 8. Diterpenoids in spruce bark hexane extract (amount, mg/g of dry bark)

Compounds	**Winter Storage**				**Summer Storage**			
	zero-sample	4 weeks	12 weeks	24 weeks	zero-sample	4 weeks	12 weeks	24 weeks
Isopimaral	0.1 ± 0.003	0.1 ± 0.02	0.1 ± 0.002	0.1 ± 0.02	0.1 ± 0.001	0.1 ± 0.01	0.1 ± 0.002	0.1 ± 0.001
Pimarol	0.3 ± 0.02	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.03	0.3 ± 0.002	0.1 ± 0.01	0.1 ± 0.002	0.1 ± 0.0003
Epimanoyl Oxide	0.1 ± 0.002	0.1 ± 0.01	0.1 ± 0.0001	0.1 ± 0.0001	0.1 ± 0.003	0.1 ± 0.002	0.1 ± 0.001	0.2 ± 0.002
Isopimarol	0.1 ± 0.002	0.1 ± 0.04	0.2 ± 0.01	0.1 ± 0.04	0.2 ± 0.004	0.1 ± 0.002	0.1 ± 0.001	0.1 ± 0.002
Cubebene	0.7 ± 0.01	0.6 ± 0.3	0.0 ± 0.0004	0.0 ± 0.003	0.0 ± 0.02	0.0 ± 0.0002	0.0 ± 0.0002	0.0 ± 0.006
Dehydroabietal	0.1 ± 0.01	0.1 ± 0.004	0.2 ± 0.001	0.1 ± 0.1	0.2 ± 0.004	0.1 ± 0.01	0.2 ± 0.002	0.2 ± 0.002
Palustral	0.2 ± 0.02	0.1 ± 0.04	0.3 ± 0.01	0.1 ± 0.001	0.2 ± 0.01	0.1 ± 0.01	0.2 ± 0.003	0.3 ± 0.01
Cis-Abienol	0.5 ± 0.01	0.4 ± 0.04	0.4 ± 0.01	0.3 ± 0.2	0.5 ± 0.0002	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01
Thunbergol	0.4 ± 0.1	0.6 ± 0.1	0.9 ± 0.01	0.1 ± 0.01	2.8 ± 0.03	0.6 ± 0.04	1.4 ± 0.04	0.2 ± 0.01
Manool	0.6 ± 0.1	0.2 ± 0.2	0.7 ± 0.1	0.5 ± 0.2	1.9 ± 0.4	0.4 ± 0.2	1.0 ± 0.1	0.7 ± 0.1
Δ13-(*trans*-)Neoabienol	0.9 ± 0.1	0.5 ± 0.1	1.0 ± 0.004	0.8 ± 0.3	1.0 ± 0.02	0.7 ± 0.02	0.9 ± 0.01	1.1 ± 0.001

Table S6 Values for Figure 9. Sterols in spruce bark hexane extract (amount, mg/g of dry bark)

Compounds	**Winter Storage**				**Summer Storage**			
	zero-	4 weeks	12 weeks	24 weeks	zero-	4 weeks	12 weeks	24 weeks

	sample				sample			
Acid 22:0 Monoglyceride	0.0 ± 0.001	0.0 ± 0.01	0.0 ± 0.004	0.0 ± 0.001	0.0	0.0	0.0	0.0
Acid 18:2 Monoglyceride	0.2 ± 0.2	0.1 ± 0.01	0.0 ± 0.001	0.0 ± 0.0001	0.0 ± 0.0001	0.0 ± 0.0003	0.0 ± 0.001	0.1 ± 0.002
24-Methylenecycloartan-3-One	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.0003	0.1 ± 0.06	0.0 ± 0.01	0.0 ± 0.0002	0.0 ± 0.003	0.0 ± 0.0003
Diglycerol	1.3 ± 0.3	0.2 ± 0.07	0.0 ± 0.002	0.0 ± 0.004	0.0 ± 0.001	0.0 ± 0.03	0.0 ± 0.002	0.0 ± 0.001
Campesterol	0.4 ± 0.01	0.4 ± 0.1	0.4 ± 0.004	0.4 ± 0.03	0.4 ± 0.03	0.4 ± 0.02	0.4 ± 0.001	0.4 ± 0.02
Sitosterol	2.0 ± 0.03	1.8 ± 0.6	2.2 ± 0.03	1.9 ± 0.3	2.2 ± 0.1	2.0 ± 0.05	1.7 ± 0.001	1.9 ± 0.1

Table S7 Values for Figure 10. Fatty alcohols in spruce bark hexane extract (amount, mg/g of dry bark)

Compounds	Winter Storage				Summer Storage			
	zero-sample	4 weeks	12 weeks	24 weeks	zero-sample	4 weeks	12 weeks	24 weeks
Alcohol 15:0	0.3 ± 0.0001	0.1 ± 0.002	0.1 ± 0.003	0.1 ± 0.02	0.1 ± 0.003	0.1 ± 0.007	0.1 ± 0.001	0.1 ± 0.001
Alcohol 18:0	0.3 ± 0.004	0.1 ± 0.01	0.1 ± 0.004	0.1 ± 0.01	0.4 ± 0.01	0.2 ± 0.02	0.2 ± 0.002	0.2 ± 0.001
Alcohol 24:0	0.4 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.2 ± 0.1	0.2 ± 0.01	0.2 ± 0.03	0.1 ± 0.01	0.3 ± 0.02
Alcohol 22:0	0.7 ± 0.02	0.3 ± 0.1	0.4 ± 0.03	0.4 ± 0.1	0.4 ± 0.001	0.4 ± 0.02	0.3 ± 0.0002	0.5 ± 0.02

Table S8 Values for Figure 11. Sugars in spruce bark water extract (amount, mg/g of dry bark)

Compounds	Winter Storage				Summer Storage			
	zero-sample	4 weeks	12 weeks	24 weeks	zero-sample	4 weeks	12 weeks	24 weeks
Other	0.7 ± 0.2	0.6 ± 0.1	0.8 ± 0.003	0.2 ± 0.01	1.2 ± 0.2	0.5 ± 0.4	0.8 ± 0.003	0.2 ± 0.02
Cellobiose	0.8 ± 0.03	0.7 ± 0.1	0.4 ± 0.02	0.1 ± 0.002	0.7 ± 0.2	0.5 ± 0.1	0.1 ± 0.01	0.1 ± 0.01
Galactose	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	1.3 ± 0.5	0.5 ± 0.03	0.5 ± 0.1
Maltose	3.5 ± 0.2	4.0 ± 1.1	2.8 ± 0.2	0.7 ± 0.1	4.2 ± 0.6	3.3 ± 0.3	0.8 ± 0.1	1.3 ± 0.1
Sucrose	26.6 ± 0.1	5.6 ± 1.0	4.5 ± 0.3	0.6 ± 0.02	8.0 ± 1.0	2.7 ± 0.1	0.6 ± 0.1	1.1 ± 0.1
Glucose	34.6 ± 0.2	36.1 ± 9.3	20.8 ± 2.2	3.8 ± 0.5	27.4 ± 0.4	14.6 ± 1.0	1.9 ± 0.1	4.0 ± 0.2

Table S9 Values for Figure 12. Organic acids in spruce bark water extract (amount, mg/g of dry bark)

Compounds	Winter Storage				Summer Storage			
	zero-sample	4 weeks	12 weeks	24 weeks	zero-sample	4 weeks	12 weeks	24 weeks
Other	0.8 ± 0.5	0.0	0.0	0.1 ± 0.002	0.6 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.5 ± 0.02
Shikmic Acid	0.4 ± 0.1	1.0 ± 0.2	0.3 ± 0.02	0.1 ± 0.01	0.3 ± 0.02	0.2 ± 0.01	0.1 ± 0.0003	0.1 ± 0.01
Malic Acid	1.4 ± 0.04	0.6 ± 0.2	0.7 ± 0.03	0.1 ± 0.01	1.0 ± 0.1	0.4 ± 0.1	0.0 ± 0.01	0.1 ± 0.0004
Quinic Aicd	5.3 ± 0.8	4.1 ± 1.0	3.5 ± 0.2	0.3 ± 0.04	5.6 ± 0.4	2.9 ± 0.1	0.3 ± 0.02	0.3 ± 0.03
Aconitic Acid	4.8 ± 0.4	5.4 ± 2.0	5.4 ± 0.1	0.6 ± 0.1	6.2 ± 0.9	3.9 ± 0.7	0.3 ± 0.01	0.4 ± 0.0002
Gluconic Acid	11.0 ± 0.1	11.6 ± 2.4	7.8 ± 0.5	1.1 ± 0.1	10.6 ± 0.7	6.3 ± 0.7	1.1 ± 0.02	2.2 ± 0.01

Table S10 Values for Figure 13. Stilbenes in spruce bark water extract (amount, mg/g of dry bark)

Compounds	Winter Storage				Summer Storage			
	zero-	4 weeks	12 weeks	24 weeks	zero-	4 weeks	12 weeks	24 weeks

	sample				sample			
Rhapontigenin	0.7 ± 0.03	0.6 ± 0.1	0.3 ± 0.03	0.4 ± 0.03	0.4 ± 0.1	0.7 ± 0.0004	0.3 ± 0.01	0.3 ± 0.03
Cis-Piceatannol	0.7 ± 0.02	0.9 ± 0.1	0.5 ± 0.004	0.3 ± 0.02	0.6 ± 0.1	0.5 ± 0.03	0.2 ± 0.04	0.2 ± 0.02
Piceid	2.0 ± 0.01	2.8 ± 0.2	1.2 ± 0.02	0.3 ± 0.2	2.8 ± 0.5	1.7 ± 0.1	0.2 ± 0.003	0.1 ± 0.01
Astringin	6.3 ± 0.1	3.5 ± 2.0	1.3 ± 0.2	0.1 ± 0.04	0.2 ± 0.01	0.6 ± 0.1	0.3 ± 0.1	0.0 ± 0.01
Isorhapontin	13.8 ± 0.04	9.3 ± 1.1	2.8 ± 0.02	0.3 ± 0.1	6.0 ± 1.3	3.1 ± 0.1	0.4 ± 0.1	0.1 ± 0.02

Table S11 Values for Figure 14. Alcohols in spruce bark water extract (amount, mg/g of dry bark)

Compounds	**Winter Storage**				**Summer Storage**			
	zero-sample	4 weeks	12 weeks	24 weeks	zero-sample	4 weeks	12 weeks	24 weeks
Coniferin	0.6 ± 0.01	0.6 ± 0.2	0.3 ± 0.01	0.1 ± 0.01	0.6 ± 0.1	0.3 ± 0.02	0.0 ± 0.001	0.1 ± 0.01
Mannitol	0.3 ± 0.01	0.3 ± 0.07	0.5 ± 0.01	0.7 ± 0.1	0.3 ± 0.03	0.5 ± 0.05	0.1 ± 0.004	0.5 ± 0.03
Arabitol	0.4 ± 0.04	0.4 ± 0.1	0.3 ± 0.01	0.6 ± 0.1	0.2 ± 0.02	0.5 ± 0.04	0.4 ± 0.01	0.6 ± 0.03
Inositol	0.5 ± 0.01	0.5 ± 0.1	0.6 ± 0.01	0.1 ± 0.02	1.0 ± 0.1	0.6 ± 0.04	0.1 ± 0.003	0.1 ± 0.002
Maltitol	0.6 ± 0.01	0.8 ± 0.1	1.5 ± 0.1	0.2 ± 0.003	1.6 ± 0.2	1.1 ± 0.04	0.2 ± 0.01	0.2 ± 0.03
Maltotriitol	1.7 ± 0.03	1.1 ± 0.2	1.1 ± 0.04	0.4 ± 0.02	1.1 ± 0.2	0.9 ± 0.05	0.2 ± 0.004	0.3 ± 0.04
Guaiacyl Glycerol	1.1 ± 0.1	1.2 ± 0.5	1.2 ± 0.1	0.4 ± 0.1	1.7 ± 0.2	1.2 ± 0.1	0.4 ± 0.02	0.2 ± 0.1
Salicin	1.9 ± 0.1	1.9 ± 0.6	1.8 ± 0.01	0.1 ± 0.02	1.5 ± 0.2	0.6 ± 0.02	0.3 ± 0.01	0.2 ± 0.01
Pinitol	4.0 ± 0.02	3.9 ± 0.9	2.6 ± 0.02	0.6 ± 0.1	4.6 ± 0.4	2.5 ± 0.2	0.3 ± 0.01	0.2 ± 0.01

Table S12 Values for Figure 15. Flavonoids in spruce bark water extract (amount, mg/g of dry bark)

Compounds	**Winter Storage**				**Summer Storage**			
	zero-sample	4 weeks	12 weeks	24 weeks	zero-sample	4 weeks	12 weeks	24 weeks
Ampelopsin	0.5 ± 0.1	0.2 ± 0.01	0.0	0.2 ± 0.002	1.0 ± 0.1	0.8 ± 0.1	0.2 ± 0.02	0.2 ± 0.04
Naringin	1.0 ± 0.1	0.9 ± 0.4	0.8 ± 0.02	0.1 ± 0.004	1.3 ± 0.3	0.5 ± 0.02	0.3 ± 0.03	0.1 ± 0.01
Catechin	1.0 ± 0.3	1.2 ± 0.3	0.5 ± 0.03	0.3 ± 0.004	0.8 ± 0.1	0.7 ± 0.1	0.3 ± 0.01	0.4 ± 0.04
Taxifolin	1.1 ± 0.02	0.9 ± 0.2	0.7 ± 0.1	0.2 ± 0.002	0.4 ± 0.1	0.7 ± 0.03	0.4 ± 0.001	0.7 ± 0.1
Taxifolin Glycoside	1.0 ± 0.1	1.8 ± 0.6	1.3 ± 0.01	0.1 ± 0.01	1.6 ± 0.2	0.5 ± 0.02	0.1 ± 0.003	0.1 ± 0.004

Table S13 Values for Figure 16. Monosaccharides in extractives-free bark (amount, % of dry matter)

Compounds	**Winter Storage**		**Summer Storage**	
	zero-sample	24 weeks	zero-sample	24 weeks
Galactose	2.8 ± 0.2	2.4 ± 0.1	1.8 ± 0.2	2.6 ± 0.2
Xylose	3.0 ± 0.2	4.0 ± 0.1	2.5 ± 0.3	3.7 ± 0.3
Mannose	3.8 ± 0.3	3.3 ± 0.1	1.4 ± 0.2	2.6 ± 0.2
Arabinose	6.5 ± 0.5	5.4 ± 0.1	6.2 ± 0.7	5.7 ± 0.4
Glucose	35.8 ± 4.2	32.7 ± 0.03	33.0 ± 1.7	29.1 ± 2.0

Table S14 Values for Figure 17. Hemicelluloses in extractives-free bark (amount, % of dry matter)

Compounds	**Winter Storage**				**Summer Storage**			
	zero-	4 weeks	12 weeks	24 weeks	zero-	4 weeks	12 weeks	24 weeks

	sample				sample			
Glucuronic Acid	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.04	0.6 ± 0.2	0.2 ± 0.02	0.9 ± 0.5	1.6 ± 0.5	1.4 ± 0.1
Rhamnose	0.7 ± 0.1	1.1 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	0.6 ± 0.02	1.1 ± 0.3	1.1 ± 0.2	1.0 ± 0.04
Mannose	2.4 ± 0.4	1.7 ± 0.4	2.1 ± 0.3	2.8 ± 0.4	0.7 ± 0.2	2.5 ± 0.2	2.0 ± 0.1	2.7 ± 0.1
Galactose	3.1 ± 0.2	3.6 ± 0.4	3.0 ± 0.3	3.8 ± 0.4	1.8 ± 0.3	3.6 ± 0.6	3.7 ± 0.5	4.2 ± 0.2
Xylose	3.9 ± 0.4	3.7 ± 0.8	3.5 ± 0.2	4.6 ± 0.3	1.9 ± 0.4	4.5 ± 1.0	3.8 ± 0.4	4.5 ± 0.5
Galacturonic Acid	6.7 ± 0.8	8.2 ± 0.4	5.7 ± 0.6	7.3 ± 1.0	3.9 ± 0.3	8.2 ± 1.1	8.9 ± 1.0	8.1 ± 1.2
Arabinose	6.3 ± 0.3	8.9 ± 1.1	6.1 ± 1.0	7.5 ± 1.0	4.9 ± 0.4	7.9 ± 1.2	8.2 ± 1.9	7.2 ± 0.6
Glucose	2.7 ± 0.1	3.3 ± 0.4	6.3 ± 0.7	5.6 ± 0.5	8.9 ± 0.8	5.6 ± 0.2	7.3 ± 1.4	7.3 ± 0.6

Table S15 Values for Figure 18. Total phenolic content (TPC) of water extracts

Compounds	**Winter Storage**				**Summer Storage**			
	zero-sample	4 weeks	12 weeks	24 weeks	zero-sample	4 weeks	12 weeks	24 weeks
TPC	111.0 ± 1.2	131.8 ± 9.1	112.9 ± 6.5	76.8 ± 5.2	89.4 ± 19.7	94.9 ± 1.7	74.7 ± 1.0	46.9 ± 0.6
Unidentified (non-phenolic)	72.8	95.1	88.4	67.1	60.7	70.0	62.6	39.3
Unidentified (polyphenols)	68.1	64.1	109.9	69.7	94.9	88.0	80.8	29.4

II

BEHAVIOUR OF EXTRACTIVES IN NORWAY SPRUCE (*PICEA ABIES*) BARK DURING PILE STORAGE

by

Halmemies, Eelis S.; Alén, Raimo; Hellström, Jarkko; Läspä, Otto; Nurmi, Juha; Hujala, Maija; Brännström, Hanna E., 10 February 2022

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Article

Behaviour of Extractives in Norway Spruce (*Picea abies*) Bark during Pile Storage

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Abstract: The current practices regarding the procurement chain of forest industry sidestreams, such as conifer bark, do not always lead to optimal conditions for preserving individual chemical compounds. This study investigates the standard way of storing bark in large piles in an open area. We mainly focus on the degradation of the most essential hydrophilic and hydrophobic extractives and carbohydrates. First, two large 450 m^3 piles of bark from Norway spruce (*Picea abies*) were formed, one of which was covered with snow. The degradation of the bark extractives was monitored for 24 weeks. Samples were taken from the middle, side and top of the pile. Each sample was extracted at 120 °C with both *n*-hexane and water, and the extracts produced were then analysed chromatographically using gas chromatography with flame ionisation or mass selective detection and high-performance liquid chromatography. The carbohydrates were next analysed using acidic hydrolysis and acidic methanolysis, followed by chromatographic separation of the monosaccharides formed and their derivatives. The results showed that the most intensive degradation occurred during the first 4 weeks of storage. The levels of hydrophilic extractives were also found to decrease drastically (69% in normal pile and 73% in snow-covered pile) during storage, whereas the decrease in hydrophobic extractives was relatively stable (15% in normal pile and 8% in snow-covered pile). The top of the piles exhibited the most significant decrease in the total level of extractives (73% in normal and snow-covered pile), whereas the bark in the middle of the pile retained the highest amount of extractives (decreased by 51% in normal pile and 47% in snow-covered pile) after 24-week storage.

Keywords: pile storage; wood extractives; condensed tannins; stilbenes; gas chromatography with mass selective detection (GC-MS); high-performance liquid chromatography (HPLC)

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1. Introduction

Bark contains the great majority of the hydrophilic extractives present in conifers, and it is produced as various forestry sidestreams annually on a massive scale. In 2016, the Finnish forest industry was estimated to produce 7.9 million tons of solid wood-based sidestreams [1]. Despite the high saturation of bark with potentially useful extractable chemicals for valorisation, conifer bark is still mainly used for purposes not directly related to extractives. Bark is primarily used (i) for the production of heat and energy (sometimes in a pelletised form), (ii) for non-energy purposes (e.g., roof material and mould manufacture) and (iii) for landscaping [1].

Among the various groups of bark extractives, tannins and stilbenes, which are categorised as polyphenolic and anti-oxidative compounds, are considered to be of particular

interest. Generally, stilbenes (especially resveratrol) and tannins have multiple commercial applications highlighting their protective and health benefits [2,3]. Therefore, extracting these crucial compounds with suitable solvents followed by purification is considered an industrially attractive approach. However, a possible bottleneck of industrial valorisation is its logistics since high-value applications also set equally high requirements for raw materials. Therefore, it stands to reason that practices that best preserve extractives must be applied before the raw material is extracted.

In general, the storage of wood, especially pile storage, can have a considerable impact on its chemical composition [4–9]. Although pile storage of bark is a standard procedure, it may result in significant material losses, leading even to fires. However, it seems practically inevitable that some forms of raw material storage must be used, and finding a solution that does not compromise the quality of the raw material ought to be considered to be of great importance. Storing bark in an intact form on saw logs has already been discussed in previous studies [10,11]. It seems evident that such a form of storage has many advantages, as compared to pile storage, in preserving extractives in bark. This is understandable, as a smaller particle size (as in pile storage) generally exposes the chemical compounds to more degrading factors. Nevertheless, the storage of whole sawlogs may not always be feasible, and for practical reasons, some form of pile storage bark needs to be used instead. Therefore, it is necessary to understand how the pile's internal thermokinetics affect the behaviour and degradation of extractives.

Bark extractives stored in piles are usually attacked both externally and internally [8]. Among the external factors that contribute to degradation are rain, wind and ultraviolet (UV) radiation, as well as heat, which causes evaporation [12–14]. On the other hand, the internal factors include bark-colonising fungi and bacteria and their enzymatic activity, as well as the self-heating of piles as a result of cellular respiration [15–17]. The main changes in extractives are polymerisation/depolymerisation reactions, oxidation reactions, hydrolysis reactions and phenoxy radical photo-degradation reactions [13,18]. In addition, extractives are also lost as a result of leaching (hydrophilic compounds, e.g., tannins and stilbene glycosides) and evaporation (e.g., monoterpenoids) [19,20].

While there are previous studies which aim at providing the overall picture of spruce bark, such as, the study by Krogell et al., to understand how that picture changes over time is also of key importance [21]. In this study, we evaluated the degradation behaviour of the lipophilic and hydrophilic extractives of Norway spruce (*Picea abies*) bark during pile storage over a period of 24 weeks. The main goal was to understand the speed, extent and nature of degradation and whether there is a significant difference between the sampling locations inside each pile (i.e., middle, side and top). We tested the following hypotheses: (i) the extractive content of bark stored in a pile depends on the physical location inside the pile, (ii) covering the bark pile with snow at the beginning of storage can better preserve the bark extractives and (iii) the degradation rate of extractives during pile storage is faster than that of intact bark on saw logs. Overall, the information gathered in this study facilitates the decision-making process regarding the optimisation of storage conditions for the preservation of extractives needed in the manufacture of value-added products.

2. Results and Discussion

2.1. Overview of the Change in the Chemical Composition of Bark during Storage

An overview of the changes in the chemical composition of the bark during storage is presented in Figure 1. In this figure, the gravimetrically determined amounts of total dissolved solids (TDSs) from hot-water and *n*-hexane extracts, the amount of lignin (both acid-soluble and acid-insoluble) and holocellulose as determined by acid hydrolysis and the amount of hemicelluloses and cellulose as determined by acidic methanolysis are presented. Here, the overall changes in the chemical composition are discussed with regard to the storage time, sampling location and pile covering. A more in-depth analysis of the changes within each extractive group is presented in Section 2.3. The exact values of the various

compound groups, individual compounds as well as their standard deviations presented in the subsequent figures are available as Supplementary Files (link at the end of the article).

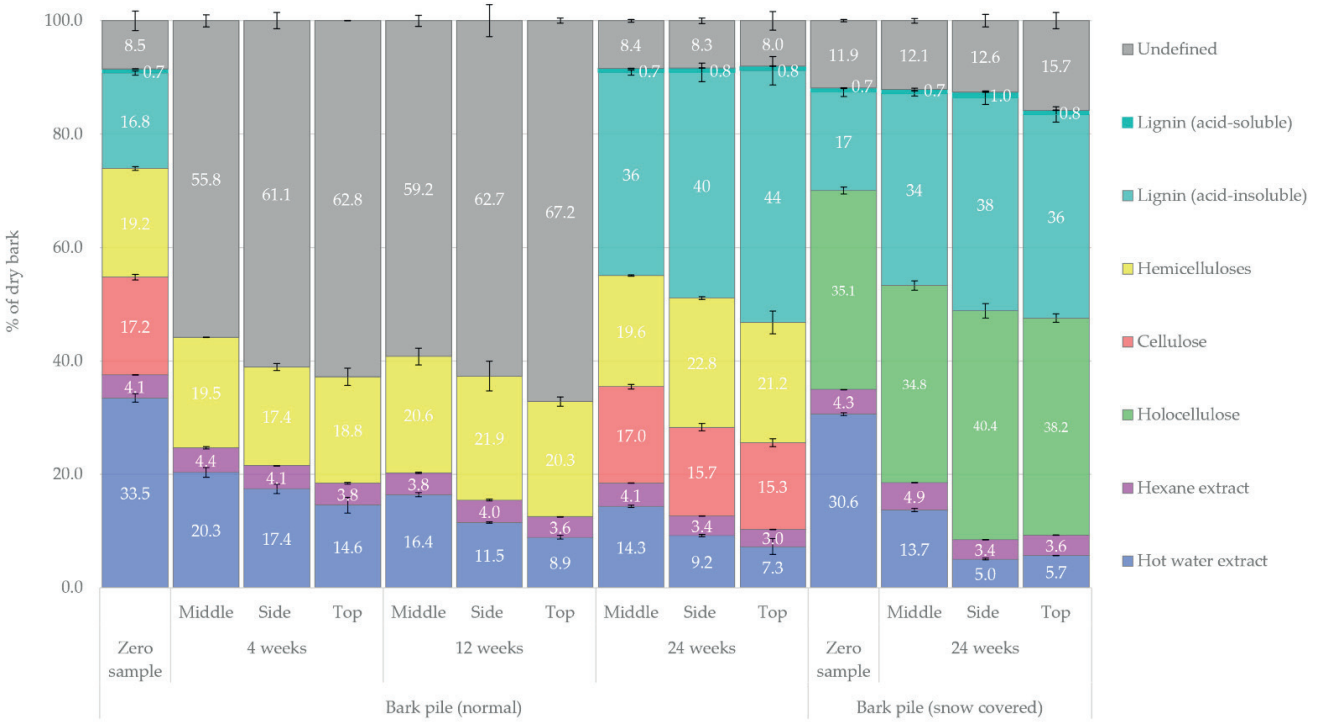


Figure 1. Overall changes in the bark samples' chemical composition during storage as % of dry bark.

2.1.1. Change in Total Dissolved Solids

The Effect of Storage Time

The approximate impact of storage on the relative amounts of chemical compounds in bark was as follows: over 24 weeks of storage, the amount of hydrophilic extractives decreased from 31–34% to 5–14%, the amount of lipophilic extractives changed from 4% to 3–5%, the amount of cellulose decreased slightly from 17% to 15–17%, the amount of hemicelluloses increased slightly from 19% to 20–23%, the amount of acid-insoluble lignin increased from 17% to 34–44%, the amount of acid-soluble lignin (determined by ultraviolet-visible [UV–Vis] spectrometry) increased from 0.7% to 0.7–1.0% and the amount of unidentified compounds changed from 9–12% to 8–16%. The major decrease in hydrophilic extractives agrees with previous storage studies of conifer bark. It has been previously reported that the extractives content in *Pinus sylvestris* chain flailing residue roughly halves during the first 4 weeks of storage, with the most significant changes showing in the hydrophilic fractions [22]. Similarly, Routa et al. studied *Pinus sylvestris* and *Picea abies* bark in pile storage and found that only 56% and 66% of the acetone-soluble extractives remained after eight weeks of storage, respectively [23,24]. Čabalova et al. also reported a significant decrease in *Picea abies* bark extractives extracted by ethanol-toluene mixture (2:1) and a relative increase in lignin and cellulose during 8 months of storage [25]. Compared to our previous study regarding *Picea abies* sawlog bark storage in winter and summer, the difference was noticeable. Although the initial chemical composition in the winter zero samples was very similar, the chemical composition of the 4-week stored piled bark was roughly comparable to that of 24-week stored sawlog bark [10].

Statistical tests revealed that, at the 10% level of significance, the storage time significantly affects the amounts of diterpenoids, unidentified lipophilic compounds, steryl esters, triglycerides, stilbenes, flavonoids, other phenolics, sesquistilbenes, distilbenes, unidentified hydrophilic compounds, proanthocyanidins and the TDSs of the hot-water extracts (Table 1).

Table 1. Results (*p*-values) obtained from testing the statistical differences among the storage duration (0, 4, 12 or 24 weeks), sampling location (middle, side or top) and snow cover (covered or not covered with snow) in terms of the amounts of lipophilic extractives, hydrophilic extractives, condensed tannins (CTs) and total dissolved solids (TDSs). The bold text indicates a statistically significant difference with a *p*-value less than 0.10.

	Storage Time	Sampling Location	Snow Cover
Lipophilic Extractive Groups			
Resin acids	0.280	0.148	**0.018**
Fatty acids	0.313	0.115	0.285
Diterpenoids	**0.058**	0.651	0.157
Sterols	0.236	0.431	0.464
Other lipophilic extractives	0.379	0.166	0.157
Unidentified	**0.022**	0.142	**0.005**
Steryl esters	**0.066**	0.446	0.255
Triglycerides	**<0.001**	0.764	0.200
Hydrophilic Extractive Groups			
Sugars	0.355	**0.078**	0.344
Organic acids	0.527	**0.010**	0.400
Sugar alcohols	0.219	0.192	0.432
Stilbenes	**0.039**	0.670	0.170
Flavonoids	**0.023**	0.430	0.176
Other phenolics	**0.031**	0.404	0.458
Alcohols	**0.076**	0.233	0.319
Lignans	0.124	0.133	0.234
Other hydrophilic extractives	0.795	**0.068**	0.472
Sesquistilbenes	**0.002**	0.862	**n/a**
Distilbenes	**<0.001**	0.805	**n/a**
Unidentified	**0.005**	0.719	0.499
Condensed Tannins			
Total concentration	**0.039**	0.733	0.827
Procyanidins	**0.039**	0.733	0.827
Prodelphinidins	**0.025**	0.424	0.436
DP	**0.039**	1.000	**0.005**
TDSs			
n-Hexane extract	0.288	0.201	0.324
Hot-water extract	**0.006**	0.161	0.364
Biofuel Properties of Stored Bark			
Ash content	0.117	0.233	0.103
Effective heating value	0.280	0.153	**0.024**

Multiple different factors affect the loss of extractives during pile storage. For example, hydrophilic compounds are readily leached by moisture and rainwater, microorganisms rapidly consume some compounds (e.g., sugars), and many extractives are oxidised (e.g., resin acids) or evaporated (e.g., monoterpenoids) [5,26–28]. However, some extractives

may be converted via heat and UV-light-induced radical chain reactions to non-extractable polymers (e.g., self-isomerisation and condensation of tannins into phlobaphenes) [20].

The Effect of Sampling Location

The sampling location in the pile (whether from the middle, side or top) appeared to have a systematic and predictable effect on the concentrations of bark components among all storage weeks. Statistical analysis showed that, at the 10% level of significance, the sampling location does not significantly affect the lipophilic extractives. However, a significant statistical result was obtained for the amounts of sugars and organic acids and for the 'other hydrophilic extractives' group (Table 1).

The degradation on the top of the pile was the most pronounced, with less degradation on the side and the most conservative degradation in the middle of the pile. These differences may largely be explained by the complex mechanics of pile storage, which differ in terms of temperature, moisture, ventilation and exposure to external forces depending on the pile formation, pile material (e.g., particle size) and the location in the pile [5,29]. The top of the pile is the part most exposed to both outside influences (e.g., wind, rain and UV light) and the pile's internal activities (steam rising from the pile as a result of self-heating, microbial degradation). Thus, it was not surprising that the top of the pile contained a low concentration of compounds that are easily affected by these factors. Interestingly, after the initial decrease in concentration at weeks 4 and 12, certain extractive groups (e.g., sugars, sugar alcohols and organic acids) experienced an increase only in the middle point of the pile. This observation suggests that the non-volatile hydrophilic extractives from the top of the pile gradually leached downwards, creating a concentrated spot in the middle. A general trend, where the lower one goes in the pile, the higher the concentration of extractives is, could not, however, be confirmed in this study. Routa et al. also looked at the effect of location in bark pile on extractives content in *Pinus sylvestris* and *Picea abies*, but they could not find similar general trends by TDS as were found in this study [23,24]. This difference may be explained by a variety of factors, such as their choice of solvent (pure acetone), difference in extraction method, pile formation and the raw material characteristics.

The Effect of Snow Cover

Minor differences were found between the results of non-covered and snow-covered bark piles. Statistical tests indicated that, at the 10% significance level, snow cover significantly affects the amounts of resin acids and unidentified lipophilic extractives, the degree of polymerisation (DP) of proanthocyanidins and the effective heating value of bark (Table 1).

Notably, the concentrations of hydrophilic TDSs in the snow-covered pile were only slightly low at the beginning and end of storage compared to those in the non-covered pile. The data shown in Figure 2a,b indicate that the snow-covered pile was frozen for 10 days since the beginning of storage, unlike the non-covered pile. This means that the snow cover must have reduced the initial degradation caused by UV light and microbes. However, once the snow melted, additional slow water extraction and consequent leaching of hydrophilic extractives towards the bottom of the pile occurred. The increased moisture also enhanced the conditions for microbial invasion. Overall, although there seemed to be some initial value in covering bark piles with snow, the material losses may have been more significant in the end. Thus, it can be concluded that the hypothesis that covering bark piles with snow can help preserve the bark extractives is invalid (at least when the storage period reaches week 24). Therefore, to study the effect of snow cover on preserving extractives, sampling should be performed before the snow melts. There is evidence that semi-permeable covering of piles can reduce moisture content, temperatures and dry matter losses in forest fuel storage piles [7,9]. However, the impact of such covering during storage on extractives still needs further investigation. Recent study found that thermal drying of *Picea abies* sawmill bark in moderate temperatures will still yield major extractive losses [30].

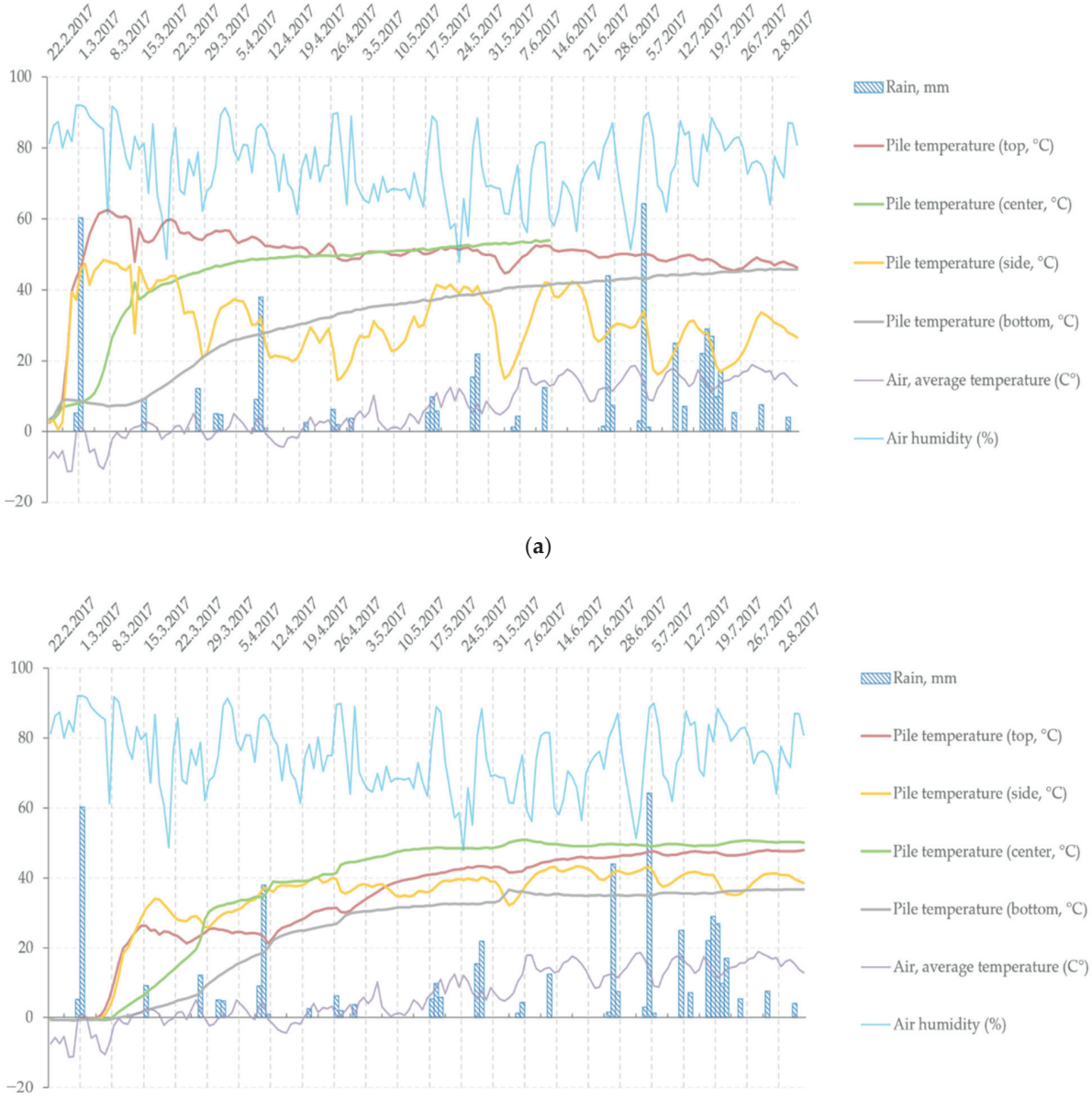


Figure 2. Temperature development inside the non-covered (**a**) and covered (**b**) bark piles according to the data gathered by thermocouples. The data shown are from sector one after 1 month of storage.

2.1.2. Changes in Carbohydrates and Lignin

Of the two studied bark piles, holocellulose was only determined from the zero samples and 24-week samples. In both piles, the holocellulose content of bark was equal at the beginning of storage (ca. 35%), and its relative proportion increased slightly towards the end of storage (because of the quicker loss of extractives). In addition, the relative total amount of lignin in bark more than doubled during storage, and the highest lignin concentrations (ca. 45%) were found at these sampling points, at which the extractive fractions were the lowest.

If no degradation occurs for hemicelluloses and cellulose, their relative proportion will increase (as in lignin). Nevertheless, the relative amounts of hemicelluloses and cellulose remained nearly the same throughout storage, indicating their slight degradation. Only on

the side and top of the pile did the relative proportion between hemicelluloses and cellulose change, resulting in an overall 4% decrease in cellulose and an increase in hemicelluloses.

Similar findings of increased lignin and carbohydrate content during storage have been reported by Čabalova et al. recently [25]. However, contrary to the results presented here, the relative amount of hemicellulose was reported to have decreased while the amount of cellulose increased. This difference may be explained by the used solvent and extraction method. Compared to the unpressurised Soxhlet extraction used by Čabalova et al. [25], our hot-water extraction at 120 °C is quite harsh and may have resulted in carbohydrates that would otherwise have been included in hemicellulose and cellulose fractions to be included in the extractives fraction.

2.2. Biofuel Properties of Stored Bark

2.2.1. Temperature Development Inside Bark Piles

The data logged from the thermocouples together with the climate conditions from a transportable weather station (air temperature, humidity and amount of rain) are displayed in Figure 2a,b. The thermocouple data revealed that the thermal activity inside the pile started almost immediately after piling the material. In general, both the centre and top of the piles experienced the highest temperatures (with a maximum at around 60 °C), whereas the side and bottom of the piles were cooler. It is also noticeable that the insides of the pile (centre and bottom) experienced a constant increase in temperature, whereas the outermost layers (top and side) experienced heavy fluctuations and correlation with rain and ambient temperature, especially on the side of the pile. Similar dependence of temperature on sampling location was also observed by Routa et al. and Krigstin et al. [23,31]. The occurrence and amount of rain was clearly most significant in June and July, towards the end of the storage period. The top of the pile was also affected by the rising steam from inside the pile. Comparing the two piles (Figure 2a,b) revealed that the snow-covered pile was initially frozen for 10 days and that the overall temperature of the pile during storage was slightly lower.

2.2.2. Heating Values of Stored Bark

The heating values of the studied bark samples, their moisture and their ash, carbon, hydrogen and nitrogen contents are presented in Table 2. The results show that the average moisture content of all bark samples was approximately 57%. The sampling location also affected the moisture content of the bark. For example, in the non-covered bark pile, the moisture content was elevated to 61% at the top of the pile, remained at its original value in the middle and decreased to 41% on the side of the pile. This increased moisture on the top samples may be explained by the steam rising from inside the pile, as microbiological and chemical reactions lead to self-heating of the pile. In the snow-covered pile, presumably because of the melting of the snow cover, the 24-week samples had a high moisture content (62–70%) at all sampling locations, especially on the side and top.

The ash content of the samples underwent a gradual increase from the zero-sample level of 3.2%, especially on the side and top of the bark piles, after storage for 24 weeks, reaching peaks of 4.2% and 8.5% on the top of the non-covered and covered piles, respectively. Similar initial ash content of *Picea abies* industrial bark has been reported previously [32]. The unusually high ash content on the top of the snow-covered pile after 24 weeks of storage is most probably explained by the inorganic impurities (e.g., sand) that were mixed in with the snow that was used for covering. After the snow melted, the inorganic material accumulated on top. Moreover, the carbon content of the dry bark samples increased slightly from an initial level of 51.4% at all sampling locations, except on the top of the snow-covered pile, reaching a maximum of 52.8% at the top of the non-covered pile. The hydrogen content of the dry bark samples decreased from an initial level of 5.8% to an average of 5.6% at all sampling points, especially on the side and top of the piles and particularly in the snow-covered pile. The nitrogen content of the dry bark samples increased from an initial level of 0.47% to an average of 0.55% at all sampling

points. This increase was most pronounced, especially on the side and top of the piles. However, the effective heating value remained very stable at approximately 19.3 MJ/kg at all sampling points. These heating values are slightly higher than those reported by Routa et al. for *Picea abies* bark at around 18.9 MJ/kg [24]. After storage for 24 weeks, the heating values decreased to 18.1 MJ/kg only on the top of the snow-covered pile due to increased ash content.

Table 2. Moisture, ash, carbon, hydrogen and nitrogen content of the studied bark samples and their effective heating values.

Storage Time, Weeks	Sampling Location	Moisture Content, %	Ash Content, %	Carbon Content [1], %	Hydrogen Content [2], %	Nitrogen Content [3], %	Effective Heating Value, MJ/kg
Normal Pile							
0		57.38 ± 0.68	3.21 ± 0.02	51.4	5.82	0.47	19.14 ± 0.02
4	Middle	59.89 ± 1.05	3.30 ± 0.01	51.3	5.80	0.53	19.10 ± 0.01
4	Side	52.20 ± 1.22	3.53 ± 0.01	52.2	5.74	0.52	19.40 ± 0.01
4	Top	56.92 ± 0.64	3.46 ± 0.02	52.1	5.78	0.53	19.56 ± 0.03
12	Middle	61.40 ± 0.86	3.45 ± 0.01	51.1	5.73	0.53	18.78 ± 0.00
12	Side	53.09 ± 0.81	3.75 ± 0.02	51.7	5.63	0.55	19.37 ± 0.01
12	Top	51.65 ± 0.32	3.74 ± 0.01	52.2	5.59	0.54	19.40 ± 0.02
24	Middle	57.83 ± 0.40	3.53 ± 0.05	52.5	5.71	0.52	19.48 ± 0.01
24	Side	40.79 ± 0.82	3.85 ± 0.00	52.5	5.50	0.56	19.47 ± 0.02
24	Top	61.01 ± 0.71	4.17 ± 0.04	52.8	5.45	0.60	19.52 ± 0.01
Snow-Covered Pile							
0		56.01 ± 0.89	3.12 ± 0.01	51.3	5.77	0.47	19.11 ± 0.01
24	Middle	62.05 ± 0.73	3.77 ± 0.12	51.8	5.65	0.50	19.36 ± 0.01
24	Side	64.33 ± 0.44	4.92 ± 0.08	51.5	5.34	0.61	19.09 ± 0.02
24	Top	69.50 ± 0.45	8.47 ± 0.35	49.9	5.27	0.56	18.13 ± 0.02

[1] Measurement uncertainty ±2%. [2] Measurement uncertainty ±4%. [3] Measurement uncertainty for values <0.3 is ±30%, and for values >0.3 is ± 15%.

2.3. Qualitative and Quantitative Results for Bark Extracts Obtained by Gas Chromatography with a Flame Ionisation Detector/Mass Selective Detector (GC-FID/MS)

2.3.1. Lipophilic and Hydrophilic Extractive Groups

The quantified lipophilic and hydrophilic extractive groups determined using GC-FID/MS methods are presented in Figures 3 and 4, respectively. The lipophilic extractives totalled 11% of all bark extractives, and their main extractive groups were resin, fatty acids, diterpenoids, sterols, steryl esters and triglycerides. In contrast, the hydrophilic extractives totalled 89% of the extractives. Their main groups were sugars, sugar alcohols, organic acids, stilbenes, sesquistilbenes and distilbenes, with the minor groups being flavonoids and other alcohols. The group defined as 'others' contained extractives that, despite being visible on the GC chromatograms, could not be identified or whose concentrations were very small. The 'unidentified' group referred to extractives that could not be detected by GC because of their low volatility or high molar weight. The relative amount of unidentified compounds increased during storage, suggesting an increase in polymerisation reactions.

As shown in Figure 3, overall, there was only a slight decrease in the total amount of lipophilic extractives over a storage period of 24 weeks. The most notable changes in the chemical composition of the lipophilic extract were as follows: a decrease in resin acids from 33% to 23%, a decrease in fatty acids from 22% to 12%, a decrease in triglycerides from 14% to 2% and an increase in unidentified compounds from 6% to 44%. Thus, the results suggest that the storage of bark increases the polymerisation reactions of lipophilic compounds. The results indicate that the rate of degradation gradually slowed as the storage progressed. The overall increase in new unidentified compounds was 2.5 mg/g/storage week after

4 weeks of storage and slowed down to 0.2 mg/g/storage week after 12 and 24 weeks of storage. The concentration of lipophilic extractives decreased on the top and side of the bark pile and increased in the middle of the pile. This finding was confirmed by comparing the results obtained on week 12 and week 24 for the zero sample of the non-covered pile and the 24-week sample of the covered pile. For a more detailed analysis of the degradation pattern of individual lipophilic compounds, see Figures 5–8. The results from our previous sawlog bark study indicate that there is much variation between individual sawlog barks, particularly in the amount of lipophilic extractives, sometimes reaching even above 70 mg/g of dry matter [10].

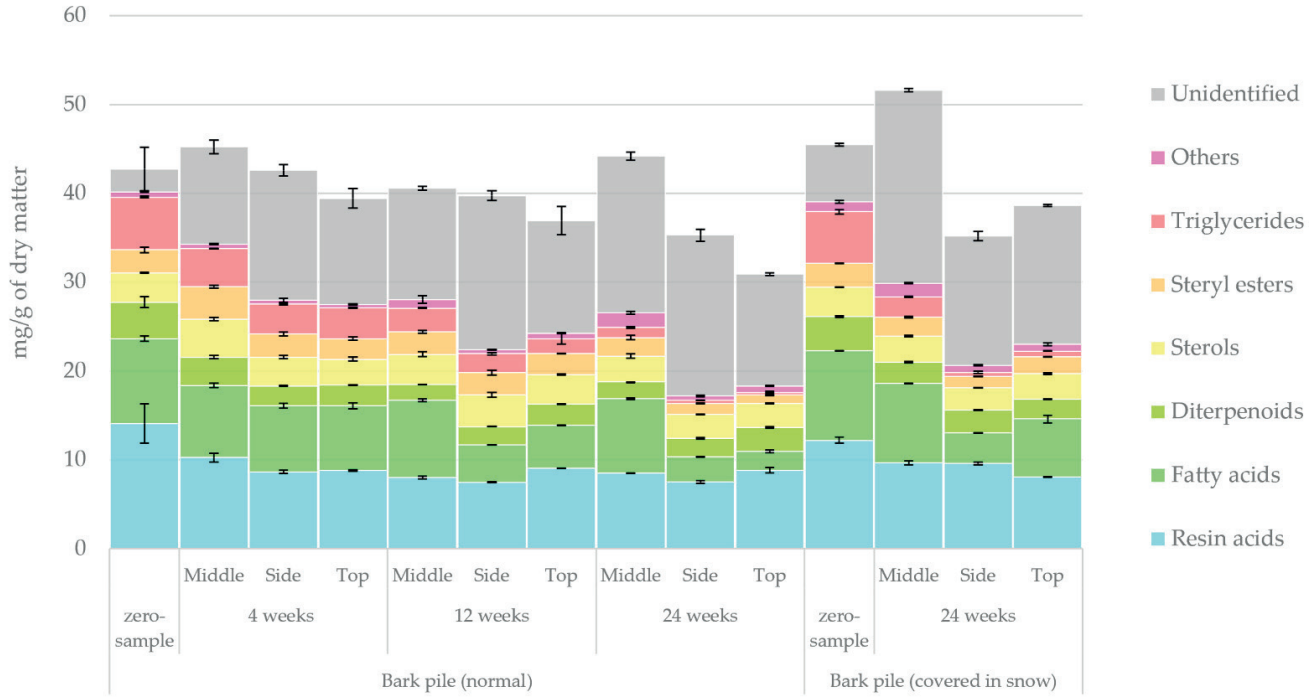


Figure 3. Lipophilic extractive groups in bark samples during pile storage.

The results outlined in Figure 4 show a clear and gradual change in the total amount of hydrophilic extractives and a dramatic decrease in the concentration of many hydrophilic extractive groups in bark resulting from pile storage. The unidentified bark extractives composed of polymeric compounds, such as condensed tannins (CTs) and oligo- and polymeric sugars, represented almost half of all hydrophilic extractives. Mono- and disaccharides represented the second-largest extractive group. The most significant changes in the relative proportion of extractives in the hydrophilic water extracts (zero sample vs. 24-week sample) were as follows: a decrease in sugars from 28% to 17% and an increase in unidentified compounds from 42% to 61%. Stilbenes, sesquistilbenes, distilbenes, flavonoids and other phenolics also experienced a major decrease in concentration, but this did not affect the total extract amount as much. Unlike with the lipophilic extractives, the relative increase in unidentified compounds seemed to result from the decrease in other compounds and not from the increase in polymerisation. For a more in-depth analysis of the hydrophilic extractive groups, see Figures 9–13. A major difference is seen here to sawlog bark, where the concentration of hydrophilics remained at the level of 300 mg/g of dry bark for up to 12 weeks of winter storage [10]. This amounted to approximately 59% less hydrophilic extractives in pile-stored bark at week 12, most likely due to microbial degradation.

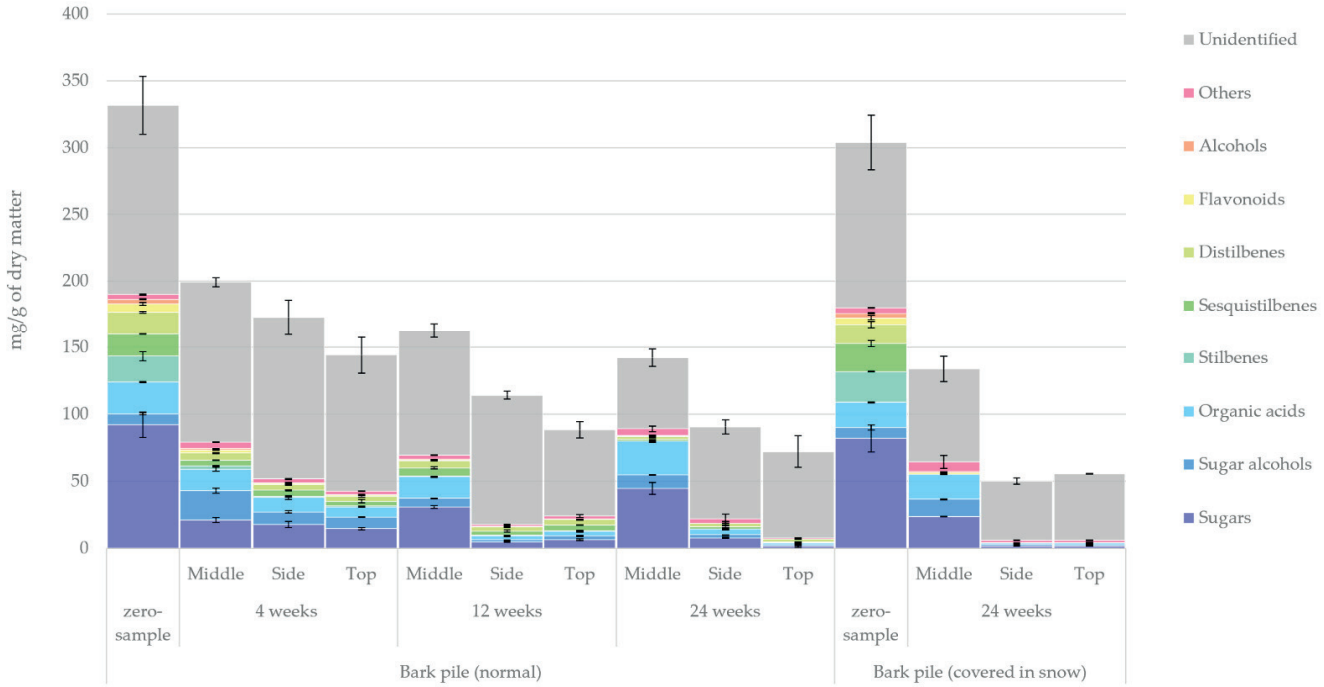


Figure 4. Hydrophilic extractive groups in bark samples during pile storage.

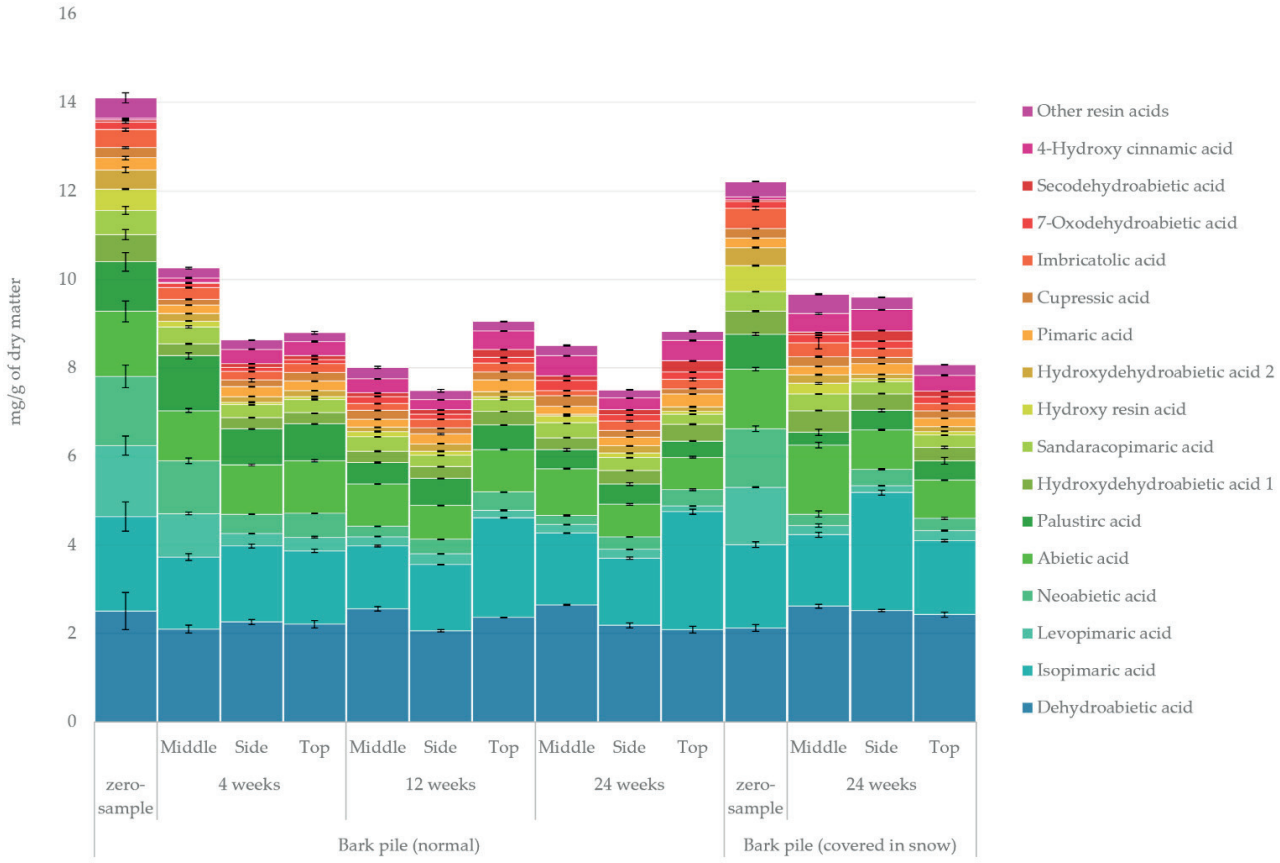


Figure 5. Quantified amounts of individual resin acids in the lipophilic extracts of stored bark.

2.3.2. Resin Acids

The quantified amount of resin acids in the lipophilic bark extracts determined using GC-FID/MS is presented in Figure 5. The results demonstrate a considerable overall decrease in the amount of resin acids during pile storage over the first 4 weeks of storage. After this initial decrease, the total amount of resin acids did not change much, and there was no apparent trend with sampling location. The general stability of resin acids has also been reported previously [10,33]. The most remarkable changes in the relative proportion of resin acids (zero sample vs. 24-week sample) were the increase in dehydroabietic acid from 18% to 28% and in isopimaric acid from 15% to 23% and the decrease in levopimaric acid from 11% to 2% and in neoabietic acid from 11% to 3%. The absolute values of the most prominent resin acids, namely dehydroabietic and isopimaric acid, remained more or less constant throughout storage. Although some reports indicate that certain fungi can reduce the amount of resin acids markedly, the way in which the degradation of resin acids halted after 4 weeks suggests that the initial drop correlated instead with the increased pile temperature [34]. This is also supported by the decrease in neoabietic and levopimaric acids, which are the most prone to thermal oxidation, Diels–Alder reaction, isomerisation and radical reactions because of their conjugated double-bond structure.

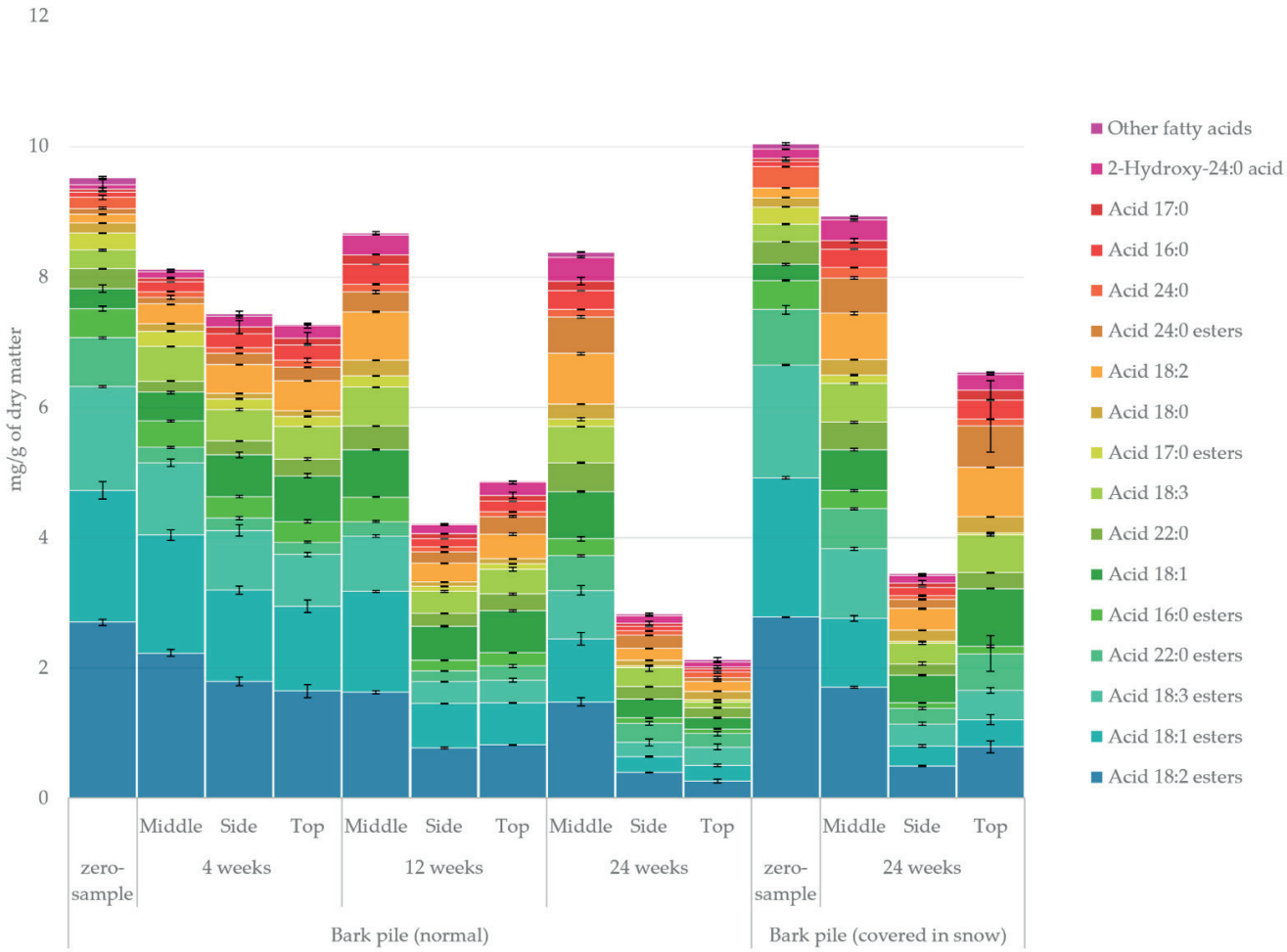


Figure 6. Quantified amounts of individual fatty acids in the lipophilic extracts of stored bark.

2.3.3. Fatty Acids

The quantified amount of fatty acids in the lipophilic bark extracts determined using GC-FID/MS is presented in Figure 6. The changes in triglycerides and fatty acids during

storage in many raw materials have been known for a long time. Fatty acids can react either by their conjugated double bonds or carboxylic acid group, leading to various different derivatives [35]. The hydrolysis of triglycerides and consequent polymerisation of the released fatty acids was reported by Ekman among the major chemical changes in wood material during storage [36]. Similarly, Nielsen et al. attributed the decrease in fatty acids during the storage of softwood chips and sawdust to polymerisation and oxidation reactions [37]. It is noteworthy that the total amount of fatty acids dropped considerably during storage, especially on the top and side of the pile, whereas the fatty acids in the middle of the pile on the other hand appeared to be remarkably well-shielded from degradation (although a change in chemical composition was observed). This clearly indicates that the degradation is connected with hydrolysation and oxidation reactions caused by external influences. Esterified fatty acids constituted the vast majority (83%) of total fatty acids at the beginning of storage. The most significant changes (zero sample vs. 24-week sample) in the relative amount of fatty acids were a decrease in fatty acid esters 18:1, 18:2 and 18:3 from 21% to 11%, from 28% to 16% and from 17% to 9%, respectively, and an increase in acids 18:1 and 18:2 and esters of acid 24:0 from 3% to 9%, from 1% to 8% and from 1% to 6%, respectively. From this, the conversion of esterified fatty acids into non-esterified fatty acids seems evident. It should be considered that the degradation of triglycerides during storage (shown in Figure 3) also releases free fatty acids. Routa et al. reported fast degradation of triglycerides during the storage of Scots pine bark, which seemingly led to an increase in the total amount of fatty acids during storage [23].

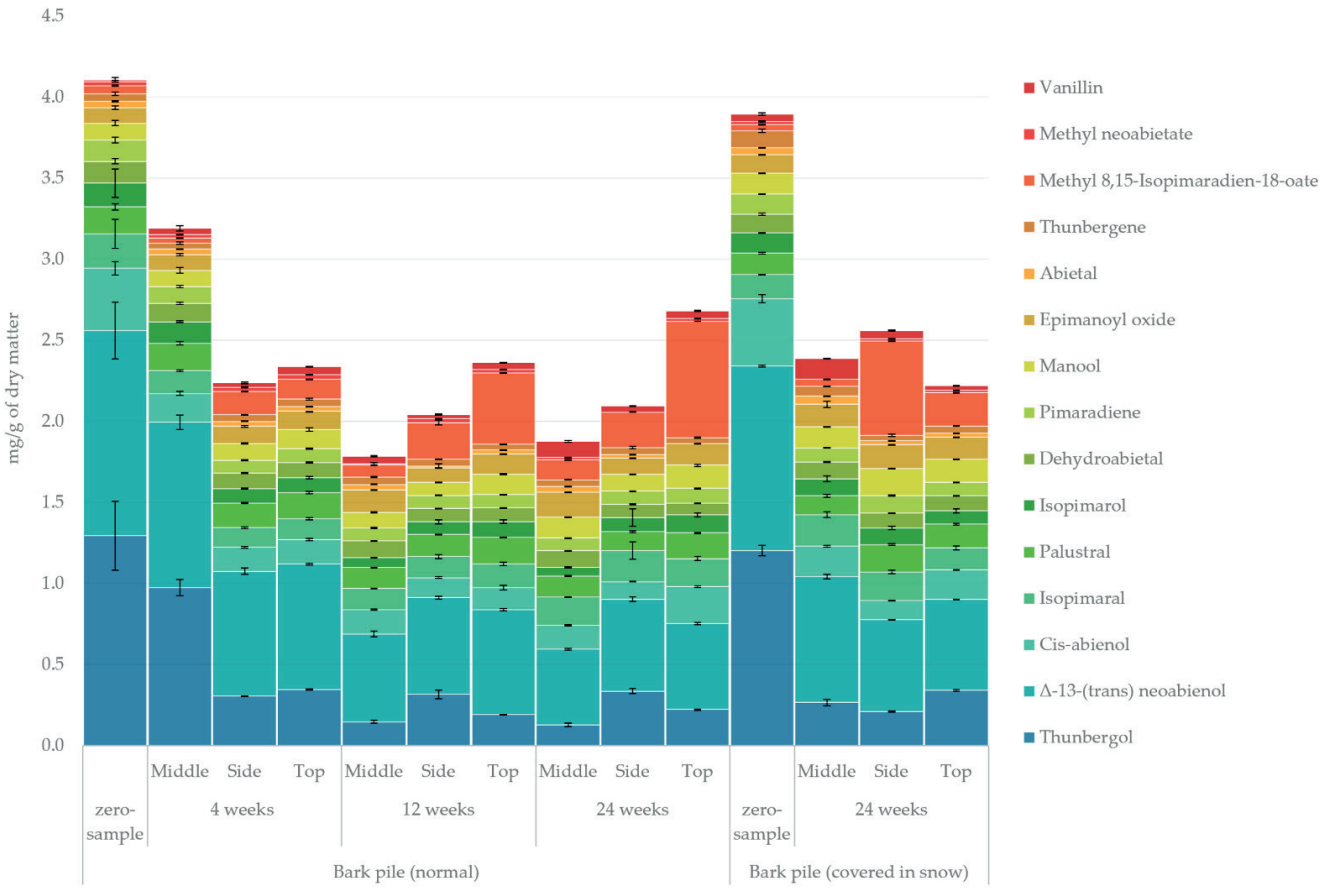


Figure 7. Quantified amounts of individual diterpenoids in the lipophilic extracts of the stored bark.

2.3.4. Diterpenoids

The quantified amount of diterpenoids in the lipophilic bark extracts determined using GC-FID/MS is presented in Figure 7. The amount of diterpenoids at the beginning of storage was slightly above the levels reported by Krogell et al. (0.7 mg/g and 3.2 mg/g in inner and outer bark, respectively) [21]. A considerable overall decrease in diterpenoids was observed during the 24-week storage. Thunbergol, which is associated with anti-fungal, anti-oxidative and anti-tumour activities, was the primary diterpenoid with Δ^{13}-(*trans*)neoabienol [38,39]. The most prominent changes (zero sample vs. 24-week sample) in the relative amount of diterpenoids were an increase in methyl 8,15-isopimaradien-18-oate from 1% to 16% and a decrease in thunbergol and Δ^{13}-(*trans*)neoabienol from 32% to 10% and from 31% to 24%, respectively. That methyl 8,15-isopimaradien-18-oate was formed primarily on the side and at the top of the piles indicates a formation through oxidation reaction. Nielsen et al. also reported that diterpenoid degradation is affected by oxidation and polymerisation reactions [37]. Thunbergol loss was expected because it is also entirely lost during tall oil distillation [40]. Our previous study regarding sawlog bark also indicated a loss of thunbergol with the increase in ambient temperature [10].

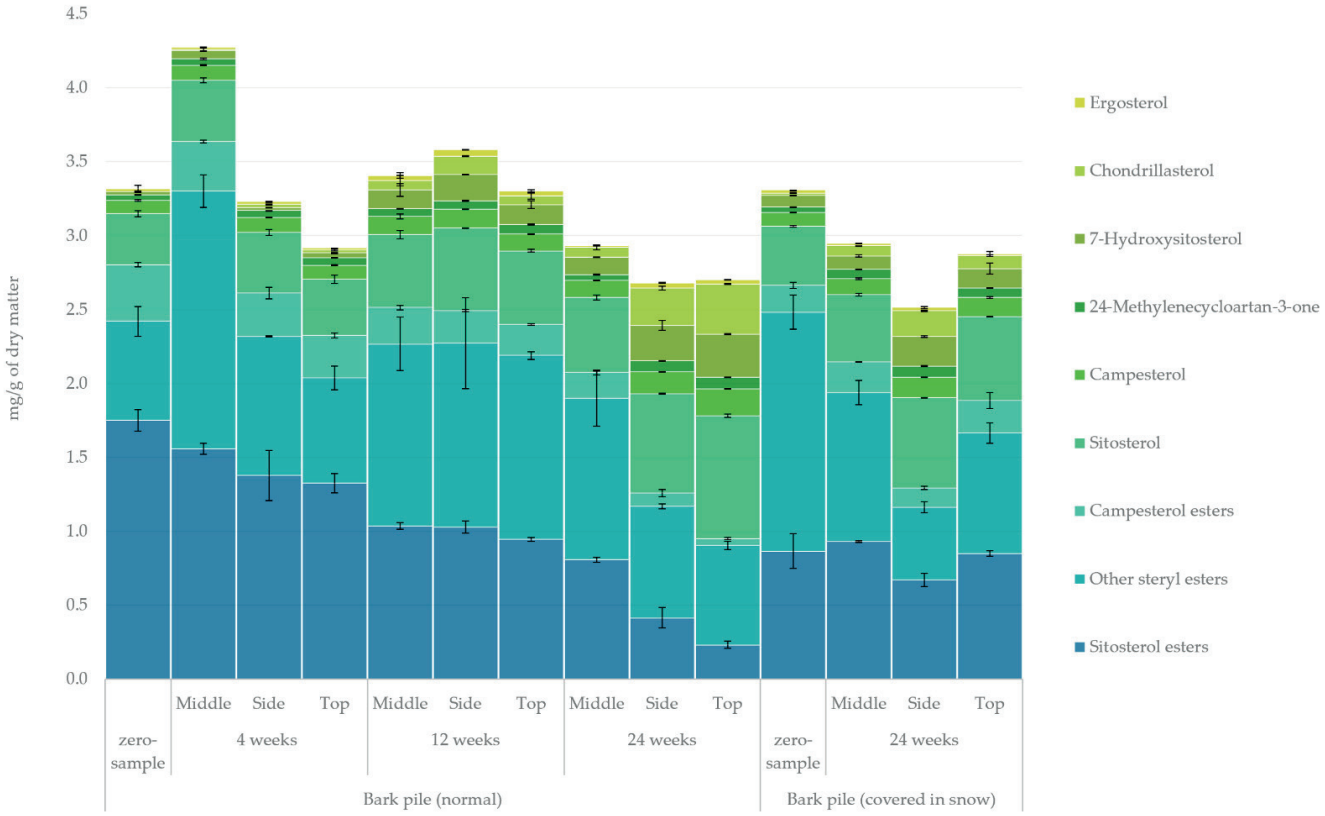


Figure 8. Quantified amounts of sterols and steryl esters in the lipophilic extracts of stored bark.

2.3.5. Sterols

The quantified amount of sterols and steryl esters in the lipophilic bark extracts determined using GC-FID/MS is presented in Figure 8. The major sterol in *Picea abies* is *β*-sitosterol, a prominent antibacterial and antioxidant agent [41]. The total amount of sterols ranged between 3.2–4.8 mg/g of dry matter and only a slight overall decrease was observed. Routa et al. reported similar sterol levels and only slight degradation during 8 weeks of Scots pine storage [23]. Assarson had reported similar resistance to degradation in unsaponifiable compounds (including sterols) in *Picea abies* chip pile storage [42]. The most prominent changes in the relative amount of sterols (zero sample vs. 24-week sample)

were a decrease in the esters of sitosterol and campesterol from 53% to 17% and from 12% to 4%, respectively, and an increase in sitosterol, chondrillasterol and 7-hydroxysitosterol from 10% to 24%, from 0% to 8% and from 1% to 8%, respectively. Given these results, it seems that esterified sterols underwent gradual conversion into free sterols during storage. In addition, ergosterol, chondrillasterol and 7-hydroxysterol were formed as a result of storage, especially on the side and at the top of the pile, again indicating a formation through oxidation reactions [43].

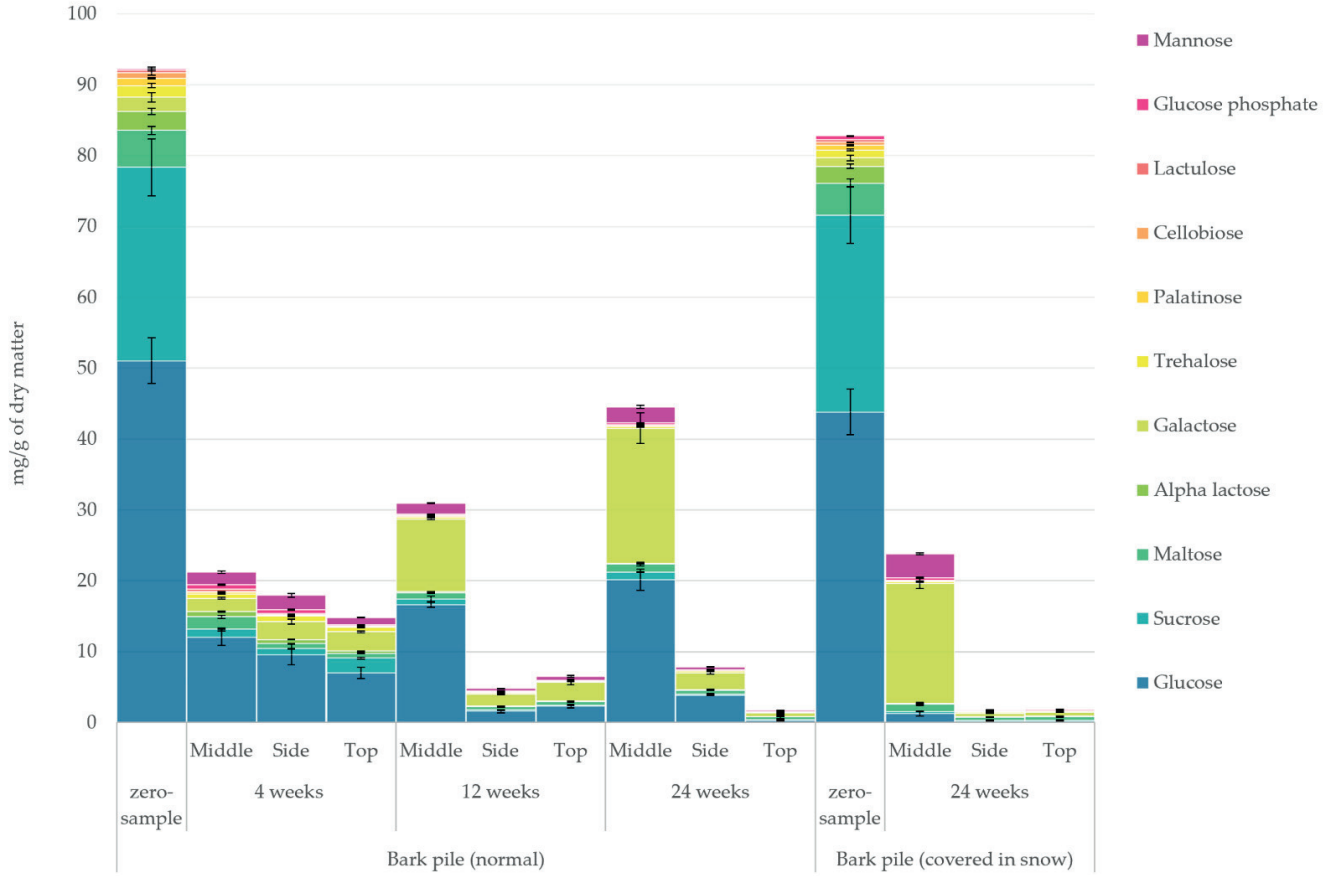


Figure 9. Quantified amounts of mono- and disaccharides in the hydrophilic extracts of stored bark.

2.3.6. Sugars

The quantified amount of simple sugars in the hydrophilic bark extracts determined using GC-FID/MS is presented in Figure 9. Mono- and disaccharides underwent major degradation during pile storage, with only approximately 20% of the sugars remaining after storage for 24 weeks. The sampling location resulted in an increasingly greater difference in the concentration of sugars. At the end of storage, the concentration of sugars at the top of the pile decreased to vanishingly low levels, with the concentration at the side of the pile being only slightly higher. The middle of the pile, on the other hand, exhibited an increased concentration after the initial decrease at week 4. The most significant changes in the relative proportion of sugars were an increase in galactose from 2% to 41% and a decrease in sucrose and glucose from 30% to 2% and from 55% to 45%, respectively. It is generally understood that the rapid loss of saccharides happens due to them being among the first to be consumed by micro-organisms [44,45]. Leaching should, however, be considered as a possibility, especially as a consequence of the steam released during pile storage [5,28,46]. Concentrations of galactose and mannose in the middle of the pile by leaching might have been observed here. In his dissertation, Sauro

Bianchi noted the prevalence of hemicellulose-derived saccharides in water extracts above 80 °C [46]. Noting that the extraction temperature that was used in this study was 120 °C, the presence of saccharides from hemicellulose should be expected. The presence of mannose after storage for 4 weeks and the increased amount of galactose may be, at least partly, explained by the degradation of galactoglucomannan, the main water-soluble hemicellulose in Norway spruce [47]. As a polymeric carbohydrate, galactoglucomannan would be included in the 'unidentified' hydrophilic extractive group (Figure 4). It is also worth noting that the degradation of lactose (4-*O*-β-D-galactopyranosyl-D-glucopyranose) released galactose units.

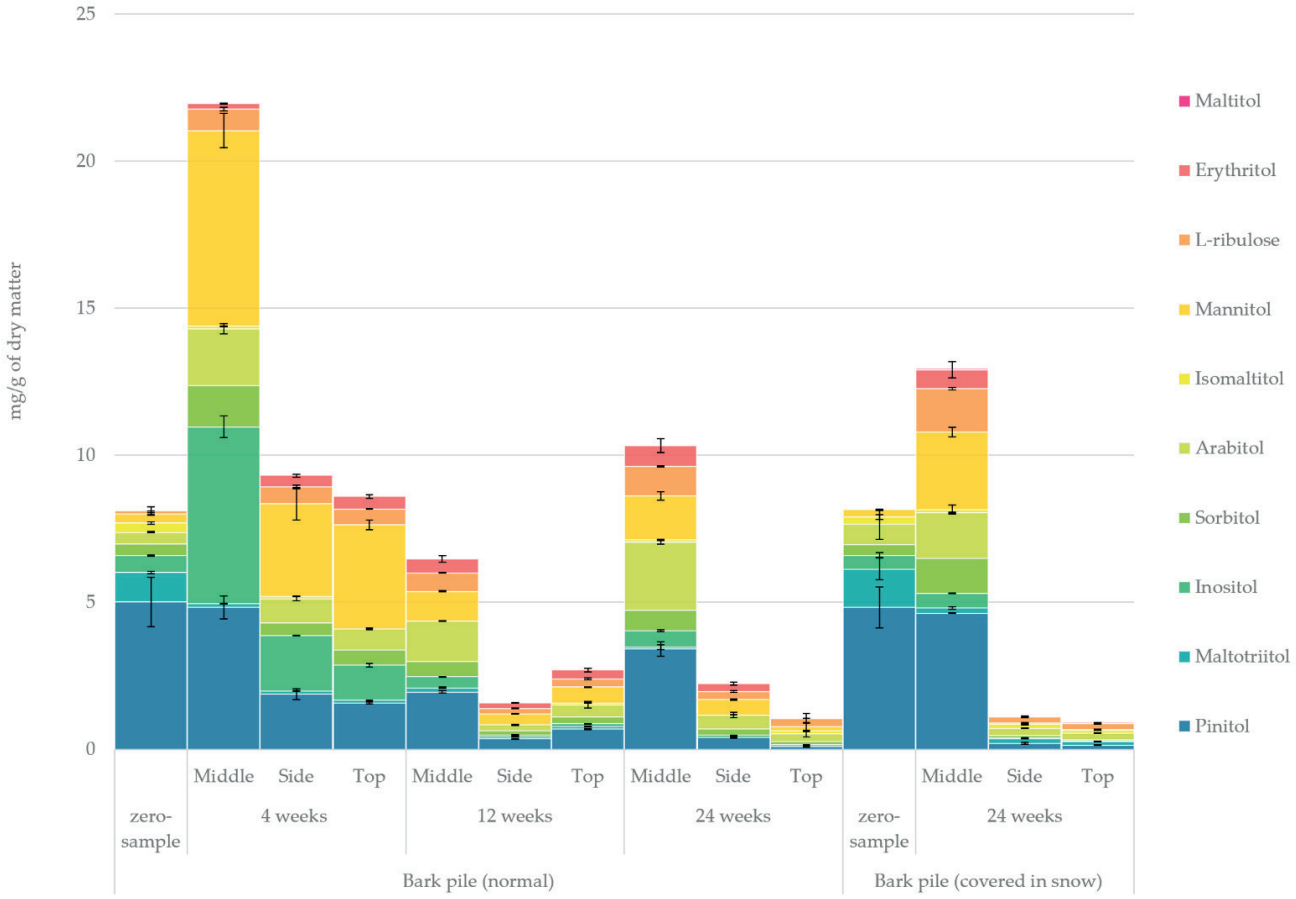


Figure 10. Quantified amounts of individual sugar alcohols in the hydrophilic extracts of stored bark.

2.3.7. Sugar Alcohols

The quantified amount of sugar alcohols in the hydrophilic bark extracts determined using GC-FID/MS is presented in Figure 10. A significant overall variation was observed in the amount of sugar alcohols during storage. After storage for 4 weeks, a sharp increase was detected in the sugar alcohol concentration in the middle of the pile, whereas on the side and at the top of the pile, the total amount remained the same. After 4 weeks, maltotriitol and isomaltitol almost disappeared, whereas inositol and maltitol dramatically increased. Moreover, L-ribulose and erythritol were produced. At the end of the 24-week storage, the amount of sugar alcohols significantly decreased, with only the middle of the pile having a slightly elevated amount of total sugar alcohols. The most significant changes in the relative amount of individual sugar alcohols in the samples (zero sample vs. 24-week storage) were an increase in arabitol, mannitol and L-ribulose from 5% to 23%, from 4% to 16% and from 1% to 11%, respectively, and a decrease in pinitol and maltotriitol from 62% to 29% and from 12% to 0%, respectively. The literature regarding the storage

of wood and forestry sidestreams does not discuss the fate of sugar alcohols much. Our previous study regarding the storage of sawlog bark found the sugar alcohol levels to remain constant (c.a. 10 mg/g level) during winter storage until week 12 and then drop to 3 mg/g at 24 weeks of storage [10]. The increase in sugar alcohols observed here, at week 4, should probably be attributed to the hydrogenation reactions of sugars—a process that has also been utilised in the production of value-added chemicals and food ingredients [48]. It is possible that the initial conversion of some sugars to sugar alcohols happened followed by their rapid leaching towards the middle of the pile. This would include the conversion of maltose to maltitol. Production of L-ribulose would, however, suggest a microbial and enzymatic conversion [49]. Similarly the formation of inositol happens through enzymatic phosphorylation of glucose to glucose phosphate (see the residues in Figure 9) followed by isomerisation of glucose phosphate to inositol-phosphate and finally dephosphorylation to inositol [50].

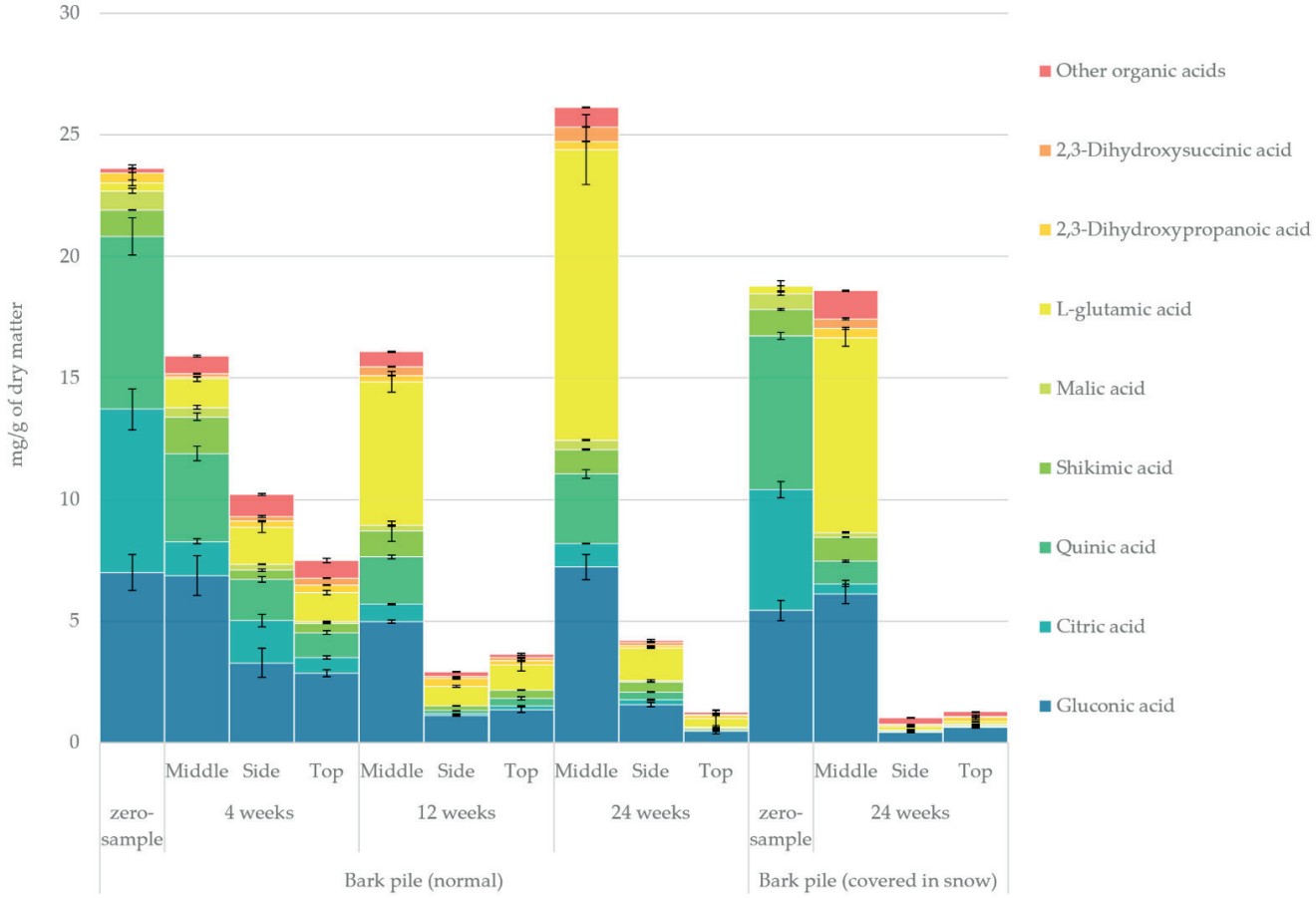


Figure 11. Quantified amounts of individual organic acids in the hydrophilic extracts of stored bark.

2.3.8. Organic Acids

The quantified amount of organic acids in the hydrophilic bark extracts determined using GC-FID/MS is presented in Figure 11. A considerable overall decrease was observed in the amount of organic acids during storage. At the beginning of storage, gluconic acid, citric acid and quinic acid constituted the vast majority of all organic acids. The presence and leaching of organic acids during wood storage has been noted several times before [28,51]. According to Fuller, the presence of even mild acetic acid in pile storage can lead to the shortening of the cellulose fragments in wood [5]. The most significant changes in the relative proportion of organic acids in the samples (zero sample vs. 24-week sample) were an increase in L-glutamic acid from 1% to 43% and a decrease in citric acid and quinic

acid from 28% to 4% and from 30% to 10%, respectively. Notably, the concentration of organic acids on the side and at the top of the pile decreased rapidly, whereas in the middle of the pile, an increase was observed from week 12 to week 24. Contrary to these results, the production of new organic acids was not observed in our previous study regarding sawlog storage of bark [10]. Generally, L-glutamic acid is an amino acid by-product of microbiological fermentation of plant proteins (e.g., gluten) with, for instance, glucose as the carbon source [52]. Thus, the significant increase observed in L-glutamic acid also indicated an increase in microbial degradation during storage. Among other degradation products, 2,3-dihydroxysuccinic acid (tartaric acid) was also formed as a fermentation product—a common degradation product in aged fruits and wines [53].

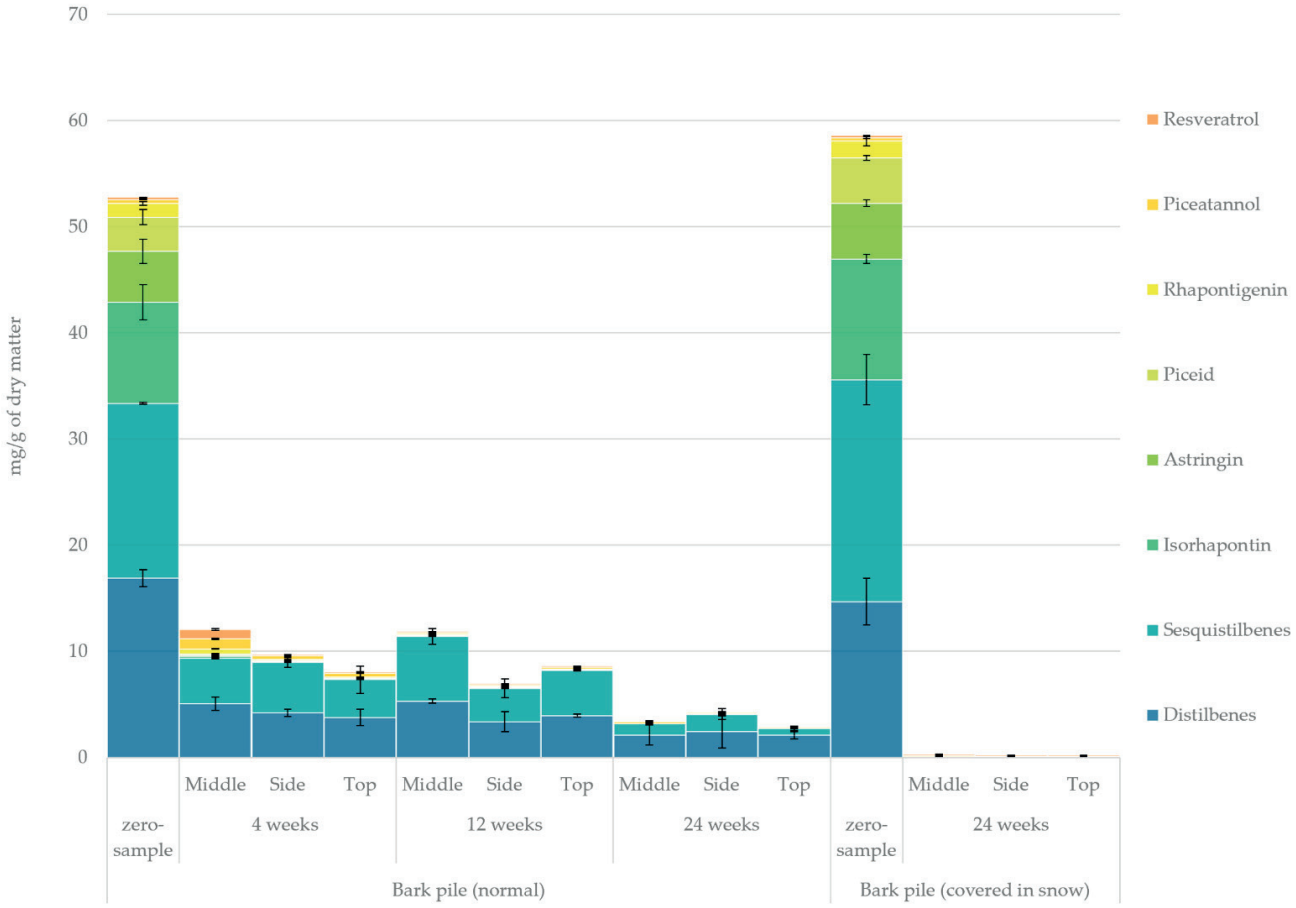


Figure 12. Quantified amounts of stilbenoids in the hydrophilic extracts of stored bark.

2.3.9. Stilbenes

Stilbenes are among the most attractive organic compounds and potential platform chemicals obtained from spruce bark. However, stilbenes are usually lost at a particularly fast rate, not only because they are hydrophilic and may be leached by rainwater but also because of their high anti-oxidative capacity and reactivity under UV light to form phenanthrene derivatives via photo-oxidative cyclisation [18].

The quantified amount of stilbenoids in the hydrophilic bark extracts determined using GC-FID/MS is presented in Figure 12. During storage, a radical overall loss of stilbenes was observed in the study samples, especially during the first few weeks of storage. After storage for 4 weeks, only 23% of the original stilbenes remained, and the stilbene monoglucosides isorhapontin, astringin and piceid, totalling 90% of the original monoglucosides, were almost completely removed. However, the concentrations of the aglycones resveratrol, piceatannol and rhapontigenin increased by 23% at week 4 as a result

of the hydrolysis reactions of the glucosides. Moreover, distilbenes and sesquistilbenes constituted 63% of the total stilbenes at the beginning of storage, but only 13% and 6% of the original distilbenes and sesquistilbenes, respectively, remained at the end of storage.

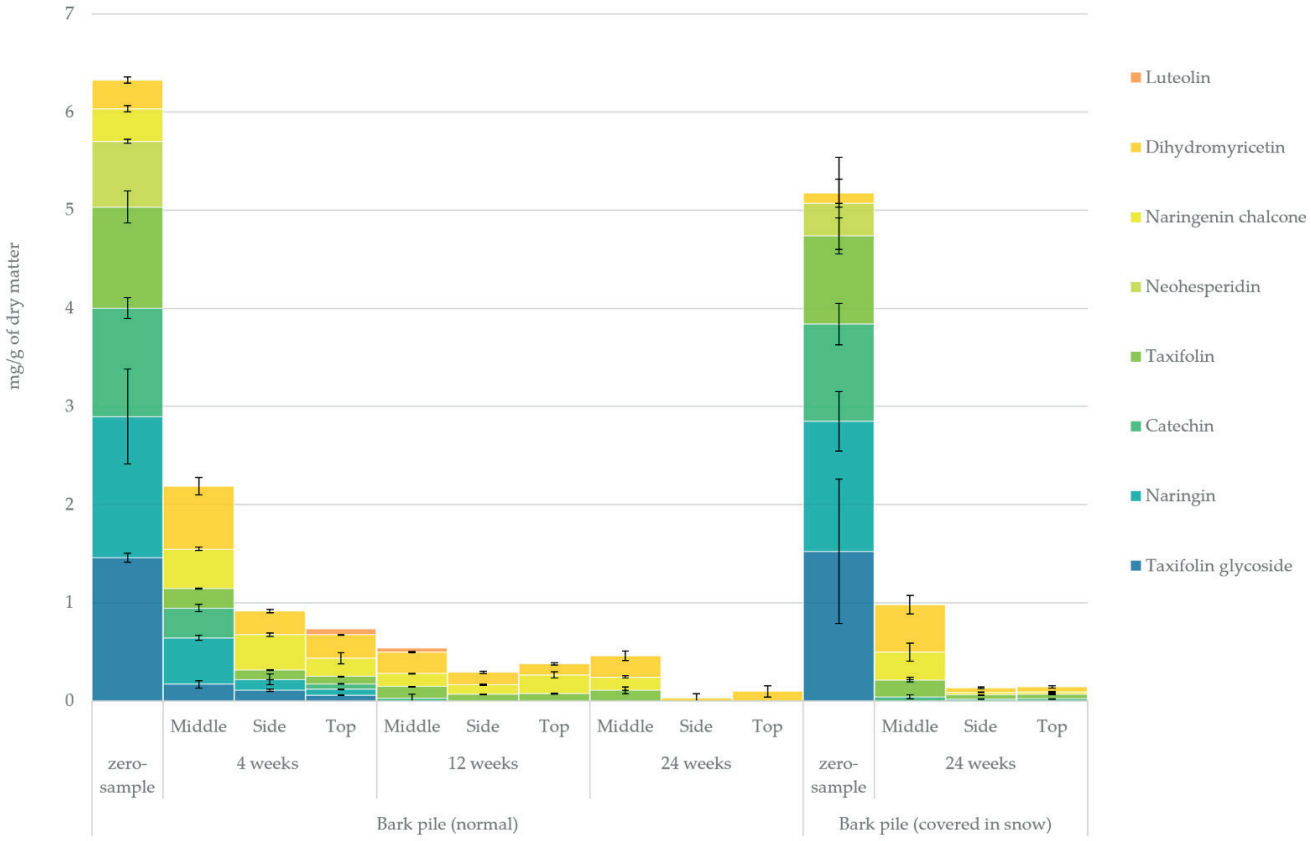


Figure 13. Quantified amounts of individual flavonoids in the hydrophilic extracts of stored bark.

The average concentrations of stilbene monoglucosides, sesquistilbenes and distilbenes in piled bark (from both covered and non-covered piles) were found to be 21.2, 18.7 and 15.8 mg/g of dry matter, respectively. On the other hand, as reported in a previous study, the average amounts of stilbene monoglucosides, sesquistilbenes and distilbenes in the bark of freshly felled (winter-stored) saw logs were found to be 23.5, 10.8 and 10.1 mg/g of dry matter, respectively [10]. Thus, it seems that while the initial amount of stilbene monoglucosides in bark pile and sawlog bark is closely paralleled, the amount of sesqui- and distilbenoids is greater in chipped and piled bark. This may be coincidental, given that the stilbene levels of individual saw logs may considerably vary. However, while the initial amount of stilbenoids was slightly greater in the piled bark, after just 4 weeks of storage, the winter-stored saw logs retained 79% more stilbenoids than those retained by the piled bark. This finding highlights the impact that the storage method can have on individual extractives. To effectively utilise piled bark for its stilbene content, either protective measures need to be taken to ensure their preservation, or the bark needs to be further processed rapidly (within days of the initial piling).

Stilbene concentrations presented here were markedly higher than those reported by Krogell et al. [21]; however, Jyske et al. have reported at least twice as high concentrations of stilbene glucosides in the bark of 18–37 year old *Picea abies* trees [54]. It should, however, be noted that while Jyske et al. [54] looked at stilbene concentration at different bark zones and heights (inner bark having highest stilbene concentrations), our results reflect more the average stilbene concentration in sidestream *Picea abies* bark from sawmills without further

distinctions. Stilbene levels similar to those presented by Jyske et al. [54] have also been reported in the root bark of Norway spruce [55].

2.3.10. Flavonoids

The quantified amount of simple flavonoids in the hydrophilic bark extracts determined using GC-FID/MS is presented in Figure 13. The initial amount of flavonoids was approximately twice as high as that reported by Krogell et al. [21].The loss of flavonoids seemed to follow a path similar to that of stilbenes, with a dramatic concentration decrease after just 4 weeks of storage. Slower flavonoid degradation was observed in our previous study regarding *Picea abies* sawlog bark [10]. The most prominent flavonoids were taxifolin glycoside, naringin, catechin, taxifolin and neohesperidin. Notably, dihydromyricetin, which has potent anti-oxidative properties, was found to be the most resilient among flavonoids [56]. Its amount was even found to be somewhat increased during storage (e.g., through the bio-conversion of other flavonoids). The most significant changes in the relative proportion of extractives in the samples (zero sample vs. 24-week sample) were an increase in dihydromyricetin and naringenin chalcone from 5% to 59% and from 5% to 23%, respectively, and a decrease in taxifolin glycoside, naringin, catechin and neohesperidin from 23%, 23%, 17% and 11% to 0%, respectively. Flavonoids (similarly to stilbenes) are lost particularly rapidly to photo-degradation because of their tendency as phenolic compounds to form unstable phenoxy radicals [12,13,18].

2.4. High-Performance Liquid Chromatography (HPLC) Analysis of Proanthocyanidins

Overall, the thiolytic degradation of spruce bark CTs (procyanidins) produced (epi)catechins and (epi)catechin thioethers as major reaction products and (epi)gallocatechins and (epi)gallocatechin thioethers as minor products, indicating that spruce bark CTs are a mixture of procyanidins and prodelphinidins, as observed in previous studies [11,57–59]. As shown in Figure 14, the initial CT content was 3.0–3.2 g/100 g, but it decreased rapidly during storage. After 4 weeks, the total content of CTs was found to exhibit a great variation (0.556–1.451 g/100 g) between the different samples, but this variation always remained below 50% of the original amount. After 12 weeks, the concentration reached 0.384–0.472 g/100 g, and only minor changes were observed for the rest of the storage duration. The final CT content in the normally stored bark pile was found to be 0.251–0.365 g/100 g after 24 weeks, which is equal to approximately 10% of the original content. A recent study on Scots pine reported a similar drastic and rapid loss in the CT content during pile storage of bark [23].

The average CT content in the snow-covered piles was somewhat higher after storage for 24 weeks, and notable differences were observed between the samples. These samples were obtained from different pile locations, which might partly explain the variations observed in the CT content. In this study, the highest CT content was determined twice in the samples taken from the middle of the pile (after storage for 4 weeks and 24 weeks for normally stored and snow-covered piles, respectively). It is possible that the bark in the middle of the pile was better protected from environmental stress than that on the side or at the top of the pile. This also means that the CTs were less exposed to detrimental reactions. Similarly, a recent study has shown that the outer bark is expected to protect the inner bark, with the CTs in the outer bark degrading much faster than in the inner bark during the summertime fresh-air storage of spruce logs [11]. However, further research is still needed to confirm the significance of location in a pile for the recovery of CTs and other constituents in spruce bark.

The average DP in spruce bark CTs was found to be the highest at the beginning of the experiment, but it decreased during storage, indicating that the polymerisation of CTs is the first step in the degradation process. However, the oxidation of CTs during storage might result in degradation and the formation of new covalent bonds between CTs and other macromolecules, producing new polymers partially resistant to thiolysis [59,60]. As a result, both the content and the DP of CTs are somewhat under-estimated with the current

determination method. Furthermore, the relative proportion of prodelphinidins in CTs was found to slightly increase during storage. The same finding was observed in the CTs of spruce logs stored in the open air [11]. This may indicate that prodelphinidins in spruce bark CTs are more resistant to environmental stress than procyanidins.

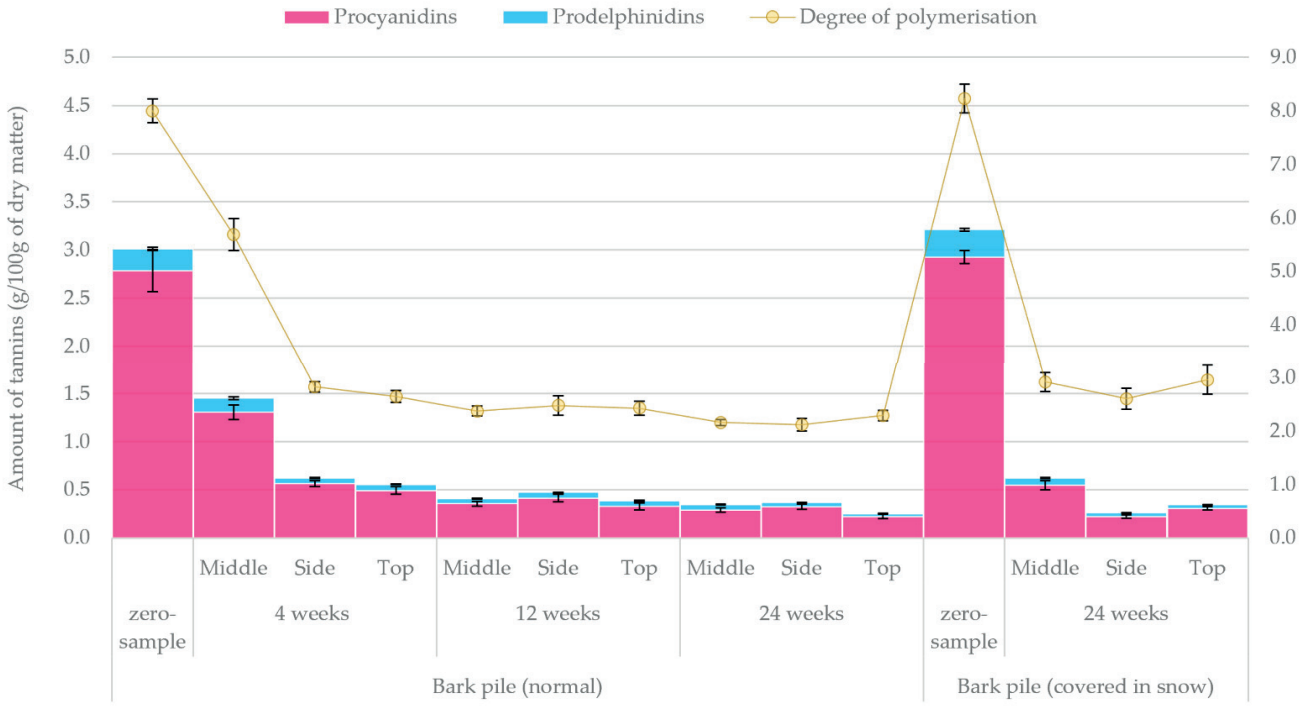


Figure 14. Quantified amounts of tannins (procyanidins, prodelphinidins) and the degree of polymerisation (DP) in freeze-dried bark samples under pile storage.

2.5. Carbohydrate Analysis

2.5.1. Acid Hydrolysis and High-Performance Anion-Exchange Chromatography (HPAEC) Analysis of Monosaccharides

The results obtained from the HPAEC analysis of extractive-free bark monosaccharides (i.e., holocellulose) are presented in Figure 15. The initial amount of holocellulosic monosaccharides in the bark samples was found to be 58% in extractives-free bark in the normal bark pile and 54% in the snow-covered pile. After a storage period of 24 weeks, the amount in both piles decreased to approximately 42% of extractives-free bark. These values, however, correlate to approximately 35.8% of the initial amount of holocellulose in dry bark (according to Figure 1) and 37.5% in dry bark after 24 weeks of storage. Thus, the total holocellulose content (as % of dry bark) increased 1.7%. In our previous study regarding *Picea abies* sawlog bark storage, the amount of holocellulose was initially 33.9% of dry bark and increased to 37.7% in 24 weeks (a 3.8% increase) [10]. Čabalova et al. also reported relatively increased cellulose content during storage for 8 months [25]. Generally, glucose was by far the most prominent monosaccharide. The notable changes in the relative proportion of monosaccharides (zero sample vs. 24-week sample) were an increase in glucose from 66% to 74% of dry matter and a decrease in arabinose from 13% to 5% of dry matter. Moreover, mannose decreased slightly more in the samples from the snow-covered pile.

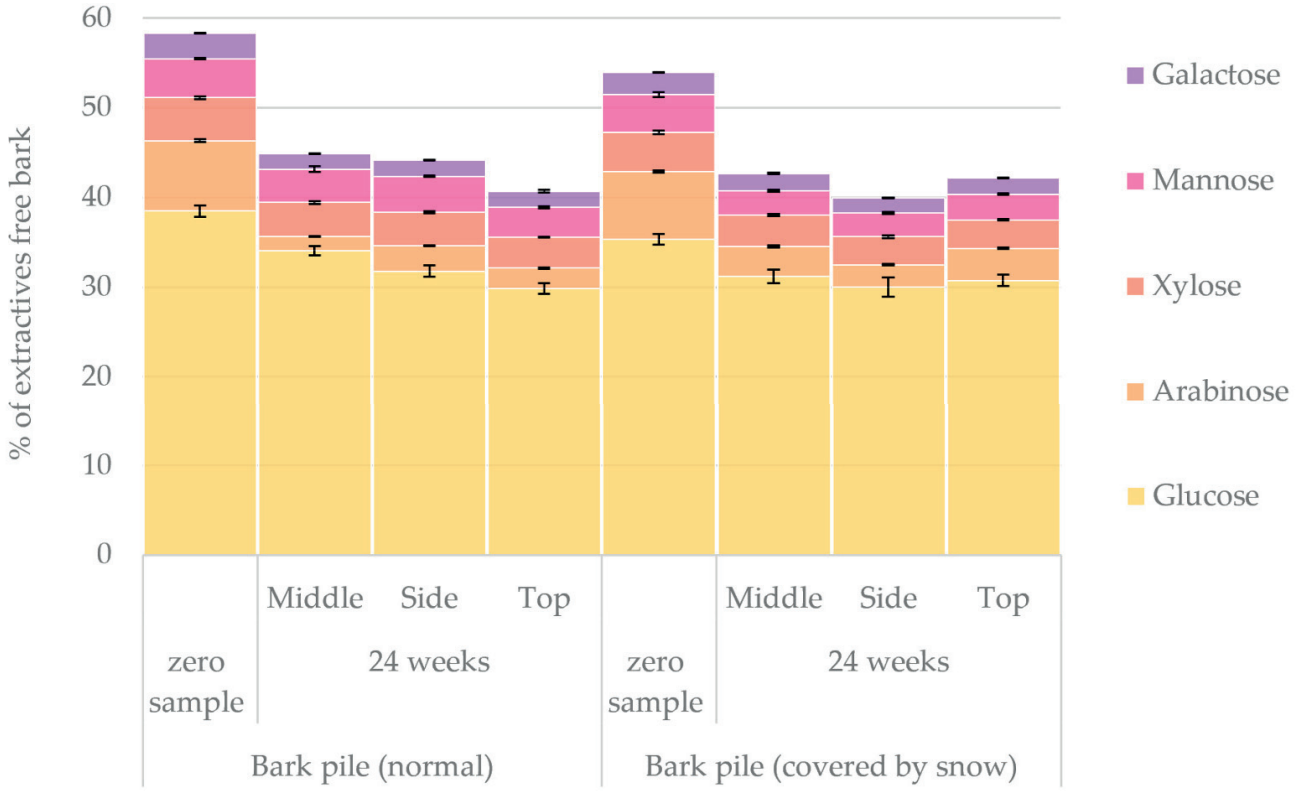


Figure 15. Quantified amounts of holocellulosic monosaccharides in bark samples at the beginning and end of normal and snow-covered pile storage.

2.5.2. Acidic Methanolysis

The results obtained from the acidic methanolysis of extractive-free bark monosaccharides (i.e., hemicelluloses) are presented in Figure 16. These results indicate that the overall amount of hemicelluloses decreased by 21%. Such a decrease occurred during the first 4 weeks of storage, and the total amount of hemicellulosic monosaccharides remained constant throughout the storage period, although changes in the composition occurred. The most notable changes in the relative proportion of hemicellulosic groups in the samples (zero sample vs. 24-week sample) were an increase in glucose and xylose from 10% to 22% and from 13% to 21%, respectively, and a decrease in galacturonic acid and arabinose from 31% to 18% and from 23% to 12%, respectively. Conversion of galacturonic acid to galactose was probably also observed. A similar trend was observed with regard to the sampling location in each pile, and the concentration of extractives was probably also observed at weeks 4 and 12. The highest concentration was found in the middle of the piles, whereas the top and side of the piles showed greater signs of degradation. Notably, the hemicellulosic monosaccharides presented here are basically a subset of the results presented in Figure 15. By comparing the results for holocellulosic and hemicellulosic monosaccharides (in the normal bark pile), we were able to observe that the cellulosic monosaccharides were primarily composed of glucose and mannose. The apparent increase in some hemicelluloses, such as glucose and xylose, could be explained (similarly to the increase in lignin in bark (see Section 2.1.2)) as a relative increase caused by the faster degradation of extractives and other carbohydrates. Relative increases in hemicelluloses were also observed in our previous study regarding single stem *Picea abies* bark storage [10]. It should also be noted that while the total amount of carbohydrates as mg/g of extractives-free bark (in Figure 15) decreased, the relative amount of carbohydrates as % of dry bark (i.e., bark containing extractives; see Figure 1) slightly increased during storage. A similar relative increase in cellulose and lignin due to short term storage of *Picea abies* bark has also been recently reported by

Čabalova et al. [25]. This effect could be likened to the concentration of carbohydrates by weight observed in dried fruits.

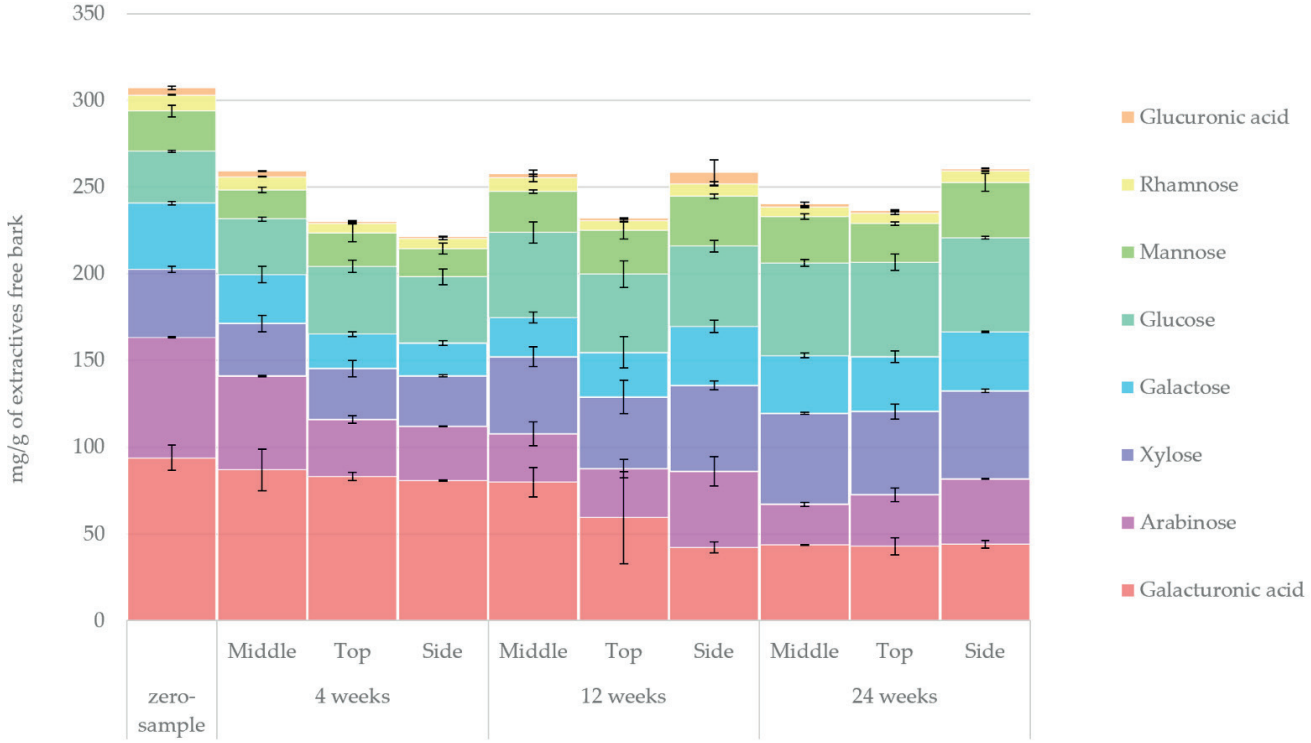
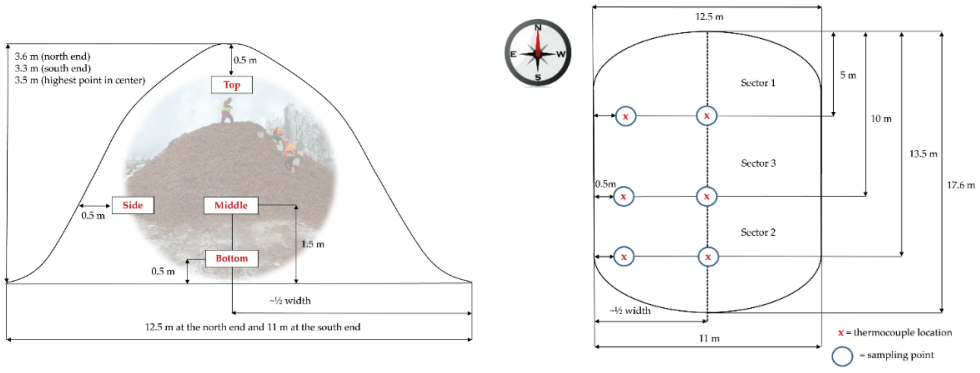


Figure 16. Quantified amounts of hemicellulosic carbohydrates in the extractive-free bark samples under normal pile storage.

3. Materials and Methods

3.1. Experimental Setup of Storage Studies and Sampling

All the bark used in this study was provided by the UPM-Kymmene Oyj sawmill in Ostrobothnia, and all the bark pile setups were located outside in the factory yard in Pietarsaari. The two 450 m^3 bark piles used in this storage study were constructed on 20 and 21 February 2017. The piles consisted of *Picea abies* bark that was debarked a maximum of 48 h before the construction of the pile. However, most of the material was even fresher. It should be noted that since the bark originated as a sidestream from a standard operating sawmill, no exact measurements of individual trees from which the bark was obtained (their height, width, age, etc.) were available. It is known that the used trees were gathered within a 200 km range from Pietarsaari, mostly from private forest owners in Ostrobothnia. The sampling points and dimensions of the bark in the non-covered pile are outlined in Figure 17. The sampling locations were chosen from areas of piles expected to have significant variations in temperature and moisture content, according to earlier storage studies [29]. The length of the constructed pile was 17.6 m, and it was divided into three sectors. Sector one was opened for sampling after 4 weeks, sector two after 12 weeks and finally sector three at the end of the storage study, after 24 weeks. Thermocouples were placed inside the pile in the locations indicated in Figure 17a, and the temperature was measured in each sector until the sector was opened for sampling. At each sampling time, bark samples were taken from the exact locations of the thermocouples, except for the bottom of the pile.



(**a**) (**b**)

Figure 17. Schematic representations of the measures of the bark pile and (**a**) the thermocouple locations inside the bark pile and (**b**) the sampling points and sectors.

3.2. Sample Pre-Treatment and Basic Characterisation

First, the bark was ground to a finer particle size with a Jens Algol System woodchipper (Jenz GmbH, Petershagen, Germany). Then, a standard method (CEN/TS 14774-2:2004) was used to determine the fresh bark samples' moisture content [61]. Next, the samples were dried at 105 °C until a constant mass was achieved. All measurements were performed in duplicate.

The bark was then lyophilised (for at least 3 days) and ground with a Retsch SM 100 cutting laboratory mill (Retsch GmbH, Haan, Germany) equipped with a bottom sieve with trapezoidal holes (perforation size < 1.0 mm) for chemical analysis. Samples were stored in a frozen state (below −20 °C). Then, the dry matter content of the lyophilised bark samples was determined by drying 1 g of bark powder at 105 °C in an oven overnight in tared crucibles.

3.3. Calorific Values and Carbon, Hydrogen and Nitrogen (CHN) Measurements of Bark Samples

First, the moisture content (on a wet basis) of the bark samples was analysed according to the same method as referred to in Section 3.2, and the ash content was determined according to the standard method SFS-EN 14775 [62]. A bomb calorimeter (IKA C 5000; IKA-Werke GmbH & Co., Staufen, Germany) was used to determine the calorific heating value (qp_{gross}) of the bark dry matter. Samples were dried, milled (Retsch SM-1 mill; Retsch GmbH, Haan, Germany) and pelletised before analysis with the bomb calorimeter. Next, the calorimetric heating values were determined and the gross calorific values were calculated using the standard method CEN/TS 14918:2005 [63]. Then, the carbon, hydrogen and nitrogen concentrations were analysed using the standard method SFS-EN ISO 16948:2015 at the laboratory of Ahma Environment Ltd. [64]. The following equation was used to calculate the effective heating value (qp_{net}):

$$qp_{net} = qp_{gross} - 2.45 \times 0.09H_2 \quad (1)$$

where qp_{net} is the effective heating value ($kJ \cdot kg^{-1}$), qp_{gross} is the calorific heating value ($kJ \cdot kg^{-1}$), 2.45 MJ kg^{-1} is the latent heat of vaporisation of water at 20 °C, 0.09 is a factor expressing that one part of hydrogen and eight parts of oxygen form nine parts of water, and H_2 is the hydrogen content of the oven-dried biomass.

3.4. Chemicals

The solvents used in the sample preparation of extractives were analytical-grade acetone (BDH), HPLC-grade *n*-hexane (VWR), methyl *tert*-butyl ether (Lab-Scan), pyridine (BDH), 95% ethanol (EtOH, >94%, ETAX A; Altia Corporation) and *n*-butanol (Merck).

Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) were obtained from Regis Technologies (Morton Grove, IL, USA) for silylation.

The compounds used as internal standards in the GC analysis of extractives were heneicosanoic acid (99%; Sigma-Aldrich Finland, Espoo, Finland) and betulinol (≥98%; Sigma-Aldrich Finland, Espoo, Finland), cholesteryl margarate (≥97%; TCI, Portland, OR, USA) and 1,3-dipalmitoyl-2-oleoylglycerol (≥99%; Sigma). NaOH (>98%; VWR), HCl (37%; VWR), Na_2CO_3 (≥99.8; Sigma-Aldrich Finland, Espoo, Finland), sulphuric acid (95–97%; Sigma-Aldrich Finland, Espoo, Finland), and bromocresol green (>95%; Sigma-Aldrich Finland, Espoo, Finland) were also used in the analysis.

Cysteamine (≥98%; Sigma-Aldrich Finland, Espoo, Finland), 37% aqueous hydrochloride (Thermo Fisher Scientific, Waltham, MA, USA) and HPLC-grade methanol (≥99.8%; VWR International, Helsinki, Finland) were used for the thiolysis of CTs. Then, CT degradation products, that is, free flavan-3-ols (terminal units) and their cysteaminyl derivatives (extension units), were quantified using external standards of catechin, epicatechin, gallocatechin and epigallocatechin (Sigma-Aldrich Finland) and thiolysed procyanidin B2 (Extrasynthese, Lyon, France). HPLC-grade acetonitrile (VWR International) and formic acid (≥98%; Sigma-Aldrich, Espoo, Finland) were used for HPLC determination of thiolysed CTs.

3.5. ASE Extraction

Bark samples were extracted with a Dionex Accelerated Solvent Extractor (Dionex, ASE 100, Sunnyvale, CA, USA) using *n*-hexane and water as solvents to extract lipophilic and hydrophilic extractives, respectively. The extraction temperature was set to 120 °C, with a static extraction time of 10 min, flush of extraction cell of 60%, nitrogen purge for 70 s and extraction pressure of 1500 psi. For each extraction procedure, 2 g of bark powder was loaded to a 34 mL extraction cell plugged with a cellulose filter. Each sample was first extracted with *n*-hexane and then with water, and the extractive-free bark was consequently lyophilised and stored for carbohydrate analysis. All extraction procedures were performed in duplicate for each sample.

3.6. Gravimetric Analysis of Total Dissolved Solids and Preparation of Stock Solutions

Overall, the TDSs of bark extracts were determined gravimetrically. The *n*-hexane extracts were evaporated to near dryness in a rotary evaporator and subsequently transferred to tared Kimax test tubes in acetone and finally evaporated to dryness under nitrogen flow. The weight of the dried extract was the TDS of the *n*-hexane extracts. A stock solution (100 mL) was then prepared by dissolving the extract in acetone.

Stock solutions of hydrophilic extracts were prepared by diluting the raw extract to 100 mL with ultra-high-quality (UHQ) water. Some of the stock solutions (10 mL) were lyophilised, and the TDS of the hydrophilic extracts was determined according to the weight of the lyophilised sample.

3.7. Analysis of Bark Extractives with Chromatographic Methods

3.7.1. Qualitative Analysis of Bark Extracts by Gas Chromatography with Mass Selective Detection (GC-MS)

To perform a qualitative analysis, 3 mg of extracts (based on dry weight) was dried (either by nitrogen flow or lyophilisation) and dissolved in 500 µL of pyridine and 300 µL of a silylation reagent (TMCS). The silylation process was accelerated by keeping the sample in a 70 °C oven for 1 h. The samples were then analysed using a Hewlett Packard 5973 GC-MS instrument equipped with an HP-5 column (30 m × 0.32 mm, with a 0.25 µm film). Next, the samples were injected at 290 °C and detected with a mass selective detector at 300 °C. Notably, the method used for the analysis was the same as in our previous study [10].

3.7.2. Quantitative Analysis of Bark Extracts by GC-FID

To perform a quantitative analysis, approximately 3 mg samples of bark extracts were dried with internal standards. The mixtures were then dissolved in 500 µL of pyridine and 300 µL of a silylation reagent and kept in an oven for 1 h.

To analyse the extractive groups, 100 µg of four internal standards was used: heneicosanoic acid, betulin, cholesteryl margarate and 1,3-dipalmitoyl-2-oleoylglycerol. An Agilent 6850 GC-FID instrument equipped with a short HP-1/simulated distillation column (7.5 m × 0.53 mm, with a 0.15 µm film) was used for the analysis. The samples were injected on-column at 90 °C and detected using FID at 320 °C. The temperature program used was the same as in our previous study [10].

To perform an individual extractive analysis, 100 µg of heneicosanoic acid and the same amount of betulin were added as internal standards. An Agilent 6850 GC-FID instrument equipped with a long HP-5 column (30 m × 0.32 mm, with a 0.25 µm film) was used for the analysis. The samples were then injected at 290 °C and detected at 300 °C. The temperature program used was the same as in our previous study [10].

To analyse the esterified lipophilic extractives, the samples were hydrolysed and derivatised for analysis as described by Halmemies et al. (2021) [10].

3.7.3. Analysis of Proanthocyanidins by High-Performance Liquid Chromatography (HPLC)

A thiolytic degradation method as described by Korkalo et al. was applied to determine CTs (proanthocyanidins) in the lyophilised bark samples [65]. First, a ground sample (10–20 mg) was mixed with 1 mL of a depolymerisation reagent (3 g of cysteamine dissolved in 56 mL of methanol acidified with 4 mL of 13 M HCl) and incubated for 60 min at 65 °C. During incubation, the samples were vortexed for a few seconds every 15 min. Thiolysis was stopped by transferring the samples into an ice bath. The cooled samples were then filtrated into HPLC vials and analysed on an Agilent 1290 Infinity UHPLC instrument equipped with a Zorbax Eclipse Plus C_{18} column (50 × 2.1 mm i.d., 1.8 m; Agilent Technologies, Santa Clara, CA, USA). The binary mobile phase consisted of 0.5% formic acid (aq.) and acetonitrile. Elution was started with 2% acetonitrile isocratically for 2 min, followed by a linear gradient to 5% in 3 min, to 15% in 7 min, to 20% in 3 min, to 35% in 5 min, to 90% in 1 min and back to the initial condition in 2 min. The post-time was 2 min before the next injection. The flow rate was 0.5 mL/min, and the injection volume was 2 µL. Elution was monitored using diode array detection (DAD; λ_1 = 270 nm, λ_2 = 280 nm) and fluorescence detection (FLD; λ_{ex} = 275 nm, λ_{em} = 324 nm).

3.8. Carbohydrate Analyses

Acid hydrolysis and acidic methanolysis were used to analyse the carbohydrate (holocellulose, cellulose and hemicelluloses) and acid-soluble and acid-insoluble lignin (see below) content of extractive-free bark. Holocellulose is defined as the sum of cellulosic and hemicellulosic carbohydrates. The holocellulose and lignin content was first determined using acid hydrolysis, and then the hemicellulose content was determined using acidic methanolysis. Then, the cellulose content of the samples was determined as the difference between holocellulose and hemicelluloses.

3.8.1. Acid Hydrolysis

Separation of holocellulose, acid-insoluble lignin and acid-soluble lignin from the extractive-free bark samples was performed according to the TAPPI standard T 222 [66]. For the acid hydrolysis samples, 200 mg of lyophilised extractive-free bark was weighed in a test tube. Then, around 4 mL of 72% cold sulphuric acid was added, and the test tubes were kept in a water bath at 30 °C for 1 h. Every 5 min, the mixtures were stirred with a glass rod. Next, the samples were transferred to 250 mL autoclave bottles, washed with 112 mL of UHQ water and then placed in an autoclave (MELAG Autoklav 23, Berlin, Germany) at a pressure of 1 bar (~121 °C) for 1 h.

Solid acid-insoluble lignin was then separated from the mixtures by filtration with a tared borosilicate glass filter (Munktell MGA 413004, Falun, Sweden) in a vacuum funnel. Insoluble lignin was gravimetrically determined by drying the residues together with the used filter papers (of known weight) in an oven at 105 °C to a constant weight. The filtrates were then diluted to 500 mL with UHQ water and consequently analysed with HPAEC for their holocellulose-derived monosaccharide content and with UV–Vis spectroscopy for their soluble lignin content.

3.8.2. High-Performance Anion-Exchange Chromatography (HPAEC) Analysis of Holocellulose-Derived Monosaccharides

First, HPAEC was used to analyse the monosaccharides formed during the acid hydrolysis from the 500 mL dilutions. Standard solutions for HPAEC were prepared using a sulphuric acid concentration corresponding to the samples' background: cold 72% sulphuric acid (3 mL) was diluted to 500 mL with UHQ water. Fucose (500 ppm) was used as an internal standard. The preparation of the standard solutions is described in detail in our previous study [10].

Bark samples (500 mL UHQ water dilution) from acid hydrolysis were analysed with HPAEC (Dionex) using 1 M sodium acetate, 0.5 M sodium acetate plus 0.1 M NaOH and 0.3 M NaOH solutions as eluents. The analytes were then separated in CarboPac PA1 + Quard PA1 columns and detected with an ED50 detector using carbohydrate pulsing. The post-column elute was pumped by an IC25 isocratic pump.

Samples for HPAEC analysis were prepared by pipetting 2 mL of an internal standard solution to a 20 mL volumetric flask and filling the flask with the diluted sample (500 mL) from the acid hydrolysis. This solution (1.0–1.5 mL) was then transferred into an HPLC vial by filtrating it through a syringe filter (Phenex-RC, 0.2 μm).

3.8.3. UV–Vis Measurement of Acid-Soluble Lignin

The amount of acid-soluble lignin was determined from the 500 mL dilution following acid hydrolysis via UV–Vis spectroscopy at 205 nm according to the TAPPI standard UM 250 using an extinction coefficient of 120 L/(g·cm) (for softwood) [67].

3.8.4. Acidic Methanolysis

The amount of hemicellulose in spruce bark samples was analysed from extractive-free lyophilised bark using acidic methanolysis. An internal standard solution was prepared by dissolving 10 mg of sorbitol into 100 mL of methanol. To prepare an external standard solution, a 10 mg mixture of arabinose, galactose, glucose, xylose, mannose, galacturonic acid and glucuronic acid was dissolved into 100 mL of UHQ water. Then, a methanolysis reagent was prepared by cooling 100 mL of methanol in an ice bath and carefully adding and mixing 16 mL of acetyl chloride into the cold methanol. Next, the reagent was stored at −20 °C.

For methanolysis, 2 mL of the methanolysis reagent was added to 2–3 mg of extractive-free bark samples and to a dried monosaccharide standard sample (1 mL). The samples were then sonicated in an ultrasound bath and kept at 100 °C in an oven for 3 h. Pyridine (80 μL) and an internal standard (1 mL) were next added to the samples, and the solvent was evaporated. Then, 80 μL of pyridine and 250 μL of a silylation reagent were added, and the samples were sonicated in an ultrasound bath and kept in a shaker at room temperature for 40 min. Next, the samples were filtrated with glass wool for GC analysis with an Agilent 6850 gas chromatograph equipped with an HP-5 column (30 m × 0.32 mm, with a 0.25 μm film). Finally, the samples were injected at 260 °C and detected by FID at 290 °C. The method used was the same as in our previous study [10].

3.9. Statistical Analysis

One-way analysis of variance (ANOVA) was used to assess the effect of storage time (0, 4, 12 or 24 weeks) and sampling location (side, middle or top of the pile) on the

concentrations of bark components, DP values of CTs, ash content and effective heating value. Logarithmic transformation was used for the variables that were sufficiently non-normal to cause concern about the validity of the normality assumption. In addition, the Kruskal–Wallis test, the non-parametric equivalent of one-way ANOVA, was used for the variables that were not normally distributed even after logarithmic transformation.

In turn, an independent-samples *t*-test was used to test the statistical differences in the concentrations, DP, ash content and net calorific value between the snow-covered and non-covered bark piles. Again, logarithmic transformation was used for the non-normal variables.

4. Conclusions

According to our study of bark storage in piles (both non-covered and covered with snow), spruce bark is a valuable raw material that is rich in hydrophilic extractives. However, it is worth noting that the material losses experienced as a result of pile storage (even during the winter) are dramatic, even after only a few weeks of storage. The loss of hydrophilic, phenolic extractive groups, such as stilbenes and tannins, was particularly notable. Significant proportions of the losses of extractives are to be attributed to the microbiological degradation and increase in pile temperature, which initiates and facilitates further degrading chemical reactions. In addition, exposure to UV light and the leaching of extractives from piles also cause losses of these compounds.

A clear trend was observed with regard to the sampling location in storage piles and the concentrations of the studied chemical compounds. In particular, both extractives and carbohydrates were found to have high concentrations in the middle of the pile with prolonged storage durations, indicating in some instances a leaching of compounds from elsewhere in the piles. Despite the covering of the other pile, no significant difference was observed between the degradation results of the non-covered and snow-covered bark piles. Other covering options and their impact on bark extractives should be further investigated.

From the results, it was evident that if piled bark material is to be valorised for its extractive content, storage periods should be as short as possible. Even four weeks of piled bark storage seems too long a time for stilbenes and tannin products. It was demonstrated that a simple hydrophilic extraction (e.g., with hot water) can effectively remove many potential platform chemicals for further purification steps. Methods, such as these, ought to be considered especially by bio-refinery plants that handle sidestream bark material. In addition, the logistics of bark material delivery to refineries needs to be planned in order to eliminate unnecessary exposure to weathering and moisture.

Supplementary Materials: The following supporting information can be downloaded online, Table S1: Values for Figure 1, Table S2: Values for Figure 3, Table S3: Values for Figure 4, Table S4: Values for Figure 5, Table S5: Values for Figure 6, Table S6: Values for Figure 7, Table S7: Values for Figure 8, Table S8: Values for Figure 9, Table S9: Values for Figure 10, Table S10: Values for Figure 11, Table S11: Values for Figure 12, Table S12: Values for Figure 13, Table S13: Values for Figure 14, Table S14: Values for Figure 15, Table S15: Values for Figure 16.

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Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples discussed in this article are not available for further study.

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Supporting material

Tabulated values of the results presented in the article.

Table S1. Values for Figure 1.

Bark Pile	UC [a]	UC	UC	UC	UC	UC	UC	UC	UC	UC	C [b]	C	C	C
Storage week	0	4M [c]	4S [d]	4T [e]	12M	12S	12T	24M	24S	24T	0	24M	24S	24T
Hot water extract (%)	33.5	20.3	17.4	14.6	16.4	11.5	8.9	14.3	9.2	7.3	30.6	13.7	5	5.7
Hot water extract SD	0.7	0.9	0.8	1.4	0.3	0.1	0.3	0.2	0.2	1.4	0.2	0.3	0.1	0
Hexane extract (%)	4.1	4.4	4.1	3.8	3.8	4	3.6	4.1	3.4	3	4.3	4.9	3.4	3.6
Hexane extract SD	0.01	0.2	0.001	0.2	0.1	0.1	0.01	0.04	0.04	0.1	0.002	0.03	0.1	0.03
Holocellulose (%)											35.1	34.8	40.4	38.2
Holocellulose SD											0.6	0.8	1.3	0.7
Cellulose (%)	17.2							17	15.7	15.3				
Cellulose SD	0.5							0.4	0.6	0.7				
Hemicellulose (%)	19.2	19.5	17.4	18.8	20.6	21.9	20.3	19.6	22.8	21.2				
Hemicellulose SD	0.3	0.004	0.6	1.5	1.5	2.6	0.8	0.1	0.2	2				
Lignin (acid-insoluble) (%)	16.8							35.8	39.8	44.4	17.4	33.8	37.6	35.9
Lignin (acid-insoluble) SD	0.4							0.4	1.6	2.5	0.8	0.4	1.2	1.3
Lignin (acid-soluble) (%)	0.7							0.7	0.8	0.8	0.7	0.7	1	0.8
Lignin (acid-soluble) SD	0.1							0.04	0.02	0.02	0.02	0.2	0.03	0.04
Unidentified (%)	8.5	55.8	61.1	62.8	59.2	62.7	67.2	8.4	8.3	8	11.9	12.1	12.6	15.7
Unidentified SD	1.7	1.1	1.4	0.04	1	2.8	0.5	0.2	0.5	1.7	0.2	0.4	1.1	1.4

[a] Uncovered [b] Covered with snow [c] Middle [d] Side [e] Top.

Table S2. Values for Figure 3.

Bark Pile	**UC** [a]	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**C** [b]	**C**	**C**	**C**
Storage week	0	4M [c]	4S [d]	4T [e]	12M	12S	12T	24M	24S	24T	0	24M	24S	24T
Resin acids (mg/g of d.m.)	14.1	10.3	8.6	8.8	8	7.5	9.1	8.5	7.5	8.8	12.2	9.7	9.6	8.1
Resin acids SD	2.2	0.5	0.2	0.1	0.2	0.1	0.03	0.02	0.2	0.3	0.3	0.2	0.2	0.01
Fatty acids (mg/g of d.m.)	9.5	8.1	7.4	7.3	8.7	4.2	4.9	8.4	2.8	2.1	10	8.9	3.4	6.5
Fatty acids SD	0.3	0.2	0.3	0.3	0.1	0.03	0.02	0.1	0.04	0.2	0.03	0.004	0.0004	0.4
Diterpenoids (mg/g of d.m.)	4.1	3.2	2.2	2.3	1.8	2	2.4	1.9	2.1	2.7	3.9	2.4	2.6	2.2
Diterpenoids SD	0.6	0.2	0.04	0.05	0.03	0.02	0.02	0.01	0.1	0.1	0.1	0.1	0.01	0.001
Sterols (mg/g of d.m.)	3.3	4.3	3.2	2.9	3.4	3.6	3.3	2.9	2.7	2.7	3.3	2.9	2.5	2.9
Sterols SD	0.1	0.2	0.2	0.2	0.3	0.3	0.1	0.3	0.1	0.1	0.02	0.1	0.01	0.1
Steryl esters (mg/g of d.m.)	0.6	0.5	0.4	0.4	1	0.5	0.6	1.7	0.5	0.7	1.1	1.5	0.8	0.7
Steryl esters SD	0.1	0.1	0.2	0.1	0.4	0.01	0.1	0.1	0.1	0.1	0.1	0.04	0.1	0.1
Triglycerides (mg/g of d.m.)	2.6	10.9	14.6	11.9	12.5	17.3	12.7	17.6	18.1	12.6	6.4	21.7	14.5	15.6
Triglycerides SD	2.5	0.7	0.7	1.1	0.2	0.5	1.6	0.4	0.7	0.2	0.2	0.2	0.5	0.1
Others (mg/g of d.m.)	2.6	3.6	2.6	2.3	2.5	2.5	2.4	2.1	1.3	0.9	2.7	2.1	1.3	1.9
Others SD	0.3	0.2	0.2	0.2	0.1	0.3	0.01	0.2	0.1	0.1	0.02	0.1	0.02	0.03
Unidentified (mg/g of d.m.)	5.9	4.3	3.4	3.5	2.7	2.1	1.7	1.1	0.3	0.3	5.8	2.3	0.4	0.7
Unidentified SD	0.1	0.001	0.1	0.1	0.02	0.1	0.6	0.1	0.01	0.1	0.2	0.05	0.1	0.03

[a] Uncovered [b] Covered with snow [c] Middle [d] Side [e] Top.

Table S3. Values for Figure 4.

Bark Pile	**UC** [a]	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**C** [b]	**C**	**C**	**C**
Storage week	0	4M [c]	4S [d]	4T [e]	12M	12S	12T	24M	24S	24T	0	24M	24S	24T
Sugars (mg/g of d.m.)	92.3	21.1	17.9	14.8	30.9	4.8	6.5	44.5	7.8	1.7	82	23.8	1.7	1.9
Sugars SD	9.5	1.9	2.3	0.9	1	0.3	0.7	4.4	0.5	1	10	0.2	0.2	0.04
Sugar alcohols (mg/g of d.m.)	8.1	22	9.3	8.6	6.5	1.6	2.7	10.3	2.2	1.1	8.1	12.7	1.1	0.9
Sugar alcohols SD	0.8	1.7	1	0.1	0.1	0.02	0.3	0.2	0.02	0.5	1.8	0.5	0.1	0.04
Organic acids (mg/g of d.m.)	23.6	15.9	10.6	7.5	16.1	2.9	3.6	25	4.2	1.3	18.8	18.6	1	1.3
Organic acids SD	0.5	1.6	1.4	0.2	0.1	0.1	0.6	0.7	0.1	0.7	0.3	0.2	0.02	0.1
Stilbenes (mg/g of d.m.)	19.4	2.7	0.7	0.7	0.5	0.4	0.4	0.3	0.2	0.2	23	0.3	0.2	0.2
Stilbenes SD	3.4	0.2	0.03	0.1	0.02	0.03	0.001	0.1	0.04	0.1	0.4	0.1	0.02	0.001
Sesquistilbenes (mg/g of d.m.)	16.4	4.3	4.8	3.6	6.1	3.1	4.3	1.1	1.6	0.6	20.9	0	0	0
Sesquistilbenes SD	0.1	0.1	0.5	1.3	0.8	0.9	0.001	0.1	0.5	0.2	2.4	0	0	0
Distilbenes (mg/g of d.m.)	16.9	5.1	4.2	3.8	5.3	3.4	3.9	2.1	2.4	2.1	14.7	0	0	0
Distilbenes SD	0.8	0.6	0.4	0.8	0.2	0.9	0.1	0.9	1.5	0.4	2.2	0	0	0
Flavonoids (mg/g of d.m.)	6.3	2.2	0.9	0.7	0.5	0.3	0.4	0.5	0.03	0.1	5.2	1	0.1	0.1
Flavonoids SD	0.9	0.1	0.1	0.1	0.03	0.02	0.02	0.004	0.04	0.1	1.8	0.2	0.02	0.02
Alcohols (mg/g of d.m.)	3.5	1.2	0.5	0.5	0.6	0.1	0.2	0.5	0.04	0.02	3.4	0.8	0.1	0.05
Alcohols SD	0.5	0.1	0.04	0.05	0.002	0.01	0.01	0.1	0.1	0.03	0.5	0.1	0.01	0.01
Others (mg/g of d.m.)	6.4	8.5	4.1	3.8	4	1.8	2.8	6	4	0.8	6.3	8.7	2	1.9
Others SD	1	0.5	0.5	0.1	0.1	0.1	1.2	2.2	3.4	0.4	0.6	5.2	0.4	0.1
Unidentified (mg/g of d.m.)	141.5	120.1	121.1	101.6	93.3	96.5	64.2	53.1	68.6	64.7	123.8	69.4	44.1	49.7
Unidentified SD	21.7	3.3	13.2	13.6	5.3	3.1	6	6.6	5.2	11.8	20.3	9.5	2.2	0.4

[a] Uncovered [b] Covered with snow [c] Middle [d] Side [e] Top

Table S4. Values for Figure 5.

Bark Pile	**UC** [a]	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**C** [b]	**C**	**C**	**C**
Storage week	0	4M [c]	4S [d]	4T [e]	12M	12S	12T	24M	24S	24T	0	24M	24S	24T
Dehydroabietic acid (mg/g of d.m.)	2.5	2.1	2.3	2.2	2.6	2.1	2.4	2.6	2.2	2.1	2.1	2.6	2.5	2.4
Dehydroabietic acid SD	0.4	0.1	0.1	0.1	0.1	0.02	0.004	0.01	0.1	0.1	0.1	0.04	0.03	0.1
Isopimaric acid (mg/g of d.m.)	2.1	1.6	1.7	1.7	1.4	1.5	2.3	1.6	1.5	2.7	1.9	1.6	2.7	1.7
Isopimaric acid SD	0.3	0.1	0.05	0.03	0.02	0.01	0.02	0.002	0.03	0.1	0.1	0.1	0.1	0.02
Levopimaric acid (mg/g of d.m.)	1.6	1	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.1	1.3	0.2	0.2	0.2
Levopimaric acid SD	0.2	0.03	0.01	0.02	0.005	0.001	0.01	0.002	0.01	0.01	0.01	0.03	0.01	0.02
Neoabietic acid (mg/g of d.m.)	1.6	1.2	0.4	0.5	0.2	0.3	0.4	0.2	0.3	0.4	1.3	0.3	0.4	0.3
Neoabietic acid SD	0.3	0.1	0.01	0.01	0.01	0.0003	0.005	0.01	0.01	0.02	0.1	0.1	0.01	0.03
Abietic acid (mg/g of d.m.)	1.5	1.1	1.1	1.2	1	0.8	1	1.1	0.7	0.7	1.3	1.6	0.9	0.9
Abietic acid SD	0.2	0.05	0.02	0.03	0.01	0.004	0.01	0.01	0.02	0.02	0.04	0.1	0.02	0.002
Palustric acid (mg/g of d.m.)	1.1	1.2	0.8	0.8	0.5	0.6	0.6	0.4	0.5	0.4	0.8	0.3	0.4	0.4
Palustric acid SD	0.2	0.1	0.01	0.01	0.01	0.005	0.0005	0.03	0.03	0.004	0.03	0.1	0.03	0.1
Hydroxydehydroabietic acid 1 (mg/g of d.m.)	0.6	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.5	0.5	0.4	0.3
Hydroxydehydroabietic acid 1 SD	0.1	0.01	0.01	0.0002	0.01	0.001	0.003	0.01	0.003	0.01	0.01	0.01	0.01	0.02
Sandaracopimaric acid (mg/g of d.m.)	0.5	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.4	0.4	0.3	0.3
Sandaracopimaric acid SD	0.1	0.02	0.01	0.01	0.01	0.002	0.001	0.001	0.01	0.01	0.01	0.01	0.002	0.002
Hydroxy resin acid (mg/g of d.m.)	0.5	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.6	0.2	0.1	0.1
Hydroxy resin acid SD	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.004	0.01	0.005	0.01	0.02	0.005	0.01
Hydroxydehydroabietic acid 2 (mg/g of d.m.)	0.4	0.2	0.1	0.1	0.1	0.2	0.1	0	0.2	0.1	0.4	0.2	0.1	0.1
Hydroxydehydroabietic acid 2 SD	0.1	0.005	0.004	0.001	0.01	0.01	0.01	0.005	0.003	0.01	0.02	0.01	0.0001	0.002
Pimaric acid (mg/g of d.m.)	0.3	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.2
Pimaric acid SD	0.03	0.01	0.003	0.01	0.004	0.0003	0.003	0.0005	0.02	0.03	0.04	0.1	0.004	0.004
Cupressic acid (mg/g of d.m.)	0.2	0.1	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.2
Cupressic acid SD	0.02	0.01	0.04	0.005	0.001	0.001	0.004	0.001	0.01	0.01	0.004	0.01	0.001	0.01
Imbricatolic acid	0.4	0.3	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.5	0.3	0.2	0.2

(mg/g of d.m.)														
Imbricatolic acid SD	0.04	0.01	0.01	0.01	0.004	0.02	0.002	0.0002	0.01	0.01	0.01	0.003	0.006	0.0001
7-Oxodehydroabietic acid (mg/g of d.m.)	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.2
7-Oxodehydroabietic acid SD	0.02	0.003	0.001	0.01	0.01	0.002	0.002	0.005	0.004	0.01	0.004	0.01	0.001	0.004
Secodehydroabietic acid (mg/g of d.m.)	0	0	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0	0.1	0.2	0.1
Secodehydroabietic acid SD	0.01	0.002	0.005	0.002	0.001	0.0002	0.001	0.001	0.002	0.01	0.002	0.001	0.01	0.002
4-Hydroxy cinnamic acid (mg/g of d.m.)	0	0.1	0.3	0.3	0.3	0.2	0.4	0.5	0.3	0.5	0.1	0.4	0.5	0.4
4-Hydroxy cinnamic acid SD	0.004	0.01	0.01	0.01	0.003	0.01	0.005	0.01	0.002	0.01	0.002	0.02	0.01	0.01
Other resin acids (mg/g of d.m.)	0.5	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.3	0.4	0.3	0.2
Other resin acids SD	0.1	0.02	0.005	0.03	0.03	0.03	0.001	0.02	0.005	0.01	0.01	0.01	0.01	0.01

[a] Uncovered [b] Covered with snow [c] Middle [d] Side [e] Top.

Table S5. Values for Figure 6.

Bark Pile	**UC** [a]	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**C** [b]	**C**	**C**	**C**
Storage week	0	4M [c]	4S [d]	4T [e]	12M	12S	12T	24M	24S	24T	0	24M	24S	24T
Acid 18:2 esters (mg/g of d.m.)	2.7	2.2	1.8	1.6	1.6	0.8	0.8	1.5	0.4	0.3	2.8	1.7	0.5	0.8
Acid 18:2 esters SD	0.05	0.1	0.1	0.1	0.02	0.01	0.002	0.1	0.01	0.03	0.005	0.02	0.01	0.1
Acid 18:1 esters (mg/g of d.m.)	2	1.8	1.4	1.3	1.6	0.7	0.6	1	0.2	0.2	2.1	1.1	0.3	0.4
Acid 18:1 esters SD	0.1	0.1	0.1	0.1	0.01	0.01	0.01	0.1	0.01	0.02	0.02	0.04	0.02	0.1
Acid 18:3 esters (mg/g of d.m.)	1.6	1.1	0.9	0.8	0.8	0.3	0.4	0.7	0.2	0.3	1.7	1.1	0.3	0.5
Acid 18:3 esters SD	0.01	0.1	0.1	0.03	0.02	0.003	0.03	0.1	0.1	0.04	0.01	0.02	0.03	0.04
Acid 22:0 esters (mg/g of d.m.)	0.8	0.2	0.2	0.2	0.2	0.2	0.2	0.5	0.3	0.2	0.9	0.6	0.2	0.6
Acid 22:0 esters SD	0.01	0.01	0.02	0.02	0.02	0.002	0.02	0.01	0.03	0.04	0.1	0.01	0.02	0.3
Acid 16:0 esters (mg/g of d.m.)	0.4	0.4	0.3	0.3	0.4	0.2	0.2	0.3	0.1	0.1	0.4	0.3	0.1	0.1
Acid 16:0 esters SD	0.04	0.02	0.02	0.02	0.004	0.001	0.002	0.03	0.002	0.01	0.01	0.01	0.01	0.02
Acid 18:1 (mg/g of d.m.)	0.3	0.4	0.6	0.7	0.7	0.5	0.6	0.7	0.3	0.2	0.2	0.6	0.4	0.9
Acid 18:1 SD	0.1	0.02	0.04	0.03	0.01	0.01	0.02	0.01	0.002	0.01	0.02	0.02	0.01	0.01
Acid 22:0 (mg/g of d.m.)	0.3	0.2	0.2	0.3	0.4	0.2	0.3	0.4	0.2	0.2	0.3	0.4	0.2	0.2
Acid 22:0 SD	0.001	0.003	0.004	0.02	0.01	0.001	0.02	0.01	0.002	0.01	0.004	0.01	0.02	0.0001
Acid 18:3 (mg/g of d.m.)	0.3	0.5	0.5	0.5	0.6	0.3	0.4	0.6	0.3	0.1	0.3	0.6	0.3	0.6
Acid 18:3 SD	0.01	0.01	0.02	0.002	0.002	0.01	0.03	0.01	0.05	0.001	0.01	0.02	0.02	0.01
Acid 17:0 esters (mg/g of d.m.)	0.3	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.03	0.04	0.3	0.1	0	0
Acid 17:0 esters SD	0.004	0.01	0.01	0.01	0.001	0.001	0.002	0.02	0.002	0.005	0.003	0.01	0.0001	0.01
Acid 18:0 (mg/g of d.m.)	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.2
Acid 18:0 SD	0.003	0.01	0.01	0.004	0.002	0.00004	0.0003	0.003	0.003	0.004	0.01	0.01	0.004	0.003
Acid 18:2 (mg/g of d.m.)	0.1	0.3	0.4	0.5	0.7	0.3	0.4	0.8	0.2	0.2	0.2	0.7	0.3	0.8
Acid 18:2 SD	0.004	0.001	0.005	0.0002	0.012	0.001	0.02	0.02	0.003	0.003	0.0002	0.02	0.01	0.002
Acid 24:0 esters (mg/g of d.m.)	0.1	0.1	0.2	0.2	0.3	0.2	0.3	0.6	0.2	0.1	0	0.5	0.1	0.6
Acid 24:0 esters	0.02	0.03	0.003	0.01	0.02	0.001	0.02	0.02	0.001	0.02	0	0.02	0.01	0.4

SD														
Acid 24:0 (mg/g of d.m.)	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.3	0.2	0.1	0.1
Acid 24:0 SD	0.037	0.004	0.002	0.03	0.01	0.002	0.0001	0.005	0.001	0.01	0.01	0.002	0.005	0.004
Acid 16:0 (mg/g of d.m.)	0.1	0.1	0.2	0.2	0.3	0.1	0.2	0.3	0.1	0.1	0.1	0.3	0.1	0.3
Acid 16:0 SD	0.002	0.01	0.01	0.001	0.005	0.002	0.005	0.01	0.001	0.003	0.001	0.01	0.002	0.001
Acid 17:0 (mg/g of d.m.)	0.04	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.05	0.03	0.04	0.1	0.1	0.2
Acid 17:0 SD	0.02	0.01	0.1	0.1	0.01	0.01	0.05	0.1	0.03	0.02	0.02	0.04	0.03	0.1
2-Hydroxy-24:0 acid (mg/g of d.m.)	0.1	0.1	0.2	0.2	0.3	0.1	0.2	0.4	0.1	0.1	0.1	0.3	0.1	0.2
2-Hydroxy-24:0 acid SD	0.1	0	0.03	0.04	0.01	0.003	0.03	0.01	0.01	0.002	0.005	0.01	0.001	0.001
Other fatty acids (mg/g of d.m.)	0.1	0.03	0.04	0.02	0.03	0.02	0.02	0.1	0.02	0.04	0.1	0.1	0.03	0.04
Other fatty acids SD	0.02	0.01	0.04	0.002	0.02	0.00003	0.0002	0.004	0.01	0.03	0.02	0.001	0.0001	0.002

[a] Uncovered [b] Covered with snow [c] Middle [d] Side [e] Top

Table S6. Values for Figure 7.

Bark Pile	**UC** [a]	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**C** [b]	**C**	**C**	**C**
Storage week	0	4M [c]	4S [d]	4T [e]	12M	12S	12T	24M	24S	24T	0	24M	24S	24T
Thunbergol (mg/g of d.m.)	1.3	1.0	0.3	0.3	0.1	0.3	0.2	0.1	0.3	0.2	1.2	0.3	0.2	0.3
Thunbergol SD	0.2	0.1	0.001	0.003	0.01	0.03	0.0003	0.01	0.02	0.004	0.03	0.02	0.003	0.01
Δ-13-(*trans)* neoabienol (mg/g of d.m.)	1.3	1.0	0.8	0.8	0.5	0.6	0.6	0.5	0.6	0.5	1.1	0.8	0.6	0.6
Δ-13-(*trans)* neoabienol SD	0.2	0.04	0.02	0.005	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.002	0.0004
Cis-abienol (mg/g of d.m.)	0.4	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.4	0.2	0.1	0.2
Cis-abienol SD	0.04	0.01	0.004	0.01	0.004	0.01	0.01	0.003	0.001	0.01	0.02	0.01	0.001	0.001
Isopimaral (mg/g of d.m.)	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.1
Isopimaral SD	0.1	0.01	0.004	0.01	0.002	0.01	0.01	0.003	0.05	0.01	0.001	0.02	0.01	0.01
Palustral (mg/g of d.m.)	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.2	0.1
Plaustral SD	0.02	0.01	0.003	0.01	0.003	0.0002	0.001	0.004	0.01	0.004	0.01	0.01	0.01	0.01
Isopimarol (mg/g of d.m.)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Isopimarol SD	0.1	0.01	0.004	0.01	0.002	0.01	0.01	0.003	0.05	0.01	0.001	0.02	0.01	0.01
Dehydroabietal (mg/g of d.m.)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Dehydroabietal SD	0.02	0.01	0.005	0.004	0.003	0.001	0.0004	0.001	0.003	0.001	0.01	0.003	0.00002	0.001
Pimaradiene (mg/g of d.m.)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Pimaradiene SD	0.02	0.01	0.003	0.004	0.004	0.002	0.001	0.002	0.0002	0.003	0.002	0.001	0.002	0.0002
Manool (mg/g of d.m.)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1
Manool SD	0.02	0.02	0.005	0.01	0.003	0.001	0.003	0.001	0.001	0.01	0.001	0.001	0.001	0.001
Epimanoyl oxide (mg/g of d.m.)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
Epimanoyl oxide SD	0.01	0.01	0.01	0.0003	0.001	0.001	0.002	0.001	0.001	0.003	0.0002	0.02	0.001	0.001
Abietal (mg/g of d.m.)	0.04	0.03	0.03	0.03	0.03	0.01	0.03	0.04	0.02	0.00	0.04	0.05	0.03	0.02
Abietal SD	0.003	0.002	0.002	0.0002	0.001	0.01	0.001	0.0001	0.002	0.0	0.001	0.003	0.00002	0.001
Thunbergene (mg/g of d.m.)	0.05	0.04	0.04	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.1	0.1	0.03	0.05
Thunbergene	0.01	0.01	0.002	0.01	0.003	0.001	0.001	0.002	0.01	0.004	0.01	0.001	0.01	0.003

SD														
Methyl-8,15-isopimaradien-18-oate (mg/g of d.m.)	0.1	0.0	0.1	0.1	0.1	0.2	0.4	0.1	0.2	0.7	0.04	0.04	0.6	0.2
Methyl-8,15-isopimaradien-18-oate SD	0.003	0.002	0.002	0.0002	0.001	0.01	0.001	0.0001	0.002	0.0	0.001	0.003	0.00002	0.001
Methyl neoabietate (mg/g of d.m.)	0.03	0.02	0.03	0.03	0.01	0.02	0.02	0.01	0.00	0.02	0.02	0.00	0.02	0.01
Methyl neoabietate SD	0.01	0.001	0.003	0.001	0.01	0.001	0.0003	0.0003	0.0	0.000	0.003	0.0	0.0001	0.003
Vanillin (mg/g of d.m.)	0.01	0.04	0.03	0.1	0.05	0.03	0.04	0.1	0.04	0.05	0.05	0.1	0.1	0.03
Vanillin SD	0.01	0.02	0.01	0.002	0.005	0.003	0.002	0.01	0.002	0.003	0.01	0.004	0.003	0.002

[a] Uncovered [b] Covered with snow [c] Middle [d] Side [e] Top

Table S7. Values for Figure 8.

Bark Pile	**UC** [a]	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**C** [b]	**C**	**C**	**C**
Storage week	0	4M [c]	4S [d]	4T [e]	12M	12S	12T	24M	24S	24T	0	24M	24S	24T
Sitosterol esters (mg/g of d.m.)	1.8	1.6	1.4	1.3	1.0	1.0	0.9	0.8	0.4	0.2	0.9	0.9	0.7	0.8
Sitosterol esters SD	0.1	0.04	0.2	0.1	0.02	0.04	0.01	0.02	0.1	0.02	0.1	0.01	0.04	0.02
Other steryl esters (mg/g of d.m.)	0.7	1.7	0.9	0.7	1.2	1.2	1.2	1.1	0.8	0.7	1.6	1.0	0.5	0.8
Other steryl esters SD	0.1	0.1	0.003	0.1	0.2	0.3	0.03	0.2	0.02	0.03	0.1	0.1	0.04	0.1
Campesterol esters (mg/g of d.m.)	0.4	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.1	0.0	0.2	0.2	0.1	0.2
Campesterol esters SD	0.02	0.01	0.04	0.02	0.02	0.01	0.01	0.02	0.02	0.01	0.02	0.001	0.01	0.1
Sitosterol (mg/g of d.m.)	0.3	0.4	0.4	0.4	0.5	0.6	0.5	0.5	0.7	0.8	0.4	0.5	0.6	0.6
Sitosterol SD	0.02	0.02	0.02	0.03	0.03	0.002	0.01	0.02	0.003	0.01	0.01	0.01	0.001	0.002
Campesterol (mg/g of d.m.)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1
Campesterol SD	0.005	0.003	0.001	0.004	0.02	0.001	0.003	0.003	0.002	0.001	0.001	0.01	0.002	0.01
24-Methylenecycloartan-3-one (mg/g of d.m.)	0.04	0.04	0.05	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.04	0.1	0.1	0.1
24-Methylenecycloartan-3-one SD	0.001	0.01	0.0004	0.001	0.001	0.002	0.003	0.003	0.002	0.002	0.0001	0.003	0.003	0.001
7-Hydroxysistosterol (mg/g of d.m.)	0.02	0.1	0.02	0.03	0.1	0.2	0.1	0.1	0.2	0.3	0.1	0.1	0.2	0.1
7-Hydroxysistosterol SD	0.005	0.001	0.0002	0.005	0.04	0.000004	0.03	0.0004	0.03	0.003	0.001	0.01	0.01	0.04
Chondrillasterol (mg/g of d.m.)	0.0	0.01	0.02	0.02	0.1	0.1	0.1	0.1	0.3	0.3	0.02	0.1	0.2	0.1
Chondrillasterol SD	0.0	0.01	0.004	0.01	0.03	0.01	0.02	0.02	0.01	0.003	0.003	0.001	0.004	0.01
Ergosterol (mg/g of d.m.)	0.02	0.02	0.02	0.01	0.03	0.04	0.03	0.01	0.04	0.03	0.02	0.02	0.02	0.01
Ergosterol SD	0.02	0.001	0.004	0.001	0.02	0.0001	0.01	0.001	0.002	0.002	0.0003	0.002	0.01	0.02

[a] Uncovered [b] Covered with snow [c] Middle [d] Side [e] Top

Table S8. Values for Figure 9.

Bark Pile	**UC** [a]	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**C** [b]	**C**	**C**	**C**
Storage week	0	4M [c]	4S [d]	4T [e]	12M	12S	12T	24M	24S	24T	0	24M	24S	24T
Glucose (mg/g of d.m.)	51.1	12.0	9.6	7.0	16.6	1.6	2.3	20.2	3.9	0.3	43.8	1.3	0.1	0.1
Glucose SD	3.2	1.2	1.4	0.8	0.4	0.2	0.3	1.5	0.01	0.1	3.2	0.3	0.01	0.01
Sucrose (mg/g of d.m.)	27.3	1.1	0.8	2.1	0.8	0.2	0.1	1.1	0.2	0.1	27.8	0.3	0.2	0.2
Sucrose SD	4.0	0.2	0.02	0.1	0.4	0.01	0.05	0.1	0.1	0.1	4.0	0.1	0.02	0.01
Maltose (mg/g of d.m.)	5.2	1.8	0.7	0.7	0.9	0.5	0.6	1.1	0.5	0.4	4.5	1.0	0.5	0.5
Maltose SD	0.6	0.2	0.1	0.02	0.1	0.01	0.01	0.3	0.05	0.2	0.6	0.2	0.1	0.004
Alpha lactose (mg/g of d.m.)	2.7	0.7	0.6	0.3	0.2	0.1	0.05	0.1	0.1	0.03	2.4	0.1	0.05	0.04
Alpha lactose SD	0.4	0.1	0.1	0.003	0.0002	0.03	0.01	0.1	0.1	0.04	0.3	0.02	0.01	0.001
Galactose (mg/g of d.m.)	2.0	1.9	2.5	2.7	10.3	1.7	2.7	19.1	2.4	0.5	1.2	17.0	0.5	0.6
Galactose SD	0.6	0.2	0.3	0.1	0.2	0.1	0.4	2.2	0.2	0.3	0.4	0.7	0.03	0.01
Trehalose (mg/g of d.m.)	1.6	0.6	0.8	0.6	0.3	0.2	0.1	0.2	0.3	0.1	1.1	0.2	0.2	0.1
Trehalose SD	0.3	0.02	0.1	0.01	0.03	0.01	0.002	0.1	0.1	0.2	0.1	0.1	0.03	0.001
Palatinose (mg/g of d.m.)	1.1	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0
Palatinose SD	0.1	0.01	0.01	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Cellobiose (mg/g of d.m.)	0.7	0.1	0.1	0.1	0.2	0.1	0.0	0.2	0.04	0.03	0.4	0.1	0.03	0.04
Cellobiose SD	0.3	0.002	0.03	0.0001	0.003	0.004	0.0	0.03	0.1	0.04	0.01	0.02	0.004	0.0005
Lactulose (mg/g of d.m.)	0.4	0.3	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.4	0.1	0.01	0.0
Lactulose SD	0.1	0.02	0.003	0.003	0.01	0.01	0.01	0.01	0.01	0.0	0.1	0.01	0.001	0.0
Glucose phosphate (mg/g of d.m.)	0.2	0.6	0.5	0.1	0.2	0.1	0.1	0.2	0.02	0.1	0.5	0.4	0.1	0.1
Glucose phosphate SD	0.3	0.1	0.1	0.01	0.002	0.01	0.01	0.002	0.04	0.01	0.04	0.1	0.004	0.003
Mannose (mg/g of d.m.)	0.0	1.8	2.1	1.0	1.5	0.4	0.5	2.3	0.4	0.1	0.0	3.4	0.1	0.1
Mannose SD	0.0	0.2	0.3	0.05	0.03	0.01	0.1	0.2	0.02	0.02	0.0	0.1	0.04	0.01

[a] Uncovered [b] Covered with snow [c] Middle [d] Side [e] Top

Table S9. Values for Figure 10.

Bark Pile	**UC** [a]	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**C** [b]	**C**	**C**	**C**
Storage week	0	4M [c]	4S [d]	4T [e]	12M	12S	12T	24M	24S	24T	0	24M	24S	24T
Pinitol (mg/g of d.m.)	5.0	4.8	1.9	1.6	2.0	0.4	0.7	3.4	0.4	0.1	4.8	4.6	0.2	0.1
Pinitol SD	0.8	0.4	0.2	0.03	0.05	0.01	0.02	0.2	0.02	0.03	0.7	0.01	0.03	0.01
Maltotriitol (mg/g of d.m.)	1.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	1.3	0.2	0.2	0.1
Maltotriitol SD	0.04	0.002	0.01	0.001	0.02	0.002	0.003	0.1	0.0	0.0	0.4	0.04	0.01	0.004
Inositol (mg/g of d.m.)	0.6	6.0	1.9	1.2	0.4	0.1	0.1	0.6	0.1	0.05	0.5	0.5	0.02	0.02
Inositol SD	0.02	0.4	0.02	0.1	0.0001	0.004	0.005	0.03	0.001	0.002	0.1	0.02	0.001	0.001
Sorbitol (mg/g of d.m.)	0.4	1.4	0.4	0.5	0.5	0.1	0.2	0.7	0.2	0.1	0.4	1.2	0.1	0.04
Sorbitol SD	0.03	0.1	0.1	0.01	0.03	0.003	0.004	0.02	0.02	0.02	0.1	0.1	0.001	0.003
Arabitol (mg/g of d.m.)	0.4	1.9	0.8	0.7	1.4	0.2	0.4	2.3	0.5	0.3	0.7	1.6	0.3	0.2
Arabitol SD	0.02	0.2	0.1	0.03	0.002	0.02	0.1	0.1	0.1	0.1	0.5	0.01	0.003	0.01
Isomaltitol (mg/g of d.m.)	0.3	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.3	0.1	0.1	0.1
Isomaltitol SD	0.04	0.0001	0.004	0.0	0.0	0.0	0.02	0.01	0.0	0.02	0.1	0.2	0.01	0.01
Mannitol (mg/g of d.m.)	0.3	6.6	3.1	3.5	1.0	0.4	0.5	1.5	0.5	0.2	0.2	2.6	0.05	0.03
Mannitol SD	0.03	0.6	0.5	0.2	0.02	0.01	0.002	0.1	0.03	0.1	0.02	0.2	0.01	0.001
L-Ribulose (mg/g of d.m.)	0.1	0.7	0.6	0.5	0.6	0.2	0.3	1.0	0.3	0.3	0.0	1.5	0.2	0.2
L-Ribulose SD	0.1	0.1	0.1	0.01	0.01	0.005	0.04	0.02	0.02	0.2	0.0	0.05	0.002	0.02
Erythritol (mg/g of d.m.)	0.0	0.2	0.4	0.4	0.5	0.2	0.3	0.7	0.3	0.0	0.0	0.6	0.03	0.04
Erythritol SD	0.0	0.02	0.1	0.1	0.1	0.01	0.1	0.2	0.1	0.0	0.0	0.3	0.001	0.006
Maltitol (mg/g of d.m.)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.04	0.01	0.02
Maltitol SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.02	0.001	0.02

[a] Uncovered [b] Covered with snow [c] Middle [d] Side [e] Top

Table S10. Values for Figure 11.

Bark Pile	**UC** [a]	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**C** [b]	**C**	**C**	**C**
Storage week	0	4M [c]	4S [d]	4T [e]	12M	12S	12T	24M	24S	24T	0	24M	24S	24T
Gluconic acid (mg/g of d.m.)	7.0	6.9	3.3	2.8	5.0	1.1	1.4	7.2	1.6	0.5	5.4	6.1	0.4	0.6
Gluconic acid SD	0.7	0.8	0.6	0.1	0.1	0.02	0.1	0.5	0.1	0.1	0.4	0.4	0.005	0.04
Citric acid (mg/g of d.m.)	6.7	1.4	1.7	0.7	0.7	0.1	0.2	1.0	0.2	0.04	5.0	0.4	0.02	0.02
Citric acid SD	0.8	0.1	0.3	0.1	0.02	0.001	0.01	0.01	0.02	0.1	0.3	0.1	0.001	0.002
Quinic acid (mg/g of d.m.)	7.1	3.6	1.7	1.0	1.9	0.1	0.3	2.8	0.3	0.0	6.3	0.9	0.003	0.0
Quinic acid SD	0.8	0.3	0.1	0.1	0.1	0.0003	0.1	0.2	0.004	0.0	0.1	0.04	0.005	0.0
Shikimic acid (mg/g of d.m.)	1.1	1.5	0.4	0.4	1.1	0.2	0.3	1.0	0.4	0.1	1.1	1.0	0.05	0.1
Shikimic acid SD	0.02	0.2	0.1	0.004	0.4	0.001	0.02	0.03	0.02	0.1	0.04	0.01	0.004	0.004
Malic acid (mg/g of d.m.)	0.8	0.4	0.2	0.1	0.2	0.0	0.0	0.4	0.03	0.05	0.7	0.2	0.04	0.1
Malic acid SD	0.1	0.1	0.02	0.0003	0.03	0.0	0.0	0.03	0.05	0.1	0.1	0.02	0.003	0.01
L-Glutamic acid (mg/g of d.m.)	0.3	1.2	1.5	1.2	5.9	0.8	1.0	11.9	1.4	0.4	0.3	8.0	0.1	0.1
L-Glutamic acid SD	0.1	0.1	0.2	0.1	0.4	0.03	0.3	1.4	0.02	0.4	0.2	0.3	0.002	0.01
2,3-Dihydroxypropanoic acid (mg/g of d.m.)	0.4	0.1	0.3	0.3	0.3	0.3	0.2	0.3	0.1	0.1	0.0	0.4	0.1	0.2
2,3-Dihydroxypropanoic acid SD	0.3	0.02	0.04	0.01	0.01	0.01	0.02	0.02	0.002	0.005	0.0	0.04	0.005	0.1
2,3-Dihydroxysuccinic acid (mg/g of d.m.)	0.0	0.1	0.2	0.3	0.4	0.1	0.1	0.6	0.1	0.03	0.0	0.4	0.02	0.02
2,3-Dihydroxysuccinic acid SD	0.0	0.01	0.03	0.01	0.01	0.002	0.03	0.003	0.001	0.04	0.0	0.04	0.001	0.002
Other (mg/g of d.m.)	0.2	0.7	0.9	0.7	0.6	0.2	0.1	0.8	0.1	0.1	0.0	1.2	0.3	0.2
Other SD	0.01	0.04	0.04	0.1	0.02	0.003	0.03	0.01	0.03	0.1	0.0	0.02	0.02	0.01

[a] Uncovered [b] Covered with snow [c] Middle [d] Side [e] Top

Table S11. Values for Figure 12.

Bark Pile	**UC** [a]	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**C** [b]	**C**	**C**	**C**
Storage week	0	4M [c]	4S [d]	4T [e]	12M	12S	12T	24M	24S	24T	0	24M	24S	24T
Distilbenes (mg/g of d.m.)	16.9	5.1	4.2	3.8	5.3	3.4	3.9	2.1	2.4	2.1	14.7	0.0	0.0	0.0
Distilbenes SD	0.8	0.6	0.4	0.8	0.2	0.9	0.1	0.9	1.5	0.4	2.2	0.0	0.0	0.0
Sesquistilbenes (mg/g of d.m.)	16.4	4.3	4.8	3.6	6.1	3.1	4.3	1.1	1.6	0.6	20.9	0.0	0.0	0.0
Sesquistilbenes SD	0.1	0.1	0.5	1.3	0.8	0.9	0.001	0.1	0.5	0.2	2.4	0.0	0.0	0.0
Isorhapontin (mg/g of d.m.)	9.5	0.2	0.1	0.05	0.04	0.1	0.03	0.0	0.0	0.0	11.4	0.0	0.0	0.0
Isorhapontin SD	1.7	0.1	0.003	0.003	0.001	0.0004	0.001	0.0	0.0	0.0	0.4	0.0	0.0	0.0
Astringin (mg/g of d.m.)	4.8	0.1	0.1	0.04	0.05	0.1	0.03	0.0	0.0	0.0	5.2	0.0	0.0	0.0
Astringin SD	1.1	0.1	0.1	0.01	0.005	0.01	0.003	0.0	0.0	0.0	0.3	0.0	0.0	0.0
Piceid (mg/g of d.m.)	3.2	0.1	0.1	0.05	0.1	0.04	0.04	0.0	0.0	0.0	4.3	0.0	0.0	0.0
Piceid SD	0.7	0.1	0.01	0.01	0.002	0.004	0.002	0.0	0.0	0.0	0.2	0.0	0.0	0.0
Rhapontigenin (mg/g of d.m.)	1.3	0.5	0.1	0.1	0.1	0.1	0.04	0.02	0.02	0.02	1.6	0.05	0.04	0.04
Rhapontigenin SD	0.2	0.02	0.001	0.001	0.005	0.01	0.002	0.03	0.03	0.03	0.4	0.02	0.01	0.003
Piceatannol (mg/g of d.m.)	0.4	0.9	0.3	0.4	0.1	0.1	0.1	0.2	0.1	0.1	0.3	0.1	0.05	0.1
Piceatannol SD	0.03	0.04	0.03	0.03	0.003	0.01	0.001	0.05	0.004	0.1	0.1	0.03	0.003	0.0002
Resveratrol (mg/g of d.m.)	0.2	0.9	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.04	0.2	0.1	0.1	0.1
Resveratrol SD	0.01	0.1	0.01	0.005	0.002	0.01	0.0003	0.02	0.01	0.1	0.02	0.02	0.01	0.003

[a] Uncovered [b] Covered with snow [c] Middle [d] Side [e] Top

Table S12. Values for Figure 13.

Bark Pile	**UC** [a]	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**C** [b]	**C**	**C**	**C**
Storage week	0	4M [c]	4S [d]	4T [e]	12M	12S	12T	24M	24S	24T	0	24M	24S	24T
Taxifolin glycoside (mg/g of d.m.)	1.5	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0
Taxifolin glycoside SD	0.05	0.04	0.01	0.003	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0
Naringin (mg/g of d.m.)	1.4	0.5	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0
Naringin SD	0.5	0.03	0.1	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0
Catechin (mg/g of d.m.)	1.1	0.3	0.0	0.1	0.03	0.0	0.0	0.0	0.0	0.0	1.0	0.04	0.02	0.02
Catechin SD	0.1	0.04	0.0	0.003	0.04	0.0	0.0	0.0	0.0	0.0	0.2	0.02	0.002	0.0002
Taxifolin (mg/g of d.m.)	1.0	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.9	0.2	0.04	0.05
Taxifolin SD	0.2	0.01	0.003	0.001	0.002	0.0002	0.01	0.04	0.0	0.0	0.2	0.02	0.003	0.004
Neohesperidin (mg/g of d.m.)	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0
Neohesperidin SD	0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0
Naringenin chalcone (mg/g of d.m.)	0.3	0.4	0.4	0.2	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.3	0.03	0.02
Naringenin chalcone SD	0.03	0.02	0.02	0.1	0.0004	0.01	0.03	0.01	0.0	0.0	0.0	0.1	0.004	0.004
Dihydromyricetin (mg/g of d.m.)	0.3	0.6	0.2	0.2	0.2	0.1	0.1	0.2	0.03	0.1	0.1	0.5	0.04	0.1
Dihydromyricetin SD	0.03	0.1	0.02	0.001	0.01	0.01	0.01	0.1	0.04	0.1	0.1	0.1	0.01	0.01
Luteolin (mg/g of d.m.)	0.0	0.0	0.0	0.1	0.04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Luteolin SD	0.0	0.0	0.0	0.0003	0.003	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Uncovered [b] Covered with snow [c] Middle [d] Side [e] Top

Table S13. Values for Figure 14.

Bark Pile	**UC** [a]	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**C** [b]	**C**	**C**	**C**
Storage week	0	4M [c]	4S [d]	4T [e]	12M	12S	12T	24M	24S	24T	0	24M	24S	24T
Procyanidins (g/100g of d.m.)	2.8	1.3	0.6	0.5	0.4	0.4	0.3	0.3	0.3	0.2	2.9	0.5	0.2	0.3
Procyanidins SD	0.2	0.1	0.03	0.04	0.02	0.04	0.03	0.02	0.03	0.02	0.1	0.05	0.02	0.02
Prodelphinidins (g/100g of d.m.)	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.04	0.03	0.3	0.1	0.04	0.04
Prodelphinidins SD	0.02	0.01	0.01	0.003	0.01	0.01	0.01	0.005	0.004	0.003	0.01	0.01	0.002	0.003
Degree of polymerisation	8.0	5.7	2.8	2.6	2.4	2.5	2.4	2.2	2.1	2.3	8.2	2.9	2.6	3.0
Degree of polymerisation SD	0.2	0.3	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.3	0.2	0.2	0.3

[a] Uncovered [b] Covered with snow [c] Middle [d] Side [e] Top

Table S14. Values for Figure 15.

Bark Pile	**UC** [a]	**UC**	**UC**	**UC**	**C** [b]	**C**	**C**	**C**
Storage week	0	24M [c]	24S [d]	24T [e]	0	24M	24S	24T
Glucose (% of d.m.)	38.5	34.1	31.8	29.9	35.3	31.2	30.0	30.7
Glucose SD	0.6	0.5	0.6	0.6	0.6	0.7	1.0	0.6
Arabinose (% of d.m.)	7.8	1.6	2.9	2.3	7.6	3.3	2.5	3.6
Arabinose SD	0.2	0.02	0.02	0.1	0.1	0.1	0.1	0.1
Xylose (% of d.m.)	4.8	3.8	3.7	3.5	4.4	3.5	3.1	3.2
Xylose SD	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.1
Mannose (% of d.m.)	4.3	3.7	4.0	3.3	4.2	2.7	2.7	2.9
Mannose SD	0.1	0.3	0.1	0.1	0.3	0.1	0.1	0.1
Galactose (% of d.m.)	2.8	1.7	1.8	1.8	2.5	2.0	1.7	1.8
Galactose SD	0.05	0.02	0.05	0.1	0.05	0.1	0.1	0.05

[a] Uncovered [b] Covered with snow [c] Middle [d] Side [e] Top

Table S15. Values for Figure 16.

Bark Pile	**UC** [a]	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**	**UC**
Storage week	0	4M [c]	4S [d]	4T [e]	12M	12S	12T	24M	24S	24T
Galacturonic acid (mg/g of d.m.)	93.9	86.9	83.1	80.8	79.8	59.4	42.2	43.6	42.9	44.0
Galacturonic acid SD	7.3	12.0	2.3	0.5	8.5	26.5	3.2	0.04	5.0	2.0
Arabinose (mg/g of d.m.)	69.4	54.1	32.9	31.3	28.1	28.3	44.0	23.4	29.6	37.6
Arabinose SD	0.4	0.2	2.0	0.3	6.9	5.3	8.3	1.3	4.0	0.2
Xylose (mg/g of d.m.)	39.2	30.3	29.3	29.1	44.4	41.3	49.4	52.6	48.0	50.9
Xylose SD	1.8	4.7	4.7	0.6	5.8	9.5	2.7	0.6	4.2	0.9
Galactose (mg/g of d.m.)	38.2	28.4	20.0	18.8	22.6	25.6	34.1	33.3	31.7	34.0
Galactose SD	0.8	4.7	1.3	1.3	3.1	9.0	3.5	1.4	3.4	0.4
Glucose (mg/g of d.m.)	30.0	31.9	39.1	38.3	49.0	45.2	46.2	53.5	54.2	54.3
Glucose SD	0.6	1.1	3.5	4.4	6.1	7.7	3.3	1.8	4.7	0.9
Mannose (mg/g of d.m.)	23.0	16.7	19.1	16.2	23.5	25.4	28.8	26.7	22.5	31.8
Mannose SD	3.3	1.6	5.1	3.2	1.0	5.0	1.2	1.4	1.0	5.0
Rhamnose (mg/g of d.m.)	9.4	7.7	5.7	6.0	7.8	5.5	7.2	5.4	6.0	6.8
Rhamnose SD	0.2	0.1	0.4	0.7	2.3	0.9	1.3	0.2	0.9	0.2
Glucuronic acid (mg/g of d.m.)	4.0	3.3	1.1	1.0	2.6	1.5	6.6	1.9	1.6	1.3
Glucuronic acid SD	0.9	0.04	0.4	0.3	1.8	0.1	7.3	1.0	0.3	0.3

[a] Uncovered [b] Covered with snow [c] Middle [d] Side [e] Top

III

AVAILABILITY OF EXTRACTIVES FROM VARIOUS NORWAY SPRUCE (*PICEA ABIES*) STUMPS ASSORTMENTS

by

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Availability of extractives from various Norway spruce (*Picea abies*) stumps assortments

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ABSTRACT

Stumps and knotwood of Norway spruce (*Picea abies*) are valuable sources of wood extractives. Although lignans from knotwood have already been utilized in value-added products, the behavior and valorization of stump-derived extractives are less studied. In this study, the composition of lipophilic and hydrophilic extractives, particularly lignans, from various spruce stump samples (stump bottom, stump heart, and crushed stump samples) stored outside were studied. Lipophilic and hydrophilic extracts were separated with an accelerated solvent extraction (ASE) apparatus using *n*-hexane and hot water, respectively. The detailed extractives content of samples was then determined by gas chromatography equipped with a flame ionization detector and a mass detector (GC-FID/MS) and high-performance liquid chromatography (HPLC). In stump bottom samples, an apparent decrease in total dissolved solids was observed in all the major extractives groups during storage: lignans, sugars, stilbene–glucosides, organic acids, resin acids, fatty acids, diterpenoids, and sterols. While a definitive decrease in extractives could not be demonstrated due to the moderately high variation of extractives among different samples, a good indication of the accessibility of important extractives in weathered stumps was obtained. Of the identified hydrophilic extractives, 79% were lignans, 53% of them being composed of 7-hydroxymatairesinol (HMR), 16% conidendric acid, and 12% todolactol. After 12 weeks of storage, the total amount of lignans was 15.3 mg/g of dry matter in stump bottom, 17.0 mg/g of dry matter in stump heart samples, and 10.2 mg/g of dry matter in crushed stump samples.

KEYWORDS

Lignan; 7-hydroxymatairesinol; high-performance liquid chromatography; gas chromatography-mass spectrometry; stump extractives; Norway spruce

Introduction

In recent years, biomaterials containing high contents of extractives, such as forest industry side-stream wood bark, are commonly used for producing energy, but their utilization for other purposes is also gradually increasing. Traditionally, the extractives from aged and pine trees (especially stumps) comprise an interesting substance group, which has been utilized, for example, to prepare tar in the Nordic countries.[1] This manufacturing process was started in the forest three to four years before the actual production in the tar pit by removing bark from pine trees, thus inducing excessive oleoresin formation of the trees.[2] In the case of pine stumps, the stump hearts may become increasingly resin-hardened and thus rot-resistant after felling.[3] This has historically made them especially favorable materials for tar production. However, this kind of resin saturation over time does not seem to occur readily in spruce stumps. Hence, the critical question is if there is a good rationale for the recovery and further utilization of spruce stump extractives, and is it worth the cost.

The knotwood of many wood species has a high concentration of valuable extractives. For example, the knot wood of aspen has been found to contain increased amounts of flavonoids,[4] and the knotwood of pine lumber has been found to contain ten times higher terpenoid concentration compared to sapwood.[5] Similarly, the knotwood of Norway spruce (*Picea abies)* is known to be saturated by the fraction of lignans, the most prominent compound being 7-hydroxymatairesinol (HMR).[6] However, *P. abies* stumps are also known to contain high levels of lignans and stilbene–glucosides.[7] Lignans are natural

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polyphenolic antioxidants that have been attributed with various health benefits, such as anti-breast cancer and prostate cancer activity.[8,9] The prospect of utilizing leftover Norway spruce stumps as feedstock materials, similarly to how spruce knotwood is already being used, for producing value-added chemicals, offers an attractive possibility.

However, the development of spruce stump extractives-based products requires a broad chemical understanding of extractable compounds, together with the influence of stump aging on the content of extractives, which are of primary importance. The degradation of spruce bark, forest residues, and stumps was studied in the EU-funded research project called BioHub. This project's general aim was to understand better the effects of storage on the chemical composition of various forest industrial sidestreams and, consequently, to find ways to improve their procurement practices.

The main goal of this study was to increase the general knowledge and provide a broad picture of extractives behavior during stump storage by investigating the effects of outside storage on the chemical composition of various stump assortments. Our primary focus was on the most prominent compound fraction of stumps, namely, lignans.

Materials and methods

Storage studies and sampling

A mature, Norway spruce-dominated stand was clear-cut in Kannus, Finland, in early May 2017. The tree stumps were extracted and split into 2–3 segments with a standard stump rake attached to an excavator. This was followed by an immediate forwarding to a landing for storage during May 15–19, 2017. Stump segments were placed in $3 \times 10 \times 2.5$ m ($W \times L \times H$) piles. Care was used not to contaminate stump sections with the underlying soil by placing them on an older set of stumps. Three types of samples were collected: (a) crushed stump, (b) stump heart, and (c) stump bottom (Figure 1). Collection times were at the initiation of the study (zero-sample, taken on May 23–24, 2017), 4, 12, and 25 weeks after the initiation. The bottom and heart samples were collected using a chain saw without bar oil at each sampling time. These samples were debarked and comminuted to a smaller particle size with a 5.6 kW Murray Mulch Maker. The crushed stump samples were stored as whole until sampling and then crushed with a 708 kW Vermeer HG6800TX horizontal grinder. After comminution, all samples were closed in plastic bags and a cooler box for immediate transport to the laboratory cold storage at −20 °C for chemical analysis.

Sample pretreatment and basic characterization

The moisture content of fresh stump samples was determined by a standard method (CEN/TS 14774-2:2004).[10] The stump samples were dried at a temperature of 105 °C until a constant mass was achieved. All the measurements were performed in duplicate.

The stumps were lyophilized (for three days) and ground with a Retsch SM 100 cutting laboratory mill (Retsch GmbH, Haan, Germany) equipped with a bottom sieve with trapezoidal holes (perforation size <1.0 mm) for chemical analysis. Samples were stored in a frozen state (<−20 °C). The dry matter content of each lyophilized stump sample was determined by drying about 1 g of stump powder in a tared crucible in an oven at 105 °C oven overnight.

Chemicals

The solvents used in the sample preparation of extractives were analytical grade acetone (BDH), *n*-butanol (Merck), high-performance liquid chromatography (HPLC)-grade *n*-hexane (VWR), methyl *tert*-butyl ether (MTBE, Lab-Scan), pyridine (BDH), and 95% ethanol (EtOH, >94%, ETAX A, Altia Corporation). The silylation reagents, bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS), were from Regis Technologies. HPLC-grade methanol (MeOH, Merck), HPLC-grade acetonitrile (VWR International), formic acid (≥98%; Sigma-Aldrich, Espoo, Finland), and *trans*-polydatin (99%, PhytoLab) were used in the HPLC analysis stump samples.

The compounds used as internal standards in the gas chromatography (GC) analysis of extractives were heneicosanoic acid (99%, Sigma), betulinol (≥98%, Sigma), cholesteryl margarate (≥97%, TCI America), and 1,3-dipalmitoyl-2-oleylglycerol (≥99%, Sigma). Other chemicals used in the analyses were NaOH (>98%, VWR), HCl (37%, VWR), Na_2CO_3 (≥99.8%, Sigma), H_2SO_4 (95–97%, Sigma), and bromocresol green (>95%, Sigma).

Separation of stump extractives

The extractions of stump samples were made via a Dionex Accelerated Solvent Extractor (ASE 100) using *n*-hexane and water as solvents to extract lipophilic and hydrophilic extractives, respectively. The extraction temperature was 120 °C, static extraction time

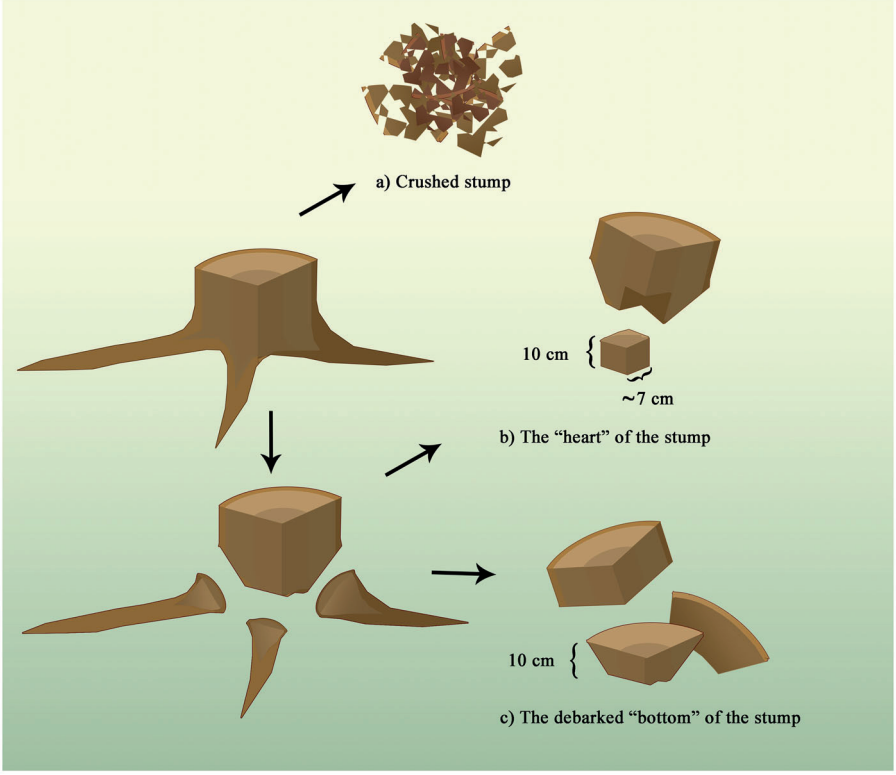

Figure 1. Preparation of the different stump samples for chemical analysis. (a) Crushed stump samples were prepared directly from pre-split stumps with roots intact. (b) The stump heart samples were cut as 10 cm × 7 cm × 7 cm pieces from the de-rooted stump, while (c) the stump bottom samples were cut at ~10 cm height from the bottom of the de-rooted stump and debarked.

was 10 min, extraction cell flush was 60%, nitrogen purge was 70 s, and extraction pressure was 1500 psi. Approximately, 2 g of dried stump powder was loaded to a 34-mL extraction cell plugged with a cellulose filter for each extraction. Each sample was first extracted with *n*-hexane and then with water. The extractions were performed in duplicates for each sample.

Gravimetric analysis of total dissolved solids and stock solutions

The total dissolved solids (TDS) of stump extracts were determined gravimetrically. The *n*-hexane extracts were evaporated to near dryness in a rotary evaporator, transferred to tared Kimax test tubes in acetone, and finally evaporated to dryness under nitrogen flow. The mass of the dried extract was the TDS of *n*-hexane extracts. Stock solution (100 mL) of the lipophilic extract was then prepared by dissolving the dried extract in acetone.

Stock solutions of the hydrophilic extract were prepared by diluting the raw extract to 100 mL with ultra-high quality (UHQ) water. Then, 10 mL of the stock solutions was lyophilized and the TDS of the hydrophilic extracts was determined based on the lyophilized sample mass.

Chromatographic analysis methods

Qualitative analysis by GC-MS

For qualitative analysis, 3 mg of extracts (based on dry mass) were dried (either by nitrogen flow or lyophilization) and dissolved in 500 μL of pyridine and 300 μL of the silylation reagent (BSTFA/TMCS (95/5, vol/vol)). The silylation was accelerated by keeping the sample in an oven at 70 °C for 1 h. The sample was then analyzed by GC-MS using an HP-5 column (30 m × 0.32 mm, with 0.25 μm film), injecting the sample at 290 °C and detecting the compounds with a mass selective detector (EI) at 300 °C. The temperature program was: at 100 °C (1.5 min), to 180 °C (6 °C/min) to 290 °C (4 °C/min), at 290 °C (13 min), to 300 °C (4 °C/min), and at 300 °C (20 min).

Quantitative analysis by GC-FID

For quantitative analysis of individual lignans, approximately 3 mg of stump extracts were dried together with 100 μg of internal standards (heneicosanoic acid and betulinol), the mixtures were dissolved in 500 μL of pyridine and 300 μL of the silylation reagent, and kept in an oven at 70 °C for 1 h. Long column GC-FID equipped with an HP-5 column (30 m × 0.32 mm, with 0.25 μm film) with injection at

290 °C and detection at 300 °C was used for the analysis. The temperature program was: at 100 °C (1.5 min), to 180 °C (6 °C/min), to 290 °C (4 °C/min), at 290 °C (13 min), to 300 °C (4 °C/min), and at 300 °C (20 min).

Analysis by HPLC

For the qualitative analysis of stump extractives with HPLC, 1 mg/mL dilutions of stump hot-water extracts were prepared in MeOH/H_2O (50/50, vol/vol) and filtrated through a 0.2-μm polytetrafluoroethylene (PTFE) filter. The HPLC analysis was performed with an Agilent 1290 LC (liquid chromatography) instrument equipped with a ZORBAX StableBond column (80 Å C18, 2.1 mm × 100 mm, 1.8 μm, 1200 bar), a ZORBAX SBC18 UHPLC guard column (2.1 mm, 1.8 μm), 1290 Infinity II Diode Array Detector, and a 6460 triple quadrupole mass spectrometer (LC/DAD/QQQ). The LC columns were maintained at 30 °C. Two solvents were used for the mobile phase: (A) 0.1% formic acid in UHQ water and (B) 0.1% formic acid in acetonitrile. The mobile phase flow rate was 0.4 mL/min. The run method was as follows: 5% B (from 0.0–20.0 min), 5–30% B (from 20.0–22.0 min), 30–80% B (from 22.0–24.0 min), and 80–5% B (from 24.0–25.0 min). Mass spectrometry analyses were performed in negative mode with a range of *m/z* 100–1200. The drying gas used was nitrogen at 10 L/min at 350 °C with a nebulizer pressure of 40 psi. The capillary voltage was 3100 V. The Bruker Data Analysis 3.2 software was used for data processing. Polydatin was used as an external standard for quantifying the hydrophilic compounds from the stump hot-water extracts.

Results and discussion

Due to an unfortunate random accident during sampling, four stump samples (weeks 4 and 25 from crushed stump, and zero-sample and 25-weeks sample from the stump heart) were lost, effectively making stump bottom the only whole stump series. Despite this, the authors of this article decided that presenting the remaining results from the other series as directive references would still be helpful.

Change in total dissolved solids

The combined TDS from *n*-hexane and water extracts in the studied stump samples are presented in Figure 2. Depending on assortment and storage time, TDS varied between 2.9% and 8.4% of dry weight. These are slightly higher concentrations to those reported by Hakkila, who found acetone extracts of *P. abies* stumps to total 2.2%–4.3% of dry matter.[11] Sjöholm has similarly reported central stump of *P. abies* to contain, on average, 2.8% and 1.5% of dry matter acetone and dichloromethane extractives.[12] It should be noted that acetone is more selective solvent than hot water and ASE-extraction (with more harsh extraction conditions) was utilized by us while Sjöholm used more mild Soxhlet-extraction.

In stump bottom, the hot-water extract totaled 70% of the extractives, 74% in crushed stump, and 73% in stump heart. In stump bottom, hydrophilic extractives ranged between 1.5% and 6.6% of dry matter, in crushed stump, 4.1%–5.8% of dry matter, and in stump heart, 1.9%–7.0% of dry matter. The total amount of *n*-hexane extractives ranged, in stump bottom, between 1.0% and 2.0% of dry matter, in crushed stump, 1.5% and 1.8% of dry matter, and in stump heart, 1.2% and 1.7% of dry matter. The crushed stump samples had consistently relatively high hydrophilic and lipophilic TDS, presumably caused by the bark material included in the samples. The root bark of Norway spruce is known for containing even up to 15%–29% of extractives.[13–15] In contrast, the lowest hydrophilic and lipophilic TDS seemed to be found in the debarked stump bottom samples. These results are comparable to those by Latva-Mäenpää, who found that *P. abies* stump wood contained 1.93% of dry matter acetone-soluble extractives.[16] Overall, there appeared to be no significant change in the total amount of lipophilic compounds in stump bottom or crushed stump samples during storage.

Of the studied lipophilic and hydrophilic compounds, about 40% and 50% were identifiable by GC-FID/MS, respectively. Unidentified compounds by GC-FID, in general, represent group of compounds that are poorly volatile and of higher molecular weight. Interestingly, unidentified hydrophilic compounds exhibited the most variation between individual stump samples. Variation between trees is not surprising by itself. The disparity in extractives content even between two trees of similar age can be high. Analogous results have also been reported regarding the extractives content between individual spruce logs.[17] Hydrophilic compounds in samples stored outside are generally lost more easily and prone to external influences, such as photodegradation via UV-light or leaching via rain.[18–21] In stump bottom, based on the identifiable (smaller molecular weight) compounds by GC, and despite the relatively low hydrophilic content of the zero-sample, an apparent

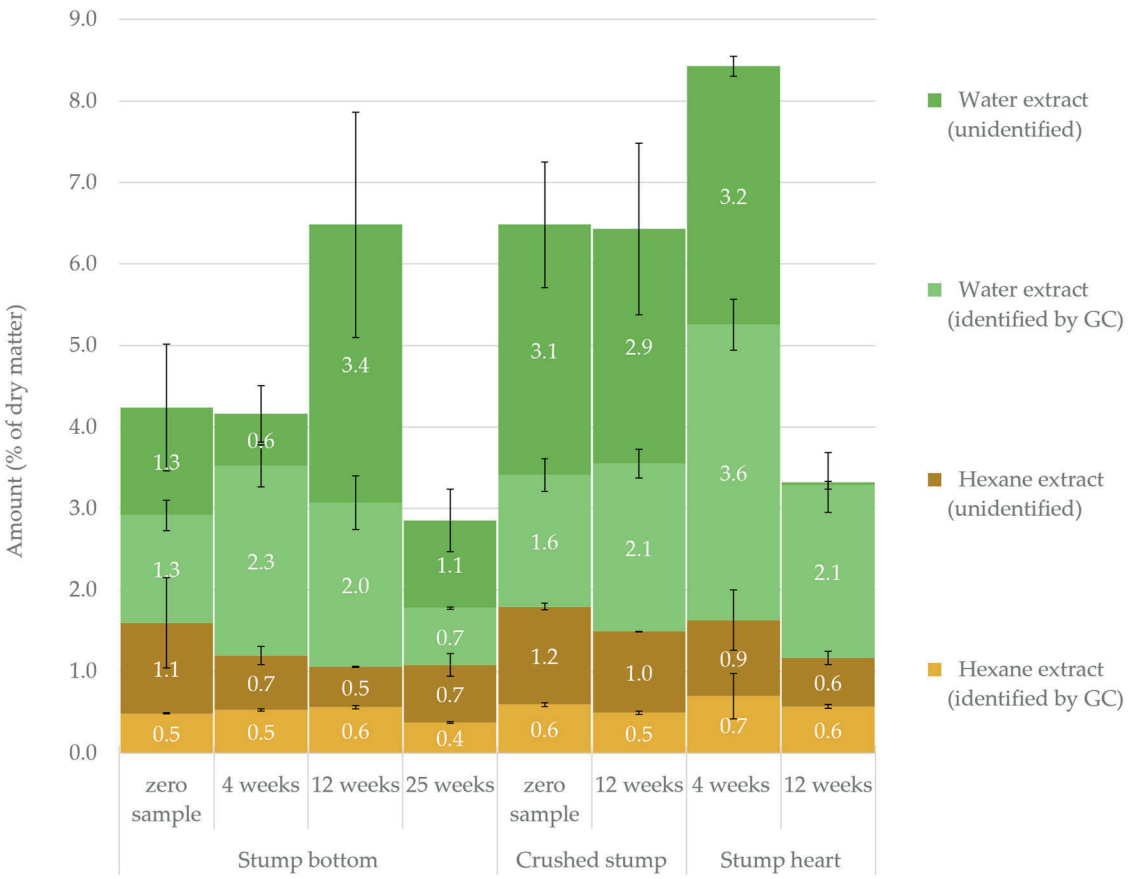

Figure 2. The total dissolved solids of the stump samples.

loss of hydrophilic extractives could be seen, aligning well with our expectations – namely, gradual loss of hydrophilic extractives. However, due to the relatively high variation between individual samples, especially in the amount of unidentifiable compounds, a definite conclusion on the decrease of stump extractives cannot be drawn based on these results.

Qualitative and quantitative results by GC-FID/GC-MS

The hydrophilic extractives groups of the stump samples are presented in Figure 3. Among the studied samples, approximately 43% of the extractives were identified, of which 79% were lignans, 8% sugars, 8% stilbene–glucosides and other aromatics, and 2% organic acids. Hydrophilic extractives, in general, seem to be most abundant in the crushed stump samples, explained by the inclusion of rootbark known for its high extractives content. *Picea abies* bark is also known to contain oligosaccharides and distilbenes unidentifiable with GC-FID.[17,22] This could also explain the abundance of unidentified hydrophilic compounds in the crushed stump samples. Lignan concentrations, on the other hand, appeared to be higher in the stump bottom and heart samples, in particular. This result would agree with the known data that lignan concentration in stumps is high, especially in the heart of the stump.[7,23] In stump bottom, despite the exceptionally high concentration of unidentified compounds in the stump bottom samples at week 12, the general trend appeared to be that the hydrophilic extractives content is decreasing during the storage. The 25-week samples from the stump bottom had, on average, 32% less hydrophilic compounds compared to the zero-sample. The high variation in unidentified compounds may reflect the fact that the excavator-cut stump pieces used for sampling were unevenly shielded in the storage pile from weathering. As has been previously demonstrated regarding bark in pile storage, it could be assumed that the stump samples in the middle of the pile have higher hydrophilic content than those in the outer layers. However, to confirm this, more studies should be conducted. For this study, assuming that all of the stumps in a given pile would be utilized for valorization, choosing between individual stump pieces regarding the location in a pile (whether in the middle or on the top) was not done. Only the stumps at the bottom of the pile were

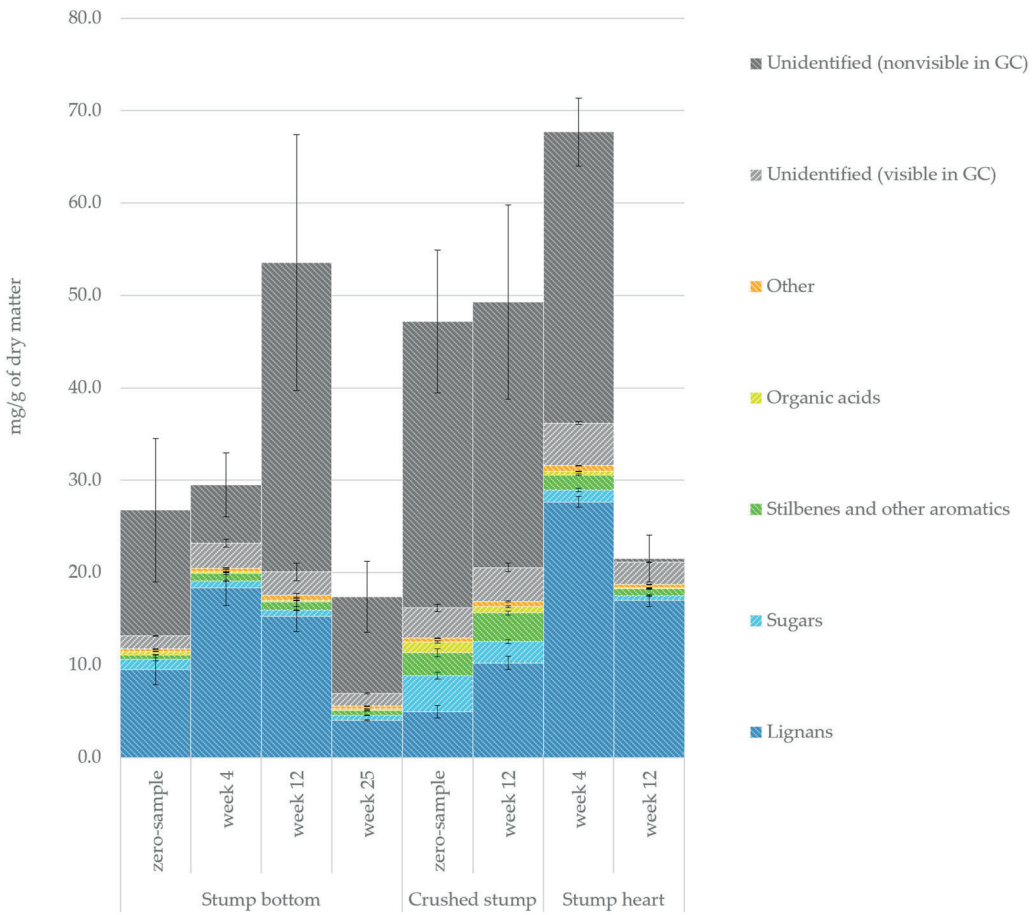

Figure 3. The hydrophilic extractives groups from the stump hot-water extracts as analyzed by GC-FID/MS.

disregarded due to a risk of contamination from the soil.

The concentration of lignans in the stump water extracts is presented in Figure 4. The greatest lignan concentration was found in spruce heart samples (on average 22 mg/g of dry matter) and the lowest in the crushed stump samples (on average 8 mg/g of dry matter). The average concentration of lignans in all the stump samples was 13.6 mg/g of dry matter. Much higher lignan concentrations have been found in the knotwood of spruce. Willför et al.[24] found as high as 6%–24% and Mansikkala et al.[25] 16% of lignans in *P. abies* knotwood by dry weight; 65%–85% of which was HMR. Latva-Mäenpää et al.[7] also found HMR lignan in some of the Norway spruce root neck samples at a concentration of ∼10% of total dry matter. Although several different variables could explain the difference, such as the soil of the growth area or the age of the trees, it appears that one of the significant reasons for the higher concentration of lignans reported by Latva-Mäenpää et al.[7] is due to their more precise way of cutting and separating the heartwood from the stump samples. Poller and Storkan[3] have demonstrated already in 1978 that the stump center is the most concentrated with extractives. Latva-Mäenpää has also demonstrated that the lowermost part of Norway spruce root neck has the highest saturation of lignans.[16] Thus, given that lignans are also localized predominately in and around the heartwood, it could be assumed that the more actual heartwood in the sample is included, the higher the lignan concentration would be.

Of the lignans discovered by us, in stump bottom, on average, 53% was HMR, 17% conidendric acid, 12% todolactol, 4% lignan A, 3% conidendrin, and 2% iso-HMR, isolariciresinol, lariciresinol, and other lignans, and 1% todolactol guiaiacyl ether, lignan A guaiacylglyceryl ether, nortrachelogenin, and oxo-matairesinol. In crushed stump, on average, 45% was HMR, 19% conidendric acid, 16% todolactol, 4% lignan A, 3% isolariciresinol and other lignans, 2% iso-HMR, lariciresinol, conidendrin, and 1% todolactol guiaiacyl ether, lignan A guaiacylglyceryl ether, nortrachelogenin, and oxo-matairesinol. In stump heart, on average, 56% was HMR, 15% conidendric acid, 10% todolactol, 4% conidendrin, 3% lignan A, 2% iso-HMR, lariciresinol, and 1% isolariciresinol, other lignans, todolactol guiaiacyl ether, lignan A

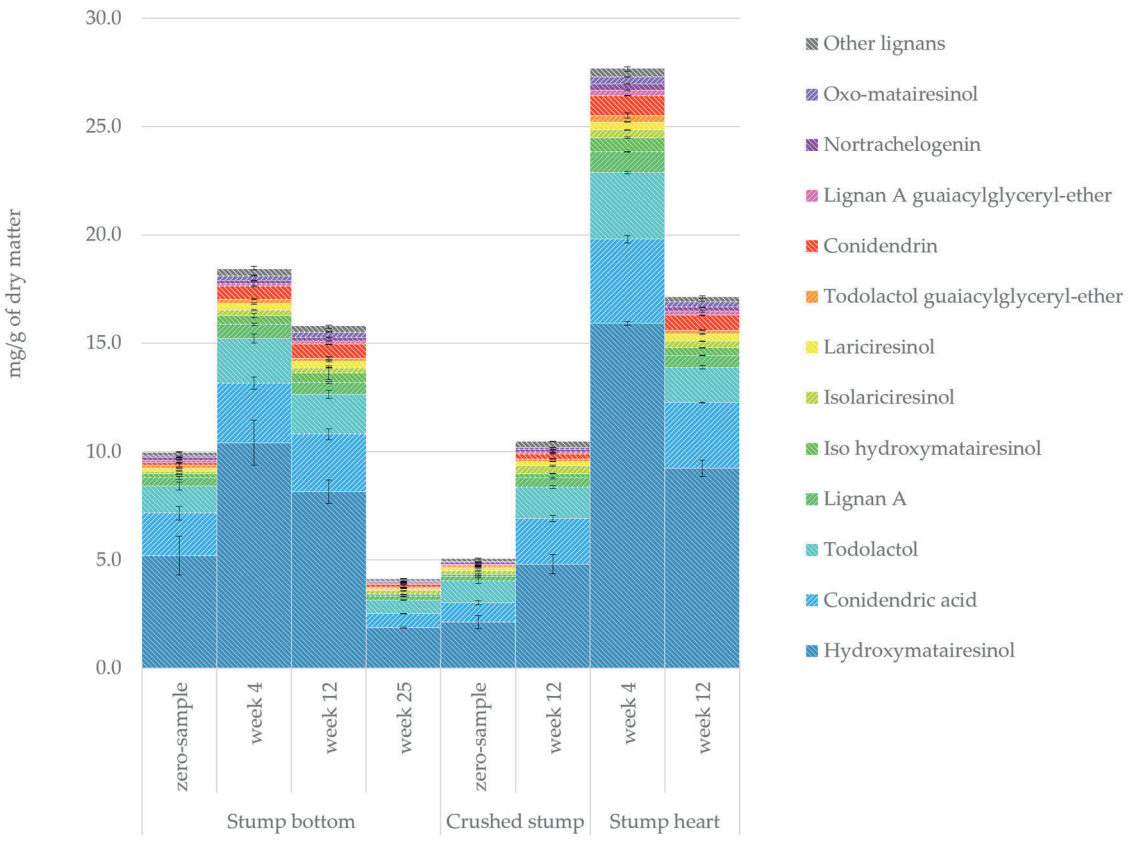

Figure 4. The lignans quantified by GC-FID/MS from stump hot-water extracts.

guaiacylglyceryl ether, nortrachelogenin, and oxo-matairesinol. It was also likely that among the unidentified hydrophilic compounds determined by GC-FID/MS, there was also a small amount of larger sesquilignans or dilignans, such as those discovered from *P. abies* knotwood by Mansikkala et al.[25] They found approximately 30% of the acetone extract of knotwood to consist of these larger lignan species.

Although the amount of lignans in the studied stump samples exhibited significant variation, indications of losses due to storage time could also be seen. Even despite the seemingly unusually low concentration at the stump bottom zero-sample, the apparent degradation of lignans after 25 weeks of storage was still 59%.

The amount of sugars in the stump hot-water extracts is presented in Figure 5. The average amount of sugars was approximately 1 mg/g of dry matter in the stump bottom and stump heart and 3 mg/g of dry matter in the crushed stump – a relatively insignificant amount compared to the amount of sugars in bark and sapwood. In *P. abies* bark, the hot-water extractable monosaccharides at 120 °C can reach up to 65 mg/g of dry matter.[17,26] The higher sugar concentration in the crushed stump can be explained by its carbohydrate-rich bark material. Of the identified sugars and their derivatives, in stump bottom, 37% was glucose, 23% galactose, 19% sucrose, 8% pinitol, 5% palatinose, and 2% inositol and other sugars, and 1% mannitol, arabitol. In crushed stump, 45% was glucose, 24% sucrose, 11% pinitol, 10% galactose, 2% inositol, mannitol and arabitol, and 1% palatinose and other sugars. In stump heart, 30% was glucose, 25% sucrose, 24% galactose, 8% pinitol, 4% palatinose, 3% other sugars, 2% arabitol, and 1% inositol and mannitol. The amount of sugars, mainly glucose and sucrose, appeared to decrease systematically during storage among all samples, while the relative proportion of galactose increased, following the pattern of degradation observed in previous studies regarding the degradation of *P. abies* bark extractives.[17,26] Interestingly, a relatively high amount of galactose and pinitol was also found in the stump samples. Pinitol is a well-researched methoxy derivative of chiro-inositol – a cyclitol with noted self-defensive and medicinal capabilities. For example, plants with high pinitol concentrations have traditionally been utilized to treat diabetes and cancer.[27]

The amount of stilbene–glucosides and other aromatic compounds from the stump samples is presented in Supplementary Figure S1. It should be noted that all of the stilbenoid species detected in the stump

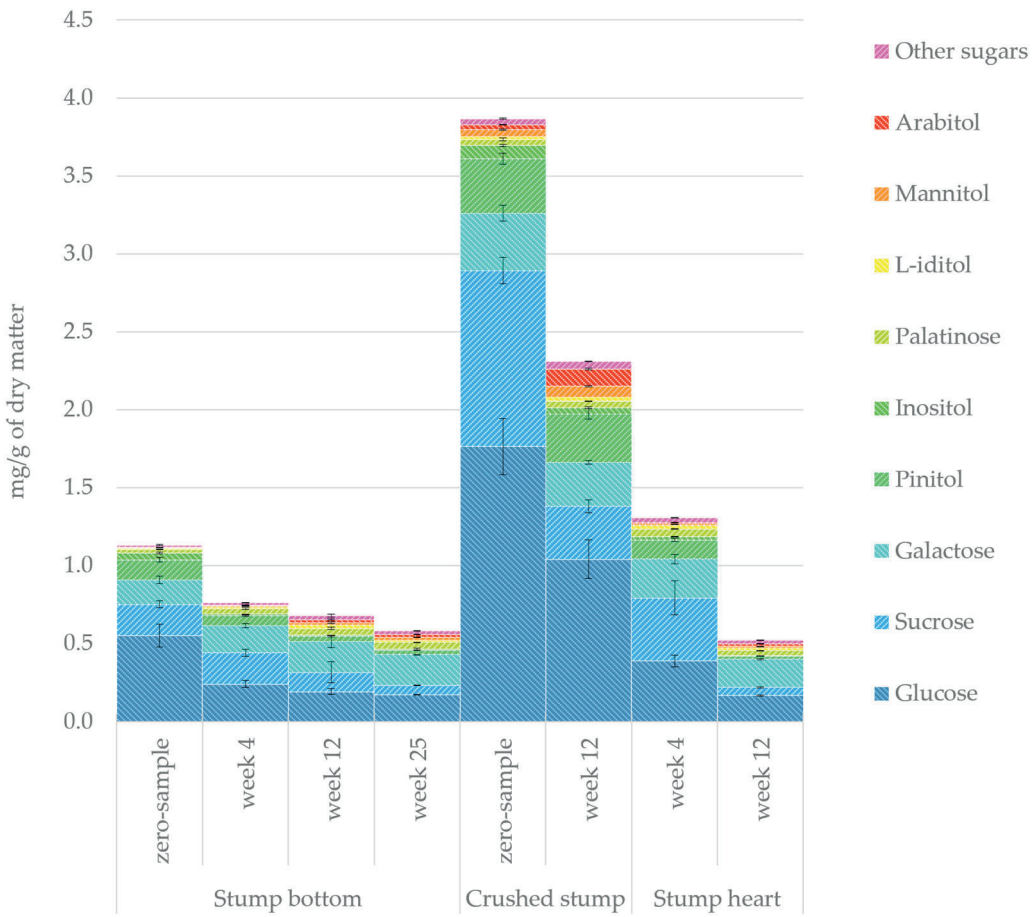

Figure 5. The sugars quantified by GC-FID/MS from stump hot-water extracts.

samples were stilbene–glucosides, namely isorhapontin, astringin, and piceid. Contrary to stump samples, aglycones of these stilbenoids have been detected in *P. abies* bark.[17,26] Among all samples, the average stilbene–glucoside concentration was 0.7 mg/g of dry matter. A slight elevation in stilbene–glucoside concentration was seen in the stump heart samples compared to the stump bottom. However, the highest concentration (2.0 mg/g of dry matter) was in the crushed stump samples. This was explained by the fact that stilbene–glucosides are concentrated primarily in the inner bark of *P. abies*, where their concentration can be as high as 24 mg/g of dry bark.[17] On the contrary, according to the results presented by Kebbi-Benkeder et al.,[28] the acetone extracts of knotwood and heartwood of *P. abies* do not contain stilbene–glucosides of any significant amount.

Of the stilbene–glucosides identified from the stump hydrophilic extracts, in stump bottom, 52% was piceid, 31% isorhapontin, and 17% astringin. In crushed stump, 75% was isorhapontin, 16% astringin, and 9% piceid. In stump heart, 41% was isorhapontin, 34% astringin, and 25% piceid. Other major (and quite resilient) aromatic compounds found in the stump samples were 1,3-(bisguaiacyl)-1,2-propandiol, 1-guaiacylglycerol, and coniferyl alcohol, with a combined concentration of 0.5 mg/g of dry matter in stump bottom, 0.6 mg/g of dry matter in crushed stump, and stump heart samples.

The amount of organic acids in the stump samples is presented in Supplementary Figure S2. The overall profile of the organic acids among the samples resembles very much the amount of sugars, as seen in Figure 5, where the crushed stump samples had the highest average concentration of 1.0 mg/g of dry matter and the stump bottom the lowest average concentration of 0.3 mg/g of dry matter – the decrease in extractives in all samples seemed to proceed in a very gradual fashion.

Of the identified organic acids, in stump bottom, 22% was citric acid, 14% L-aspartic acid, 13% gluconic acid, 11% malic acid, 9% quinic acid, 8% other acids, 7% vanillic acid, 5% shikimic acid, and 5% vanillic acid, and 2% threonic acid. In crushed stump, 31% was citric acid, 25% quinic acid, 9% malic acid, 7% gluconic acid and shikimic acid, 6% other acids, 4% L-aspartic acid, 3% vanillic acid, and 1% threonic acid. In stump heart, 21% was citric acid, 14% L-aspartic

acid and quinic acid, 12% gluconic acid, 9% malic acid, 8% vanillic acid, 7% other acids, 6% shikimic acid, and 1% threonic acid. Previous studies regarding *P. abies* bark extractives have demonstrated that the relative amount of gluconic acid, the most common organic acid in bark, is approximately equal to that of the combined amount of citric and quinic acids.[26] Citric acid has been utilized industrially, especially for its fermentation capabilities.[29] Compared to *P. abies* bark, the relative decrease in the prominence of gluconic acid in stump samples is most likely because the amount of gluconic acid depends on the amount of glucose in the samples, which is markedly lower in stumps. It has been demonstrated that gluconic acid may be obtained from glucose via enzymatic oxidation.[30] However, the formation of new organic acids due to microbial activity is very limited in the pile storage of stumps, unlike in the pile storage of bark, due to larger particle size and, thus, a lack of degradation caused by internal heating.[26,31,32] Among the different stump assortments, the relative increase in the amount of some important acids in the crushed stumps, such as the shikimate pathway acids, shikimic acid, and quinic acid (used in the synthesis of phenylpropanoid amino acids), is likely correlated to the inclusion of bark material in the crushed stump.

The lipophilic extractives groups of the stump samples are presented in Figure 6. Only approximately 32% of the lipophilic extractives were identified, of which in stump bottom, 54% was resin acids, 17% fatty acids, 11% diterpenoids, 10% lignans, 8% sterols, in crushed stump, 66% was resin acids, 14% fatty acids, 9% diterpenoids, 6% sterols, 4% lignans, and 1% other lipophilic compounds, and in stump heart, 54% was resin acids, 16% fatty acids, 12% diterpenoids, 10% lignans, 7% sterols, and 1% other lipophilic compounds. The lipophilic extractives remained relatively stable during 12 weeks of storage. The total amount of lipophilics divergence among the samples was only 13.0–16.5 mg/g of dry matter – a relatively small difference. However, in the stump bottom, unidentified compounds appeared to decrease by ~38% after 12 weeks of storage, while the overall drop in lipophilic extractives was 28.3%.

The slightly increased amount of fatty acids, diterpenoids, lignans, and sterols after 12 weeks of storage suggested that the observed decrease in unidentified compounds might be explained by hydrolysis reactions of triglycerides, steryl esters, oligomeric lignans, and diterpenoids. The hydrolysis of oligomeric lignans into simple lignans in alkaline and acidic conditions has been demonstrated with flax seed oligomeric lignans, namely secoisolariciresinol diglucosides.[33] As in the case of the hydrophilic extracts, the largest lignan concentration was found in the stump heart samples.

The amount of resin acids in the stump *n*-hexane extracts is presented in Figure 7. The average amount of resin acids in all samples was approximately 2.5 mg/g of dry matter, 4–5 times lower than the initial amount of resin acids in *P. abies* bark.[26] Of the identified resin acids, in stump bottom, 29% was dehydroabietic acid, 13% palustric acid, 10% levopimaric acid, isopimaric acid, and hydroxydehydroabietic acid, 7% abietic acid, 5% neoabietic acid and sandaracopimaric acid, 4% hydroxy resin acid, 3% pimaric acid, and 2% other resin acids. In crushed stump, 33% dehydroabietic acid, 14% hydroxydehydroabietic acid, 11% isopimaric acid, 10% hydroxy resin acid, 9% abietic acid, 5% palustric acid and levopimaric acid, 4% pimaric acid and sandaracopimaric acid, and 2% neoabietic acid and other resin acids. In stump heart, 29% was dehydroabietic acid, 12% palustric acid, 10% isopimaric acid and hydroxy dehydroabietic acid, 9% levopimaric acid, 8% abietic acid, 7% hydroxy resin acid, 5% neoabietic acid and sandaracopimaric acid, 3% pimaric acid, and 2% other resin acids. From zero-sample to 25 weeks of storage, the amount of resin acids in stump bottom decreased by 36%. In crushed stump, the apparent decrease was 21% after 12 weeks of storage. In stump heart, there was a similar 19% decrease in resin acids from week 4 to week 12 of storage. The total amount of dehydroabietic acid in samples remained relatively stable. In the stump bottom, dehydroabietic acid decreased only 14% in 25 weeks and hydroxydehydroabietic acid increased by 15%. A significant decrease was shown in palustric acid (74%) and levopimaric acid (76%), probably due to their having conjugated double bonds in their structure, which increase the reactivity (Diels–Alder reactions). The decrease in palustric acid and levopimaric acid has also been noted during bark storage.[26] In crushed stump, the most significant changes were the 73% decrease in neoabietic acid and 59% decrease in pimaric acid, while in stump heart 25% increase in palustric acid and 27% increase in levopimaric acid was observed and hydroxy resin acid decreased by 54%.

The amount of fatty acids in the stump samples is presented in Supplementary Figure S3. The total amount of fatty acids was 0.7 mg/g of dry matter in stump bottom and crushed stump and 0.9 mg/g of dry matter in stump heart. Prominent fatty acids in stump bottom were acid 18:2 (22%), acid 18:3 (17%), acid

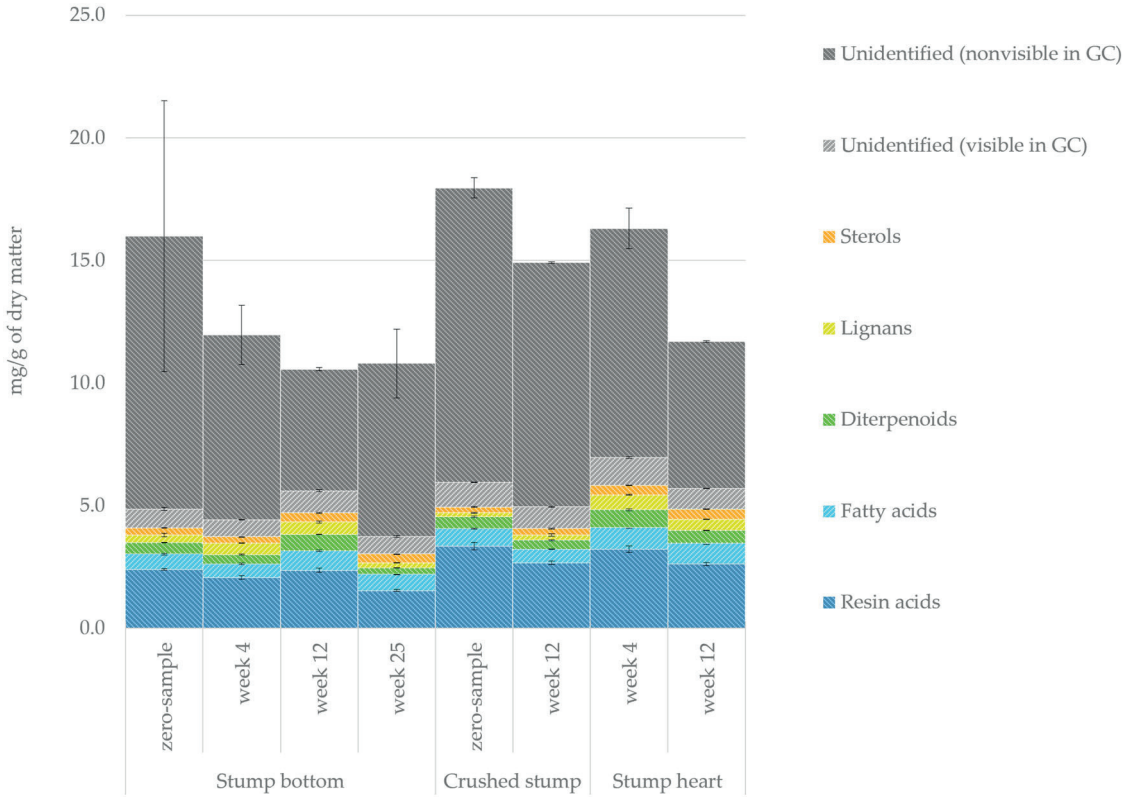

Figure 6. The lipophilic extractives groups from the stump *n*-hexane extracts as analyzed by GC-FID/MS.

18:1 (13%), acid 22:0 (9%), acid 16:0 (7%), acid 17:0 (6%), and acids 18:0 and 20:0 (5%). In crushed stump, acid 18:2 (19%), acid 18:1 (14%), acid 18:3 (13%), acid 22:0 (12%), acid 16:0 (7%), and acids 17:0 and 18:0 (5%). In stump heart, acid 18:2 (25%), acid 18:3 (17%), acid 18:1 (13%), acid 22:0 (9%), acid 16:0 (6%), acid 17:0, and acid 20:0 (5%). No significant change occurred during storage in the total amount of fatty acids. The maximum variation between the highest and the lowest fatty acid concentration was only 0.2 mg/g of dry matter. This result suggested that the 20% increase and the 23% decrease in the stump bottom and the crushed stump fatty acid content, respectively, at week 12 should be seen as part of the natural variation in stump lipophilic extractives rather than pure degradation related to storage.

The fatty acids are preserved much better in stump storage and sawlog bark[17] than in pile storage of bark.[26] This was probably due to the lack of thermal degradation associated with pile storage. However, it should be noted that there may have been degradation in the esterified fatty acids, which were not studied here (and thus were probably included among the unidentified lipophilic extractives group (see Figure 6).

The amount of diterpenoids in stump samples is shown in Supplementary Figure S4. The average amount of diterpenoids was 0.5 mg/g of dry matter in stump bottom, 0.4 mg/g of dry matter in crushed stump, and 0.6 mg/g of dry matter in stump heart. Of the identified diterpenoids, in stump bottom, 38% was thunbergol, 19% Δ^{13}-(*trans*)-neoabienol, 12% palustrol, 8% palustral, 5% pimarol, 4% pimaral and isopimarol and *cis*-abienol, and 3% epimanoyl oxide, 2% methyl neoabietate, and isopimaradiene, and 1% methyl abietatetraenoate. In crushed stump, 27% was thunbergol, 24% Δ^{13}-(*trans*)-neoabienol, 10% palustrol, 7% pimaral, 6% pimarol, 5% isopimarol and *cis*-abienol, 4% palustral, 3% methyl neoabietate and methyl abietatetraenoate, and 2% epimanoyl oxide and isopimaradiene. In stump heart, 39% was thunbergol, 14% Δ^{13}-(*trans*)-neoabienol, 12% palustrol, 7% palustral, 6% pimarol, 5% isopimarol, 4% pimaral and *cis*-abienol, 3% epimanoyl oxide, 2% methyl neoabietate and isopimaradiene, and 1% and methyl abietatetraenoate. Diterpenoids were more easily affected by storage than resin and fatty acids. In 25 weeks, diterpenoids appeared to decrease 43% in the stump bottom and 20% in the crushed stumps in 12 weeks. As in the case of fatty acids, the amount of diterpenoids in the stump bottom week 12 sample was notably higher than in previous weeks. Hence, it was interesting to note that looking at the total amount of lipophilic extractives (Figure 8), the amount of

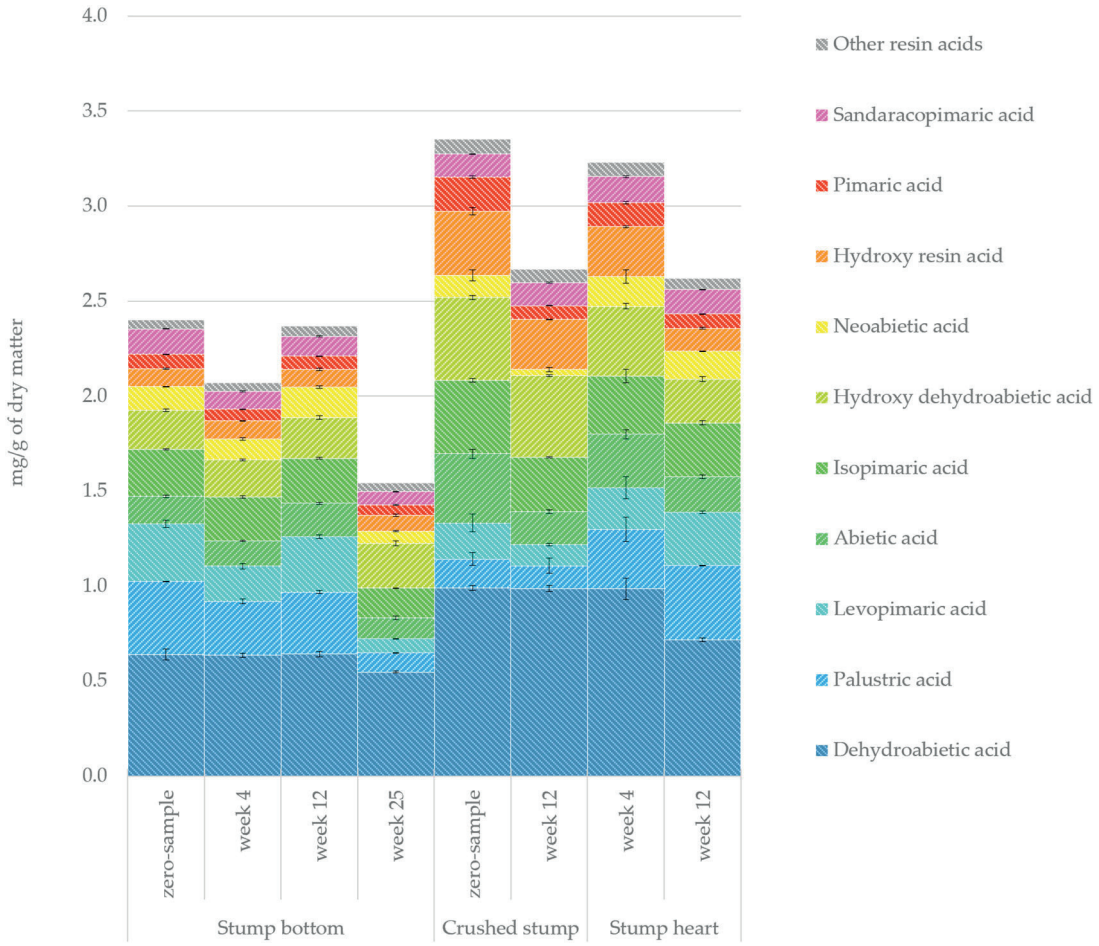

Figure 7. The resin acids quantified by GC-FID/MS from stump hexane extracts.

unidentified lipophilic compounds in the stump bottom at week 12 was also ~35% lower than what would be expected; the amount of unidentified compounds would be expected to follow a linear line of degression. Thus, the results indicated that the apparent increase in identified lipophilic compounds at the stump bottom week 12 was related to the simultaneous decrease of the unidentified compounds. An increase in fatty acids could be explained by increased hydrolysis of triglycerides among the unidentified compounds. Likewise, the observed increase in diterpenoids could be explained by the degradation of tri-, tetra-, or polyterpenoids among the unidentified compounds. The results suggested that the stump bottom samples at week 12 were particularly exposed to degrading conditions (direct sunlight or rainwater). Similar degradation divergence was observed in the bark samples of individual sawlogs.[17]

The total amount of lignans in the *n*-hexane extracts is presented in Supplementary Figure S5. It should be pointed out that, as also seen in Figures 3 and 4, lignans were by far the most prominent extractives group in the stump samples. While most (~97%) of the extracted and identified lignans were extracted *via* hot water, a small fraction (0.4 mg/g of dry matter on average) was also extractable *via* hexane. The *n*-hexane extracted lignan profile looked very similar to the hot-water extracted lignan profile in Figure 4, with HMR being the major lignan and constituting 48% of the *n*-hexane-extracted lignans in stump bottom, 52% in crushed stump and 51% in stump heart. The profiles of lariciresinol, conidendrin, and oxomatairesinol appeared especially similar, however the *n*-hexane extract contained roughly ten times less of them. Notably, some highly hydrophilic lignans were absent in the *n*-hexane extract, such as conidendric acid, todolactol, and lignan A, while other hydrophobic lignans not found in the water extracts, such as pinoresinol, were included. Interestingly, as the storage proceeds, the amount of pinoresinol appears to increase (at least proportionally to other lignans). This could be explained by monolignol radicals formed during the storage with a similar reaction as was demonstrated by Davin et al.[34] For the discussion about the degradation pattern of lignans, see above.

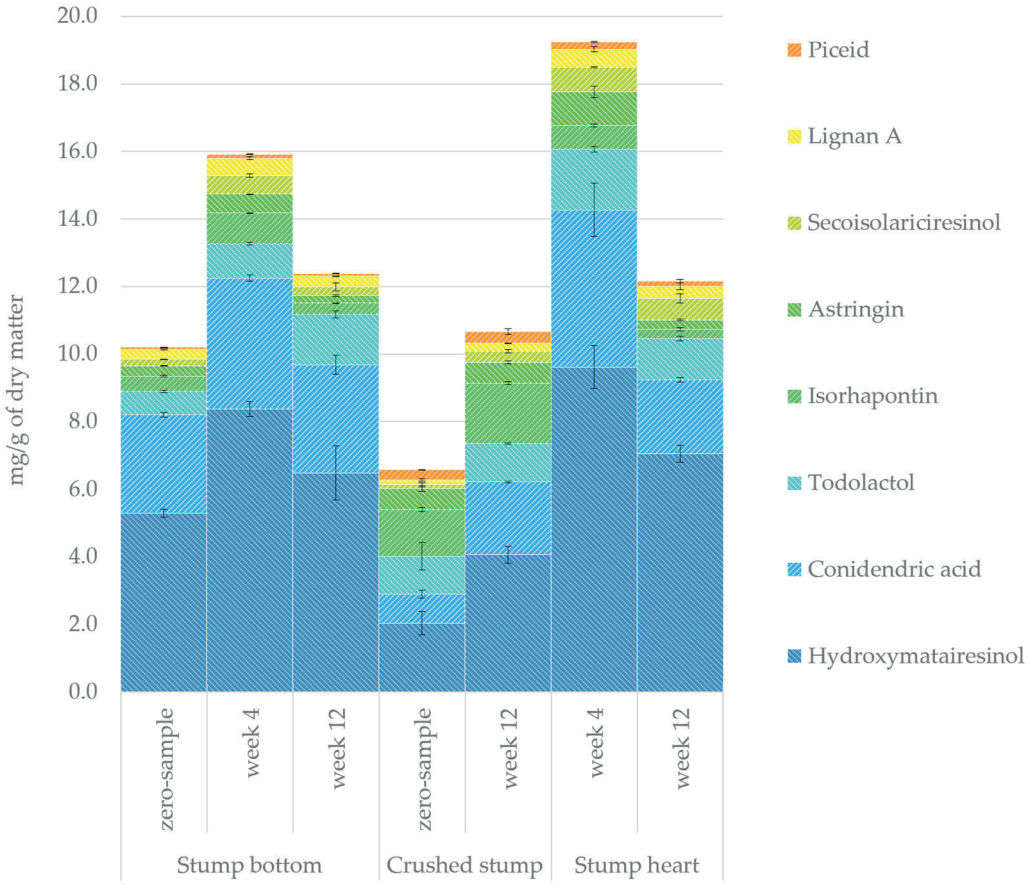

Figure 8. The hydrophilic compounds quantified by HPLC from stump hot-water extracts.

The quantified amount of sterols in the *n*-hexane extracts of the stump samples is presented in Supplementary Figure S6. The amount of sterols appeared to be relatively low (in general below 0.4 mg/g of dry matter). However, it should be noted that these sterols represented only the 'free sterols' in the *n*-hexane extract. The group of lipophilic compounds, which remained unidentified and invisible by the used GC-FID method, is presented in Figure 8. It could also be assumed that esterified sterols are included in that compound group. In the *n*-hexane extracts of *P. abies* bark, the amount of esterified sterols has been shown to be more significant than the amount of free sterols.[17,26] As in spruce stem bark, sitosterol and campesterol were the most prominent sterols in stumps. Of the identified sterols, in stump bottom, 52% was sitosterol, 21% campesterol, 11% 7-hydroxysitosterol, 7% sitostanol, 6% sitostadien-7-one, and 2% acid 24:0 monoglyceride and cholesteryl stearate. In crushed stump, 56% was sitosterol, 19% campesterol, 7% 7-hydroxysitosterol and sitostadien-7-one, 5% acid 24:0 monoglyceride, 4% sitostanol and 2% cholesteryl stearate. In stump heart, 56% was sitosterol, 19% campesterol, 9% 7-hydroxysitosterol, 6% sitostadien-7-one and sitostanol, 3% acid 24:0 monoglyceride and 2% cholesteryl stearate. Overall, the amount of sterols appeared to be very stable throughout the storage periods and stump parts. Non-esterified sterols derived from Norway spruce bark have also been shown to resist degradation, while the loss of esterified sterols is more significant.[26]

The results of the HPLC analysis of stump hot-water extracts are presented in Figure 8, in which the main identified phenolic compounds (lignans and stilbene–glucosides) are presented. Except for secoisolariciresinol, all these compounds were also found and quantified by GC-FID (Figure 4 and Supplementary Figure S2). By comparison, the GC-FID method yielded an average 10.5% higher concentration for the extractives than the HPLC method. However, the average divergence in the concentration was markedly dependent on the stump sample. In the stump bottom samples, the GC-FID results were only an average of 1.0% higher than those from HPLC. Similarly, the GC-FID results were only 1.7% higher in the crushed stump samples. However, in the case of the stump heart samples, the GC-FID results were 26.9% higher than those presented in Figure 8 and focused heavily on the quantified amount of HMR in the 4-weeks stump storage sample.

The differences between the GC and HPLC results could generally be attributed to the differences in the analysis method itself, sample preparation, and the used standards. The HPLC samples were filtered prior to analysis, while the GC-FID samples were not. The HPLC samples were also quantified against the polydatin standard, while the GC samples were quantified by the internal standards, heneicosanoic acid and betulin. However, the unusually significant difference in the HMR concentration in the stump heart sample at week 4 between GC-FID and HPLC appears to be more related to the nature of the sample matrix. The HPLC samples were analyzed from different extracts than those used for the GC analyses. However, the same ground stump heart material was used in both cases. This indicated that even after grinding, the used stump powder remains inhomogeneous with respect to lignan concentration and should have undergone more thorough mixing. Concerning lignan concentration, the inhomogeneity was highly localized toward the pith of the stump; thus, the acquired stump heart material would also contain material with varying lignan concentrations.

The stilbene–glucoside concentrations (isorhapontin, astringin, and piceid) of the HPLC samples were, on average, 3.8 mg/g of dry matter greater than in GC-FID samples. Similar observations have also been made in a previous study regarding GC-FID and HPLC analyzed Norway spruce bark.[17] The four most prominent lignans identified by GC-FID, namely, hydroxymatairesinol, conidendric acid, todolactol, and lignan A were also identified by the HPLC method. It should be noted that the compounds that could not be reliably identified were left out of Figure 8.

The gained chromatographic results confirm that HMR is the most prominent lignan in Norway spruce. Commercial HMR products, such as HMRlignan™ made from Norway spruce knotwood by a Swiss company Linnea, have been available as dietary supplement capsules since 2006.[2] In addition, US company Swanson Health Products also sells HMR extracted from spruce knotwood in 60 mg × 40 mg HMR capsules.[35] According to the GC-FID results of the crushed stump, the average amount of HMR was 3.5 mg/g of dry matter, in stump bottom 6.4 mg/g of dry matter, and in stump heart 12.6 mg/g of dry matter. The pattern of these results agrees with the idea that lignans are mainly concentrated in the heartwood near the pith of the stump.

To conservatively estimate the potential of producing HMR capsules (as described earlier) from *P. abies* stumps, it can be concluded that if the dry mass of a single spruce stump is assumed to be 30 kg and the extractable HMR concentration of crushed bark 3 mg/g of dry matter, a single crushed stump will yield 90 g of pure HMR lignan, which would translate to 37.5 packages of 60 mg × 40 mg HMR capsules. If one package were sold for 10 USD, this would place a 12.5 USD/kg value for the stumps, only based on the HMR concentration.

Conclusions

Based on this study, it can be concluded that *P. abies* stump material presents an exciting option for the isolation and further purification of extractives, especially lignans. Higher intensity in lignan concentration seems to be located, especially at the bottom and heart of the stump material; however, the selective isolation of lignan-rich parts of the stump might prove to be impractical for upscaling. Precut and piled stumps exhibit significant variation in their extractives content. Lipophilic stump extractives are minor components and quite stable in all samples, even at the stump bottom stored for 25 weeks. Hydrophilic extractives are predominant; however, storage appears to decrease their amount in stump bottom and stump heart. However, because of the high extractives variation between samples, the perceived effects of storage may be coincidental.

It seems, nevertheless, reasonable that if stumps are stored for extractives utilization, they should be stored as intact as possible because any cutting of the stump material would dramatically increase the likelihood of oxidation, UV-light, and potential microbial attack on the stump extractives. While inconclusive, the results from the crushed whole stump, retaining much of its initial value in all samples in terms of total extractives content, certainly support this assumption (Figures 2–4). However, further studies would need to be conducted to confirm this as well as the possibility and effect of shielding stump material from UV-light and weathering during storage. In addition, the viability of collecting the hydrophilic extractives easily lost due to leaching from the storage piles is also worth future investigation.

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Supporting material

Figures presenting the GC-FID results of various extractives groups

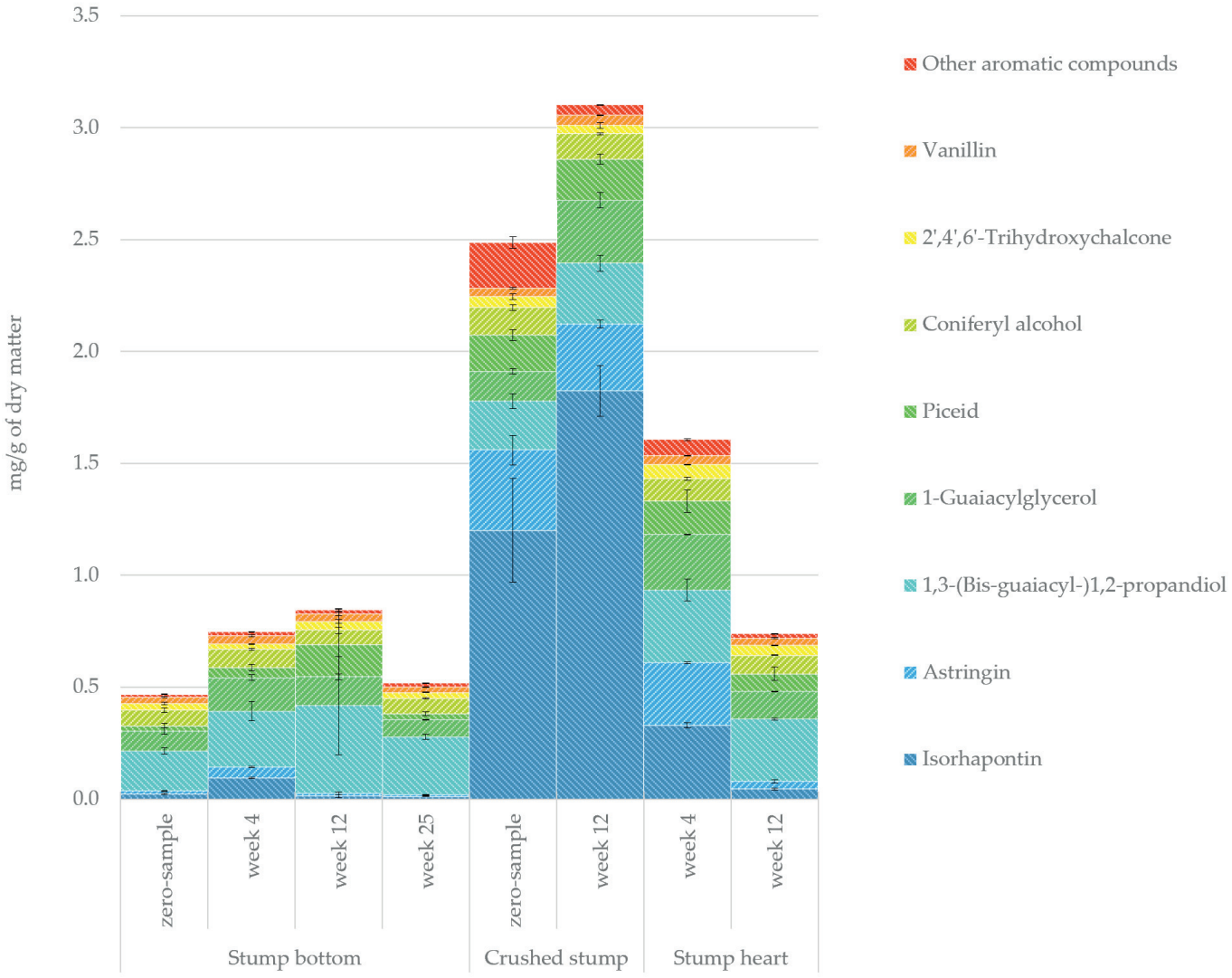

Figure S1. The stilbenes and other aromatic compounds quantified by GC-FID/MS from stump hot-water extracts.

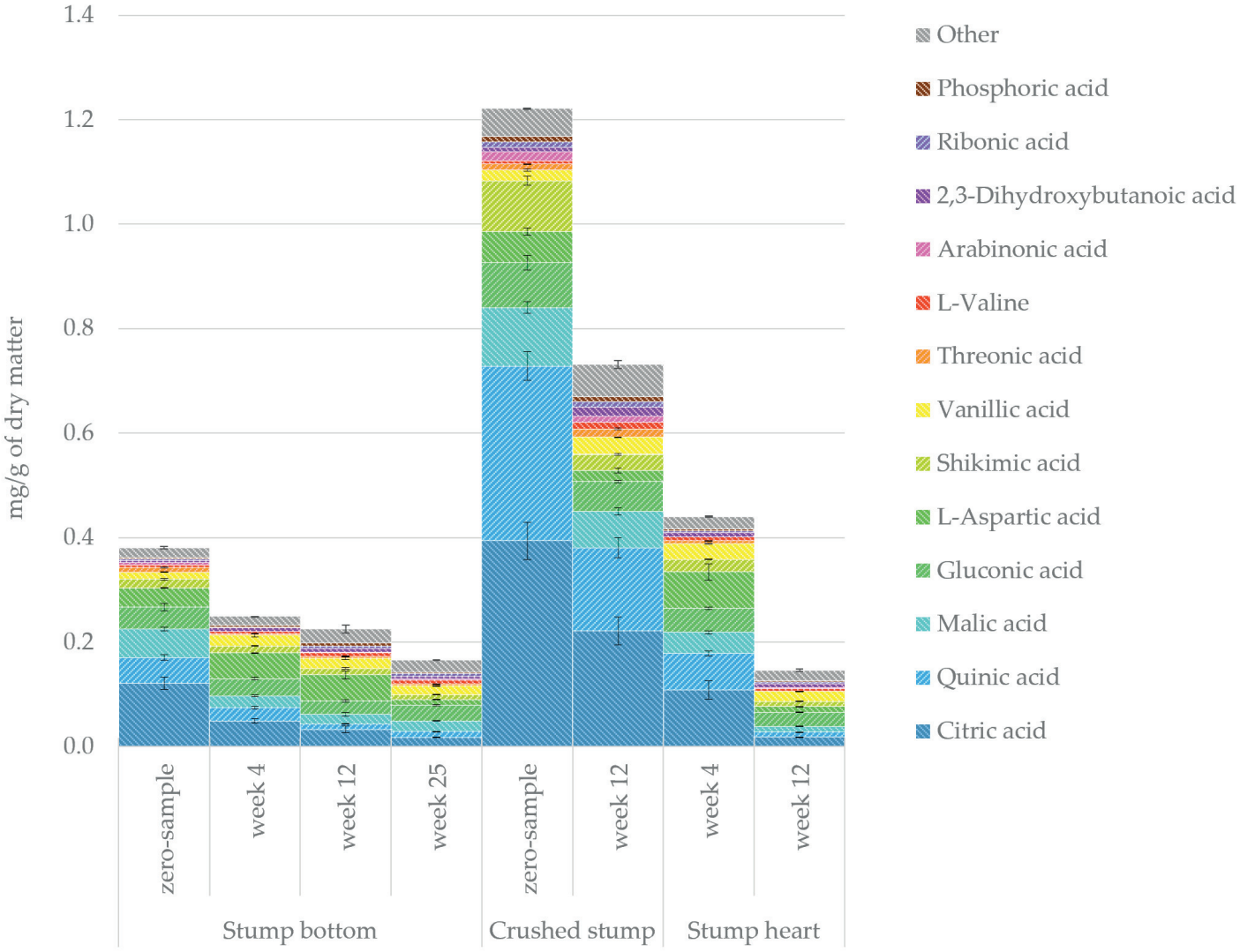

Figure S2. The organic acids quantified by GC-FID/MS from stump hot-water extracts.

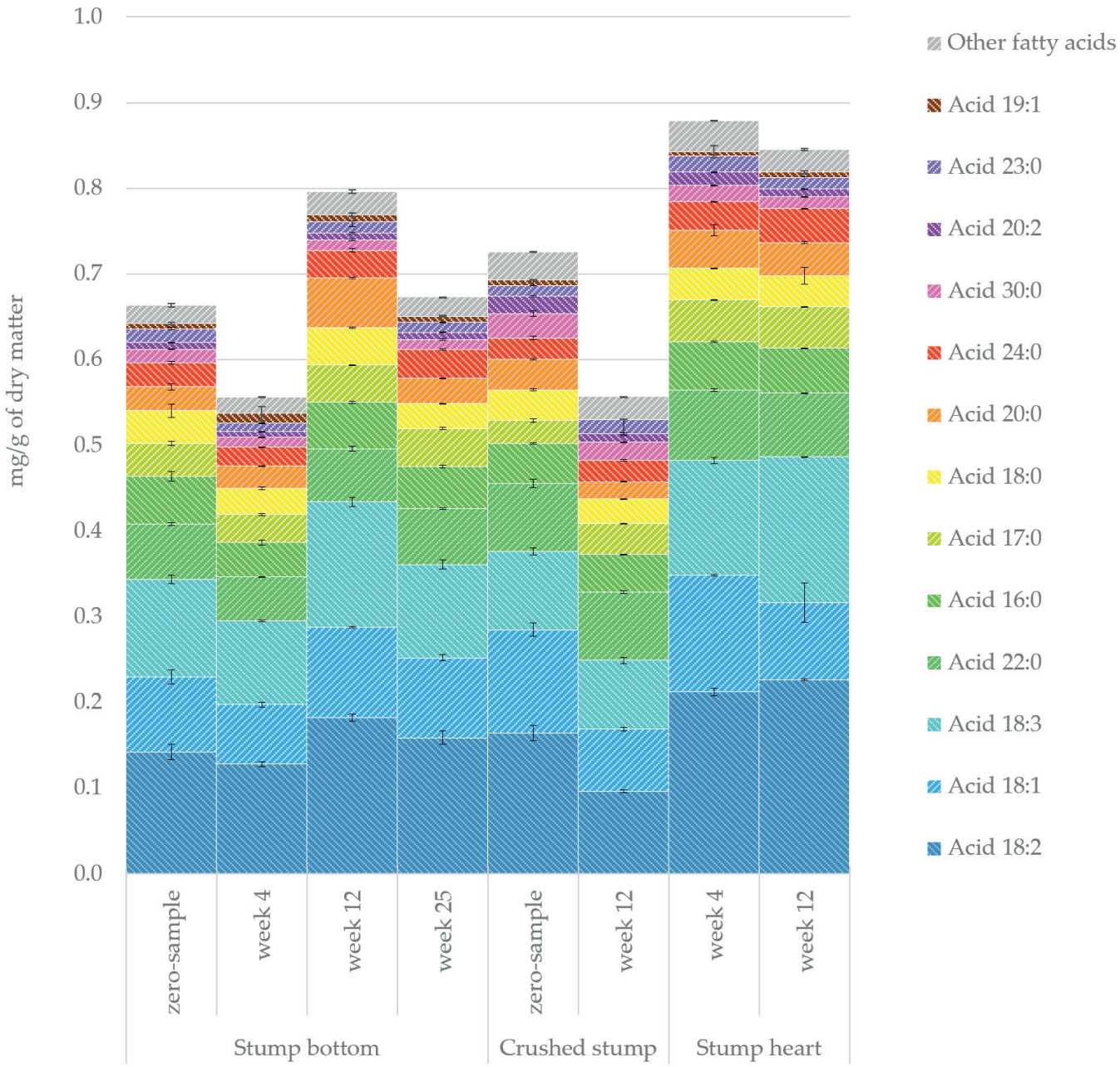

Figure S3. The fatty acids quantified by GC-FID/MS from stump *n*-hexane extracts. The explanation of the symbols used for fatty acids is: the first number after the designation *acid* and before the colon tells the chain length of a given fatty acid, the number after the colon tells the amount of double bonds in the fatty acid chain. Alkanoic acids have no double bonds while alkenoic acids have one or more.

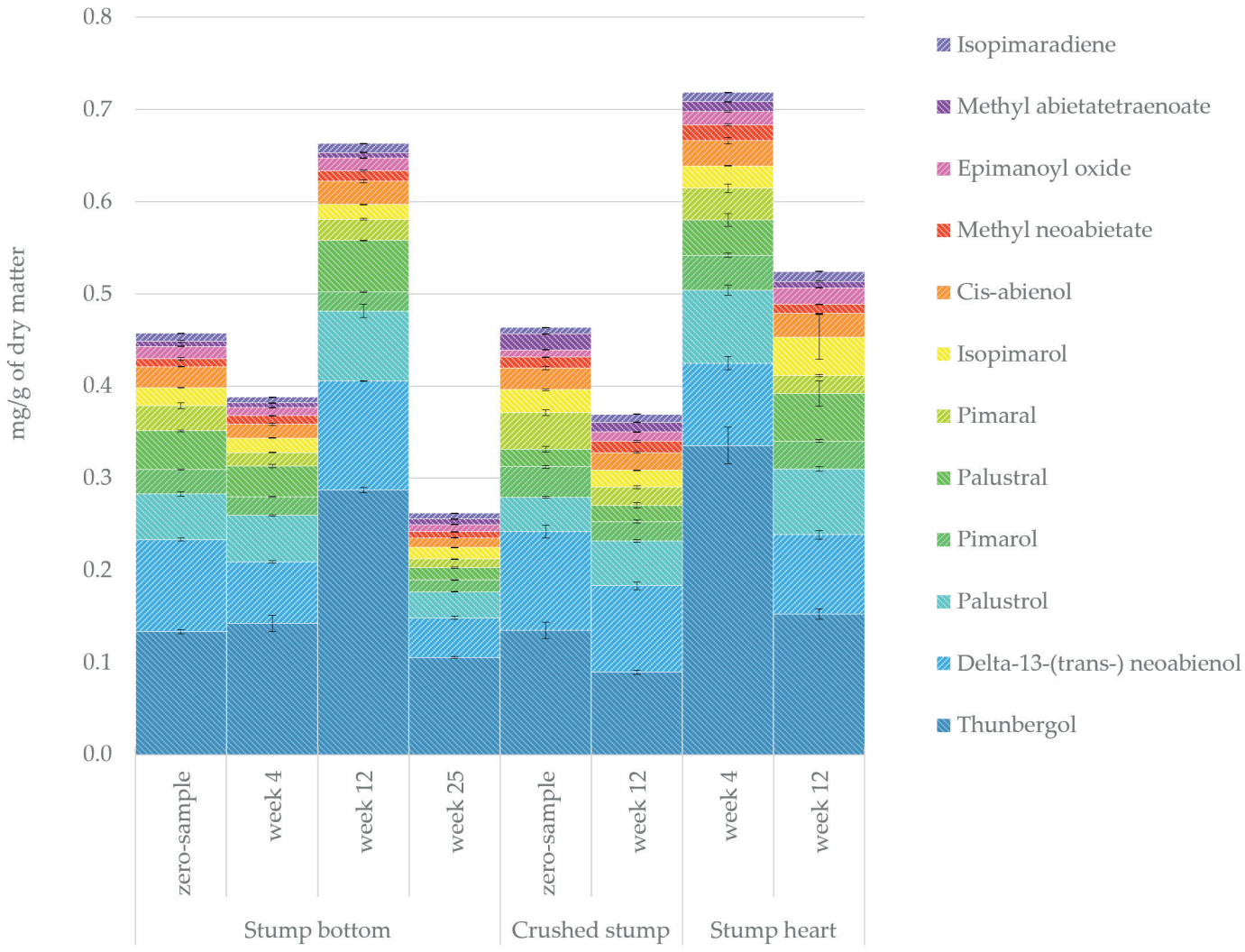

Figure S4. The diterpenoids quantified by GC-FID/MS from stump *n*-hexane extracts.

Figure S5. The lignans quantified by GC-FID/MS from stump *n*-hexane extracts.

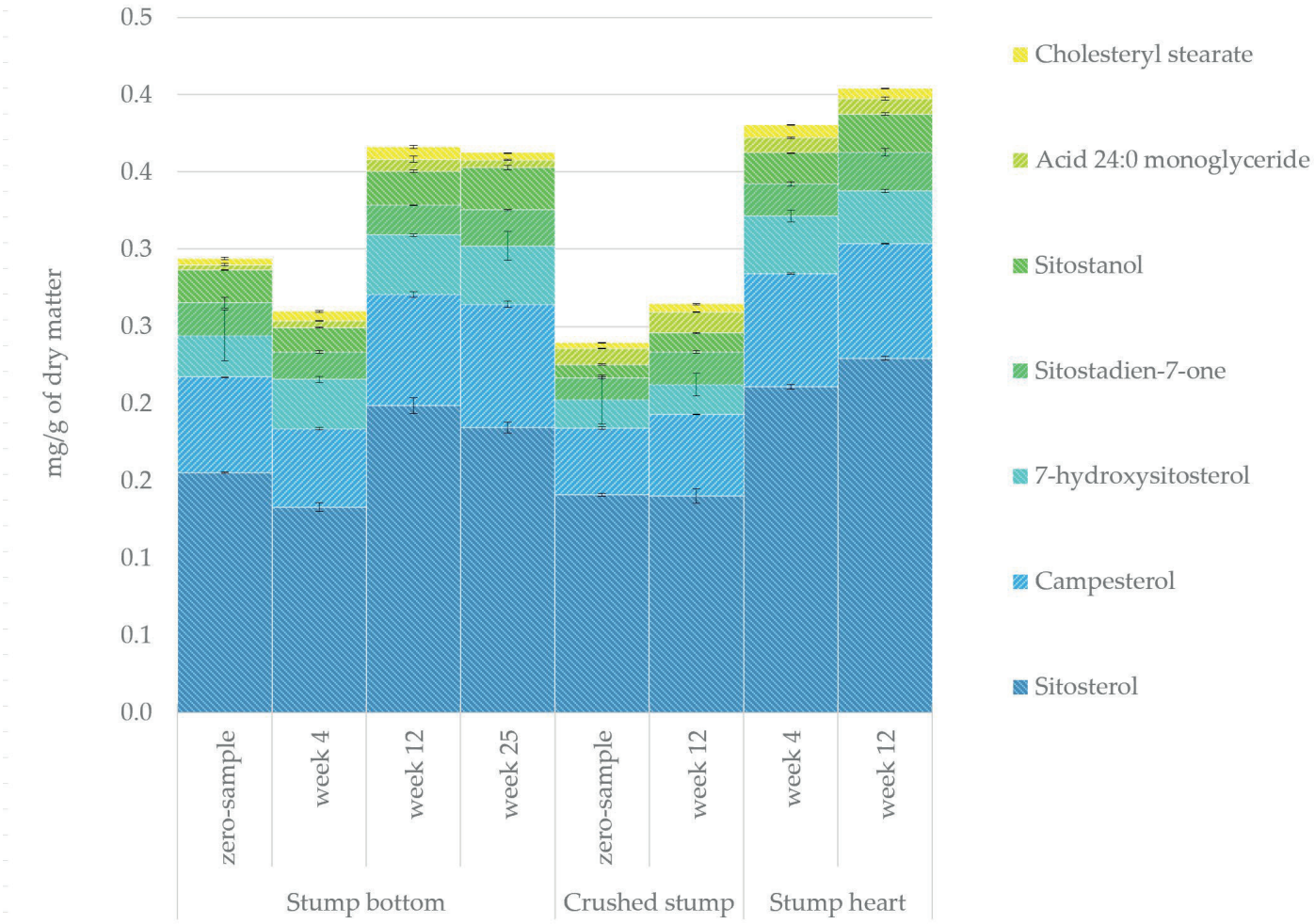

Figure S6. The sterols quantified by GC-FID/MS from stump *n*-hexane extracts.

Tabulated values of the results presented in the article

Table S1. Values for Figure 2.

Total dissolved solids (% of dry matter)	Stump bottom				Crushed stump		Stump heart	
	zero sample	4 weeks	12 weeks	25 weeks	zero sample	12 weeks	4 weeks	12 weeks
Hexane extract (identified by GC)	0.49	0.53	0.56	0.37	0.59	0.50	0.70	0.57
Hexane extract (identified by GC) SD[a]	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.01
Hexane extract (unidentified)	1.11	0.67	0.50	0.71	1.20	1.00	0.93	0.60
Hexane extract (unidentified) SD	0.53	0.11	0.01	0.14	0.04	0.00	0.08	0.01
Water extract (identified by GC)	1.32	2.32	2.01	0.70	1.61	2.06	3.62	2.11

Water extract (identified by GC) SD	0.19	0.26	0.33	0.01	0.20	0.17	0.05	0.06
Water extract (unidentified)	1.32	0.64	3.41	1.07	3.07	2.88	3.17	0.04
Water extract (unidentified) SD	0.78	0.35	1.38	0.38	0.77	1.05	0.37	0.26

[a] Standard deviation

Table S2. Values for Figure 3.

Hydrophilic extractives (mg/g of dry matter)	Stump bottom				Crushed stump		Stump heart	
	zero sample	4 weeks	12 weeks	25 weeks	zero sample	12 weeks	4 weeks	12 weeks
Lignans	9.51	18.39	15.29	4.02	4.98	10.23	27.66	16.99
Lignans SD	1.62	1.95	1.65	0.06	0.68	0.70	0.58	0.62
Sugars	1.12	0.75	0.66	0.56	3.86	2.30	1.30	0.51
Sugars SD	0.15	0.07	0.01	0.00	0.38	0.23	0.19	0.02
Stilbenes and other aromatics	0.47	0.75	0.85	0.52	2.49	3.10	1.61	0.74
Stilbenes and other aromatics SD	0.04	0.08	0.44	0.02	0.43	0.22	0.10	0.04
Organic acids	0.37	0.23	0.20	0.14	1.18	0.67	0.41	0.13
Organic acids SD	0.03	0.02	0.02	0.003	0.11	0.07	0.01	0.004
Other	0.29	0.39	0.56	0.34	0.41	0.59	0.61	0.40
Other SD	0.03	0.04	0.22	0.01	0.04	0.08	0.02	0.02
Unidentified (visible in GC)	1.45	2.72	2.52	1.37	3.26	3.66	4.61	2.39
Unidentified (visible in GC) SD	0.001	0.43	0.98	0.06	0.39	0.46	0.15	0.05
Unidentified (nonvisible in GC)	13.56	6.27	33.47	10.44	30.98	28.73	31.48	0.38
Unidentified (nonvisible in GC) SD	7.77	3.47	13.83	3.84	7.72	10.50	3.69	2.55

Table S3. Values for Figure 4.

Lignans (mg/g of dry matter)	Stump bottom				Crushed stump		Stump heart	
	zero sample	4 weeks	12 weeks	25 weeks	zero sample	12 weeks	4 weeks	12 weeks
Hydroxymatairesinol	5.20	10.41	8.15	1.86	2.13	4.80	15.91	9.24
Hydroxymatairesinol SD	0.90	1.03	0.54	0.03	0.30	0.45	0.08	0.38

Conidendric acid	1.96	2.74	2.65	0.67	0.88	2.11	3.89	3.02
Conidendric acid SD	0.32	0.28	0.25	0.001	0.10	0.14	0.18	0.003
Todolactol	1.25	2.07	1.84	0.62	1.02	1.46	3.07	1.63
Todolactol SD	0.19	0.21	0.20	0.003	0.13	0.08	0.06	0.08
Lignan A	0.38	0.65	0.54	0.17	0.19	0.41	0.96	0.54
Lignan A SD	0.06	0.06	0.04	0.001	0.02	0.04	0.02	0.02
Iso hydroxymatairesinol	0.18	0.40	0.45	0.09	0.11	0.21	0.65	0.36
Iso hydroxymatairesinol SD	0.01	0.09	0.31	0.01	0.03	0.01	0.04	0.02
Isolariciresinol	0.14	0.25	0.24	0.16	0.15	0.36	0.37	0.30
Isolariciresinol SD	0.02	0.02	0.01	0.002	0.02	0.04	0.004	0.002
Lariciresinol	0.14	0.31	0.30	0.11	0.14	0.19	0.37	0.34
Lariciresinol SD	0.02	0.03	0.04	0.003	0.02	0.01	0.003	0.01
Todolactol guaiacylglyc-eryl-ether	0.13	0.20	0.15	0.05	0.07	0.12	0.29	0.17
Todolactol guaiacylglyc-eryl-ether SD	0.05	0.01	0.06	0.002	0.002	0.01	0.11	0.01
Conidendrin	0.12	0.61	0.64	0.13	0.04	0.24	0.92	0.69
Conidendrin SD	0.02	0.03	0.01	0.003	0.01	0.01	0.01	0.01
Lignan A guaiacylglyc-eryl-ether	0.11	0.11	0.15	0.08	0.06	0.07	0.25	0.19
Lignan A guaiacylglyc-eryl-ether SD	0.02	0.09	0.02	0.001	0.01	0.02	0.02	0.03
Nortrachelogenin	0.10	0.15	0.17	0.04	0.08	0.12	0.28	0.20
Nortrachelogenin SD	0.01	0.02	0.004	0.0004	0.01	0.005	0.02	0.004
Oxo-matairesinol	0.07	0.21	0.22	0.05	0.02	0.11	0.34	0.23
Oxo-matairesinol SD	0.01	0.03	0.05	0.01	0.01	0.001	0.01	0.02
Other lignans	0.17	0.31	0.31	0.09	0.15	0.27	0.39	0.24
Other lignans SD	0.004	0.05	0.12	0.004	0.02	0.01	0.14	0.07

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Table S4. Values for Figure 5. 30

Sugars (mg/g of dry matter)	Stump bottom				Crushed stump		Stump heart	
	zero sample	4 weeks	12 weeks	25 weeks	zero sample	12 weeks	4 weeks	12 weeks
Glucose	0.55	0.24	0.19	0.17	1.76	1.04	0.39	0.17
Glucose SD	0.07	0.02	0.02	0.003	0.18	0.12	0.04	0.004
Sucrose	0.20	0.20	0.12	0.06	1.13	0.34	0.40	0.05
Sucrose SD	0.02	0.02	0.07	0.0004	0.08	0.04	0.11	0.004
Galactose	0.16	0.17	0.20	0.20	0.37	0.28	0.25	0.18
Galactose SD	0.02	0.01	0.04	0.001	0.05	0.01	0.03	0.01
Pinitol	0.13	0.06	0.03	0.03	0.35	0.31	0.12	0.02
Pinitol SD	0.02	0.005	0.003	0.001	0.04	0.03	0.01	0.001
Inositol	0.04	0.01	0.01	0.01	0.08	0.04	0.02	0.003
Inositol SD	0.01	0.001	0.001	0.01	0.01	0.005	0.002	0.0002
Palatinose	0.02	0.04	0.04	0.04	0.04	0.04	0.05	0.032
Palatinose SD	0.002	0.01	0.01	0.001	0.01	0.002	0.003	0.0001
L-iditol	0.01	0.01	0.02	0.01	0.02	0.03	0.02	0.015
L-iditol SD	0.002	0.001	0.01	0.00001	0.001	0.01	0.001	0.0004
Mannitol	0.004	0.01	0.01	0.02	0.04	0.07	0.01	0.011
Mannitol SD	0.001	0.0002	0.005	0.001	0.004	0.003	0.0001	0.001
Arabitol	0.001	0.01	0.02	0.02	0.03	0.11	0.01	0.018
Arabitol SD	0.00003	0.001	0.004	0.001	0.002	0.01	0.002	0.001
Other sugars	0.01	0.01	0.02	0.02	0.04	0.05	0.03	0.022
Other sugars SD	0.004	0.001	0.01	0.001	0.004	0.001	0.001	0.002

Table S5. Values for Figure 6.

Lipophilic extractives (mg/g of dry matter)	Stump bottom				Crushed stump		Stump heart	
	zero sample	4 weeks	12 weeks	25 weeks	zero sample	12 weeks	4 weeks	12 weeks

Resin acids	2.40	2.07	2.37	1.54	3.35	2.67	3.23	2.62
Resin acids SD	0.04	0.06	0.08	0.04	0.15	0.07	0.13	0.07
Fatty acids	0.63	0.55	0.80	0.67	0.73	0.56	0.88	0.85
Fatty acids SD	0.05	0.02	0.03	0.02	0.03	0.0002	0.02	0.04
Diterpenoids	0.46	0.39	0.66	0.26	0.46	0.37	0.72	0.52
Diterpenoids SD	0.01	0.01	0.02	0.002	0.03	0.02	0.04	0.001
Lignans	0.31	0.47	0.51	0.20	0.17	0.21	0.61	0.46
Lignans SD	0.07	0.004	0.04	0.01	0.02	0.06	0.02	0.01
Sterols	0.29	0.26	0.37	0.36	0.24	0.26	0.38	0.40
Sterols SD	0.01	0.003	0.01	0.003	0.02	0.01	0.01	0.004
Unidentified (visible in GC)	0.76	0.69	0.91	0.71	1.00	0.89	1.15	0.85
Unidentified (visible in GC) SD	0.07	0.01	0.05	0.02	0.01	0.02	0.04	0.02
Unidentified (nonvisible in GC)	11.13	7.54	4.95	7.05	12.01	9.96	9.34	5.99
Unidentified (nonvisible in GC) SD	5.52	1.21	0.06	1.40	0.41	0.02	0.82	0.04

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Table S6. Values for Figure 7. 34

Resin acids (mg/g of dry matter)	Stump bottom				Crushed stump		Stump heart	
	zero sample	4 weeks	12 weeks	25 weeks	zero sample	12 weeks	4 weeks	12 weeks
Dehydroabietic acid	0.64	0.64	0.64	0.55	0.99	0.99	0.99	0.72
Dehydroabietic acid SD	0.03	0.01	0.02	0.01	0.01	0.02	0.06	0.01
Palustric acid	0.38	0.29	0.33	0.10	0.15	0.12	0.31	0.39
Palustric acid SD	0.001	0.01	0.01	0.002	0.03	0.04	0.06	0.001
Levopimaric acid	0.30	0.18	0.29	0.07	0.19	0.11	0.22	0.28
Levopimaric acid SD	0.02	0.01	0.01	0.002	0.05	0.01	0.06	0.01
Abietic acid	0.15	0.13	0.18	0.11	0.36	0.17	0.28	0.19

Abietic acid SD	0.01	0.003	0.01	0.01	0.02	0.01	0.02	0.01
Isopimaric acid	0.25	0.23	0.24	0.16	0.39	0.29	0.31	0.28
Isopimaric acid SD	0.003	0.01	0.005	0.001	0.01	0.003	0.03	0.01
Hydroxy dehydroabietic acid	0.21	0.19	0.22	0.24	0.44	0.43	0.37	0.23
Hydroxy dehydroabietic acid SD	0.01	0.004	0.01	0.01	0.01	0.003	0.01	0.01
Neoabietic acid	0.12	0.11	0.16	0.06	0.12	0.03	0.16	0.15
Neoabietic acid SD	0.001	0.01	0.01	0.002	0.03	0.01	0.04	0.002
Hydroxy resin acid	0.09	0.10	0.09	0.08	0.34	0.26	0.26	0.12
Hydroxy resin acid SD	0.004	0.002	0.01	0.01	0.02	0.0003	0.004	0.005
Pimaric acid	0.08	0.06	0.07	0.05	0.18	0.07	0.13	0.08
Pimaric acid SD	0.003	0.001	0.001	0.004	0.01	0.001	0.01	0.002
Sandaracopimaric acid	0.13	0.09	0.10	0.07	0.12	0.12	0.14	0.13
Sandaracopimaric acid SD	0.001	0.004	0.004	0.001	0.0004	0.003	0.004	0.002
Other resin acids	0.05	0.04	0.06	0.04	0.08	0.07	0.08	0.06
Other resin acids SD	0.003	0.0004	0.005	0.001	0.004	0.001	0.003	0.004

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Table S7. Values for Figure 8. 36

Hydrophilic extractives (mg/g of dry matter)	Stump bottom			Crushed stump		Stump heart	
	zero sample	4 weeks	12 weeks	zero sample	12 weeks	4 weeks	12 weeks
Hydroxymatairesinol	5.29	8.36	6.48	2.04	4.07	9.61	7.05
Hydroxymatairesinol SD	0.11	0.22	0.80	0.34	0.24	0.64	0.25
Conidendric acid	2.91	3.89	3.20	0.86	2.16	4.66	2.19
Conidendric acid SD	0.07	0.09	0.27	0.11	0.03	0.78	0.06
Todolactol	0.69	1.02	1.49	1.12	1.12	1.80	1.22
Todolactol SD	0.03	0.04	0.10	0.40	0.02	0.09	0.07
Isorhapontin	0.45	0.91	0.33	1.38	1.79	0.70	0.27

Isorhapontin SD	0.02	0.003	0.005	0.06	0.04	0.04	0.06
Astringin	0.31	0.55	0.22	0.63	0.61	1.00	0.28
Astringin SD	0.01	0.00	0.02	0.09	0.04	0.17	0.02
Secoisolariciresinol	0.19	0.54	0.26	0.11	0.34	0.73	0.64
Secoisolariciresinol SD	0.004	0.05	0.12	0.06	0.06	0.01	0.14
Lignan A	0.29	0.52	0.32	0.15	0.23	0.54	0.35
Lignan A SD	0.02	0.04	0.002	0.03	0.01	0.08	0.10
Piceid	0.07	0.12	0.08	0.30	0.34	0.22	0.16
Piceid SD	0.004	0.01	0.003	0.01	0.08	0.01	0.05

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Table S8. Values for Figure S1. 38

Stilbenes and other aromatic compounds (mg/g of dry matter)	Stump bottom				Crushed stump		Stump heart	
	zero sample	4 weeks	12 weeks	25 weeks	zero sample	12 weeks	4 weeks	12 weeks
Isorhapontin	0.02	0.10	0.01	0.01	1.20	2.35	0.33	0.04
Isorhapontin SD	0.003	0.003	0.01	0.001	0.23	0.11	0.01	0.004
Astringin	0.01	0.05	0.01	0.01	0.36	0.30	0.28	0.03
Astringin SD	0.001	0.003	0.01	0.0001	0.07	0.02	0.004	0.01
1,3-(Bis-guaiacyl-)1,2-propandiol	0.18	0.25	0.39	0.26	0.22	0.27	0.32	0.28
1,3-(Bis-guaiacyl-)1,2-propandiol SD	0.01	0.04	0.22	0.01	0.03	0.04	0.05	0.004
1-Guaiacylglycerol	0.09	0.15	0.13	0.08	0.13	0.28	0.25	0.12
1-Guaiacylglycerol SD	0.01	0.01	0.01	0.001	0.01	0.04	0.0001	0.002
Piceid	0.02	0.05	0.14	0.03	0.16	0.18	0.15	0.08
Piceid SD	0.01	0.01	0.16	0.01	0.02	0.02	0.05	0.03
Coniferyl alcohol	0.07	0.08	0.06	0.07	0.12	0.11	0.10	0.08
Coniferyl alcohol SD	0.01	0.004	0.01	0.002	0.01	0.004	0.01	0.002
2',4',6'-Trihydroxychalcone	0.03	0.03	0.04	0.03	0.05	0.04	0.06	0.05

2',4',6'-Trihydroxychalcone SD	0.01	0.0002	0.01	0.001	0.01	0.01	0.001	0.001
Vanillin	0.03	0.04	0.03	0.02	0.04	0.05	0.04	0.03
Vanillin SD	0.003	0.004	0.01	0.001	0.004	0.001	0.0003	0.002
Other aromatic compounds	0.01	0.02	0.02	0.02	0.21	0.05	0.07	0.02
Other aromatic compounds SD	0.002	0.001	0.01	0.0005	0.03	0.0004	0.003	0.001

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Table S9. Values for Figure S2. 40

Organic acids (mg/g of dry matter)	Stump bottom				Crushed stump		Stump heart	
	zero sample	4 weeks	12 weeks	25 weeks	zero sample	12 weeks	4 weeks	12 weeks
Citric acid	0.12	0.05	0.03	0.02	0.39	0.22	0.11	0.02
Citric acid SD	0.01	0.004	0.01	0.001	0.04	0.03	0.02	0.001
Quinic acid	0.05	0.02	0.01	0.01	0.33	0.16	0.07	0.01
Quinic acid SD	0.01	0.002	0.0002	0.0003	0.03	0.02	0.005	0.0003
Malic acid	0.05	0.02	0.02	0.02	0.11	0.07	0.04	0.01
Malic acid SD	0.004	0.002	0.003	0.0005	0.01	0.01	0.003	0.001
Gluconic acid	0.04	0.03	0.03	0.03	0.09	0.06	0.05	0.03
Gluconic acid SD	0.01	0.002	0.002	0.001	0.01	0.002	0.002	0.0002
L-Aspartic acid	0.04	0.05	0.05	0.01	0.06	0.02	0.07	0.01
L-Aspartic acid SD	0.001	0.0002	0.01	0.001	0.01	0.005	0.02	0.0005
Shikimic acid	0.02	0.01	0.01	0.01	0.10	0.03	0.02	0.01
Shikimic acid SD	0.002	0.001	0.002	0.0001	0.01	0.002	0.001	0.0004
Vanillic acid	0.01	0.02	0.02	0.02	0.02	0.03	0.03	0.02
Vanillic acid SD	0.0003	0.003	0.004	0.0002	0.002	0.001	0.001	0.0002
Threonic acid	0.01	0.003	0.003	0.004	0.01	0.02	0.01	-
Threonic acid SD	0.001	0.0001	0.001	0.00004	0.001	0.001	0.0004	-
Other	0.02	0.02	0.03	0.02	0.05	0.06	0.02	0.02

Other SD	0.002	0.00004	0.01	0.0002	0.001	0.01	0.001	0.002

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Table S10. Values for Figure S3. 42

Fatty acids (mg/g of dry matter)	Stump bottom				Crushed stump		Stump heart	
	zero sample	4 weeks	12 weeks	25 weeks	zero sample	12 weeks	4 weeks	12 weeks
Acid 18:2	0.14	0.13	0.18	0.16	0.16	0.10	0.21	0.23
Acid 18:2 SD	0.01	0.003	0.004	0.01	0.01	0.002	0.004	0.001
Acid 18:1	0.09	0.07	0.11	0.09	0.12	0.07	0.14	0.09
Acid 18:1 SD	0.01	0.003	0.001	0.004	0.01	0.002	0.001	0.02
Acid 18:3	0.11	0.10	0.15	0.11	0.09	0.08	0.13	0.17
Acid 18:3 SD	0.005	0.001	0.005	0.01	0.004	0.004	0.004	0.0004
Acid 22:0	0.06	0.05	0.06	0.07	0.08	0.08	0.08	0.07
Acid 22:0 SD	0.002	0.0001	0.003	0.001	0.005	0.002	0.001	0.0005
Acid 16:0	0.06	0.04	0.05	0.05	0.05	0.04	0.06	0.05
Acid 16:0 SD	0.01	0.003	0.001	0.002	0.001	0.0001	0.001	0.0002
Acid 17:0	0.04	0.03	0.04	0.04	0.03	0.04	0.05	0.05
Acid 17:0 SD	0.003	0.001	0.001	0.001	0.002	0.0001	0.00004	0.0003
Acid 18:0	0.04	0.03	0.04	0.03	0.04	0.03	0.04	0.04
Acid 18:0 SD	0.01	0.002	0.001	0.0001	0.001	0.0001	0.0002	0.01
Acid 20:0	0.03	0.03	0.06	0.03	0.04	0.02	0.04	0.04
Acid 20:0 SD	0.004	0.0005	0.001	0.0003	0.001	0.0003	0.01	0.002
Acid 24:0	0.03	0.02	0.03	0.03	0.02	0.02	0.03	0.04
Acid 24:0 SD	0.001	0.0001	0.002	0.001	0.002	0.001	0.0002	0.0004
Acid 30:0	0.02	0.01	0.01	0.01	0.03	0.02	0.02	0.01
Acid 30:0 SD	0.0001	0.001	0.001	0.001	0.003	0.0001	0.0005	0.0001
Acid 20:2	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.01

Acid 20:2 SD	0.0004	0.0003	0.0005	0.0004	0.001	0.001	0.0002	0.0002
Acid 23:0	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.01
Acid 23:0 SD	0.002	0.00003	0.001	0.0004	0.01	0.00005	0.001	0.0001
Acid 19:1	0.01	0.01	0.01	0.01	0.01	-	0.005	0.01
Acid 19:1 SD	0.001	0.01	0.002	0.001	0.001	-	0.01	0.001
Other fatty acids	0.02	0.02	0.03	0.02	0.03	0.03	0.04	0.03
Other fatty acids SD	0.002	0.0005	0.002	0.0000005	0.0003	0.0003	0.0002	0.001

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Table S11. Values for Figure S4. 44

Diterpenoids (mg/g of dry matter)	Stump bottom				Crushed stump		Stump heart	
	zero sample	4 weeks	12 weeks	25 weeks	zero sample	12 weeks	4 weeks	12 weeks
Thunbergol	0.13	0.14	0.29	0.11	0.13	0.09	0.34	0.15
Thunbergol SD	0.002	0.01	0.003	0.001	0.01	0.002	0.02	0.01
Delta-13-(*trans*-) neoabienol	0.10	0.07	0.12	0.04	0.11	0.09	0.09	0.09
Delta-13-(*trans*-) neoabienol SD	0.002	0.001	0.0002	0.002	0.01	0.004	0.01	0.005
Palustrol	0.05	0.05	0.08	0.03	0.04	0.05	0.08	0.07
Palustrol SD	0.003	0.001	0.01	0.0003	0.001	0.001	0.01	0.003
Pimarol	0.03	0.02	0.02	0.01	0.03	0.02	0.04	0.03
Pimarol SD	0.001	0.001	0.001	0.0002	0.002	0.001	0.002	0.001
Palustral	0.04	0.03	0.06	0.01	0.02	0.02	0.04	0.05
Palustral SD	0.001	0.002	0.001	0.0004	0.003	0.003	0.01	0.01
Pimaral	0.03	0.01	0.02	0.01	0.04	0.02	0.03	0.02
Pimaral SD	0.003	0.001	0.001	0.0001	0.003	0.001	0.005	0.001
Isopimarol	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.04
Isopimarol SD	0.001	0.0003	0.0004	0.00004	0.001	0.0003	0.0003	0.02
Cis-abienol	0.02	0.01	0.03	0.01	0.02	0.02	0.03	0.03

Cis-abienol SD	0.0003	0.001	0.001	0.0003	0.002	0.001	0.003	0.0002
Methyl neoabietate	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01
Methyl neoabietate SD	0.002	0.0002	0.001	0.0001	0.0003	0.001	0.001	0.0003
Epimanoyl oxide	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
Epimanoyl oxide SD	0.0003	0.0003	0.0003	0.0001	0.00003	0.0003	0.001	0.0003
Methyl abietatetraenoate	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01
Methyl abietatetraenoate SD	0.001	0.0001	0.0002	0.0003	0.00002	0.0002	0.0002	0.001
Isopimaradiene	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Isopimaradiene SD	0.0002	0.0001	0.0003	0.0001	0.0002	0.0001	0.0002	0.0002

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Table S12. Values for Figure S5. 46

Lignans (mg/g of dry matter)	Stump bottom				Crushed stump		Stump heart	
	zero sample	4 weeks	12 weeks	25 weeks	zero sample	12 weeks	4 weeks	12 weeks
Hydroxymatairesinol	0.19	0.25	0.23	0.05	0.08	0.12	0.33	0.21
Hydroxymatairesinol SD	0.05	0.01	0.02	0.01	0.003	0.03	0.04	0.01
Pinoresinol	0.04	0.07	0.08	0.08	0.02	0.02	0.05	0.08
Pinoresinol SD	0.02	0.02	0.01	0.02	0.01	0.01	0.03	0.01
Conidendrin	0.02	0.05	0.08	0.02	0.01	0.01	0.09	0.06
Conidendrin SD	0.001	0.003	0.01	0.002	0.0004	0.003	0.0005	0.003
Oxo-matairesinol	0.02	0.03	0.03	0.01	0.01	0.01	0.05	0.03
Oxo-matairesinol SD	0.001	0.003	0.002	0.001	0.0002	0.001	0.001	0.0001
Lariciresinol	0.01	0.02	0.03	0.01	0.01	0.01	0.03	0.02
Lariciresinol SD	0.001	0.000002	0.003	0.001	0.0001	0.003	0.001	0.001
Todolactol epimer	0.02	0.02	0.02	0.01	0.02	0.01	0.03	0.02
Todolactol epimer SD	0.001	0.0003	0.002	0.001	0.001	0.003	0.001	0.0004
Secoisolariciresinol	0.01	0.01	0.01	0.004	0.01	0.01	0.01	0.03

Secoisolariciresinol SD	0.001	0.001	0.002	0.0002	0.001	0.004	0.001	0.001
Todolactol guaiacylglyceryl-ether	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Todolactol guaiacylglyceryl-ether SD	0.001	0.0002	0.002	0.00004	0.001	0.001	0.0001	0.0001
Matairesinol	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Matairesinol SD	0.001	0.001	0.001	0.00001	0.001	0.001	0.0004	0.00005

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Table S13. Values for Figure S6. 48

Sterols (mg/g of dry matter)	Stump bottom				Crushed stump		Stump heart	
	zero sample	4 weeks	12 weeks	25 weeks	zero sample	12 weeks	4 weeks	12 weeks
Sitosterol	0.16	0.13	0.20	0.18	0.14	0.14	0.21	0.23
Sitosterol SD	0.0002	0.003	0.01	0.003	0.001	0.005	0.001	0.001
Campesterol	0.06	0.05	0.07	0.08	0.04	0.05	0.07	0.07
Campesterol SD	0.00004	0.001	0.002	0.002	0.001	0.0002	0.001	0.0002
7-hydroxysitosterol	0.03	0.03	0.04	0.04	0.02	0.02	0.04	0.03
7-hydroxysitosterol SD	0.02	0.002	0.001	0.01	0.02	0.01	0.004	0.001
Sitostadien-7-one	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.03
Sitostadien-7-one SD	0.004	0.001	0.0004	0.0004	0.0005	0.001	0.001	0.002
Sitostanol	0.02	0.02	0.02	0.03	0.01	0.01	0.02	0.02
Sitostanol SD	0.0002	0.0004	0.001	0.001	0.0003	0.0005	0.000002	0.001
Acid 24:0 monoglyceride	0.003	0.004	0.01	0.01	0.01	0.01	0.01	0.01
Acid 24:0 monoglyceride SD	0.001	0.0002	0.002	0.0004	0.0003	0.0001	0.0005	0.001
Cholesteryl stearate	0.004	0.01	0.01	0.005	0.004	0.01	0.01	0.01
Cholesteryl stearate SD	0.001	0.001	0.001	0.00001	0.0001	0.0004	0.0001	0.00004

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