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Valorization of long-neglected spruce bark and bark-press effluents through integrated utilization of stilbenoids and pectin

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ABSTRACT

Wood bark has exclusively been used for energy at pulp mills, while long-neglected, this huge biomass resource has great potential for valorization. To maximize its value in new ways, here we propose using both fresh (industrial) spruce bark and its bark-press effluents as sustainable sources of natural stilbenoids and pectin. Ultrafiltration removed approximately 90% of the high molecular weight polyphenols as well as enriched stilbenoids from bark-press effluents. Based on a techno-economic analysis, the forest industry would obtain greater revenue potential up to €100 per ton of bark by processing bark side streams compared to their traditional combustion-based strategy if implementing the improved debarking technology. Overall the valorization of stilbenoids and pectin from bark-press effluents or spruce bark offers a promising strategy to produce bio-based, high-value UV-filter ingredients as alternatives to fossil-based ones, and high-grade pectin in an economic feasible and potentially environmentally friendly bolt-on process to the existing mills.

1. Introduction

Land-based mitigation [1], a forest-based bioeconomy [2], and valorisation of cellulose-enriched waste [3] are pathways toward a circular bioeconomy [4] and climate stabilization [5]. Forests are considered to be a giant carbon sink since they absorb more carbon than is released through downstream applications, such as pulp and paper production. [6] Carbon sequestration through sustainable forest management and efficient raw material utilization could partially replace the fossil-based bulk chemicals by resource-wise use of the long-neglected spruce bark from forest industries [6], which would play an important role in tackling climate and environmental issues and building the circular bioeconomy (Fig. 1).

Here we recommend a sustainable process to recover stilbenoids and pectin from undervalued industrial bark and its bark-press effluents. Using a life cycle assessment (LCA) and techno-economic analysis (TEA), the proposed biorefinery strategy significantly improves carbon efficiency and economic viability of bark use when compared to the traditional strategy of burning bark to generate energy. The proposed biorefinery strategy should be considered when designing next-generation pulp and paper mills. This study offers new opportunities to re-visit the concept of “bark biorefinery” for this resource and other abundantly available lignocellulose biomasses in the industry. Most importantly the presented novelty and strategy here could be tailored to other pectin- and stilbene enriched wood bark. If all these active components of the wood bark be utilized, the value of bark is likely to be comparable to that of wood.

In the production of pulp, paper, and packaging products by the forest industry, bark represents 10–15 wt% of spruce (Picea abies) logs [7] which is a long-neglected source of valuable raw materials. In Finland alone, roughly 0.9–1.3 million tons per year of spruce bark is used for energy, despite its untapped resources for other purposes. Spruce wood itself is chemically and morphologically different from the bark. Apart from the traditional chemical constituents found in wood (cellulose, hemicellulose, lignin), spruce bark also contains a significant number of hydrophilic extractives (20 w/w%) and non-cellulosic polysaccharide pectin (10 w/w%). [8–10] Stilbenoids (e.g., astringin, polydatin, isorhapontin) and polyflavonoid tannins [7] are the main constituents of the hydrophilic extractives from bark. By calculation, roughly 6.8 w/w% stilbenoid units are integrally incorporated into spruce bark lignin. [11,12] Stilbene glucosides are naturally synthesized as secondary metabolites by the shikimate pathway and have been
shown to be an effective natural anti-UV agents [13] and to have pharmacological activities (e.g., antioxidant, anti-aging, treatment for Alzheimer’-s-related activities) [14]. Although many such established activities have been reported, trans-isomer glucoside can be isomerized to its cis-isomer form under light, and cis-isomers are prone to undergo a Mallory reaction (also known as electrocyclization), yielding polycyclic aromatic hydrocarbons. [15,16] The global demand for E-stilbene is expected to grow at an almost double-digit rate during 2020–2026 with a global market size exceeding 1.8 billion US dollars (2019) [17], further, the manufacture of stilbenes is currently exclusively synthetic-based [18]. Stilbene-derivatives are widely used as fluorescent brighteners for synthetic dyes [19], liquid-crystals [20], and optical LEDs [21] due to their established photophysical and photochemical properties. A breakthrough in substituting the chemical synthesis approach with the wood bark-derived stilbenes from the forest industry would make spruce bark an attractive sustainable source for the biosynthesis of stilbenes, replacing or reducing the need for petroleum-based bulk chemicals in the current synthesis.

The main structural representatives of pectin polysaccharides include three major fragments in primary cell walls: homogalacturonan (HG), xylogalacturonan, and rhamnogalacturonan I (RG-I). HG is a linear homopolymer chain consisting of 1,4-linked α-D-galacturonic acid (GalA) units. However the skeleton of RG-I is considered to be the “hairy region” of pectin, consisting of alternating 1,4-linked GalA and 1,2-linked rhamnose units, the side chains (e.g., single (or polymeric) unit of galactosyl and arabinosyl residues) are attached to the O-4 of the L-rhamnosyl residues. [22] Pectin has been widely used as a thickener, stabilizer, and gelling agent in the food industry and by pharmaceutical companies. Pectin is mainly produced from fruit waste (i.e., citrus peels, apple pomace) [22]. It is crucial to find additional sustainable ways to supply high-quality pectin (with a high degree of methylation and GalA content) that can meet the growth in demand of the $1.9 billion market (by 2025), which is expected to have a 7.1% annual growth rate during 2016–2025 [23].

2. Materials and methods

2.1. Materials and chemicals

The inner bark (S1) was manually stripped from fifty-year old spruce stems on 30 October 2021, at Inkoo (Finland). The industrial bark samples (S2-S4) and the bark-press effluents S5 were supplied by a Finnish pulp and paper mill. S2 was sampled at the mill yard before the debarking process. S3 was sampled after debarking and before pressing. S4 was sampled after both debarking and pressing. Tiny pieces of wood chips were manually screened from S3 and S4, their yields were neglected and not included in the study. Bark-press effluents S5 were collected in two different seasons, and they were named S5-November (collected in November 2021) and S5-May (collected in May 2017), respectively. The detailed sampling points of the industrial barks (S2-S4) are shown in Fig. 4a. All the collected spruce (inner) bark was freeze-dried and ground into 1 mm powder. The ground samples were stored with aluminum foil at −20 °C before further use. Filtration under the Büchner funnel (qualitative filter paper VWR, particle retention 12 µm) was implemented to remove any water-insoluble particles (yield of 1.33 ± 0.3 mg/ml from S5. Then the filtrates were further concentrated using the rotavapor (BUCHI Labortechnik AG, model R210) before further freeze drying. The lyophilized S5 was preserved at the desiccator with aluminium foil at −20 °C for further use. The mechanical separation was conducted using the ultrasonic cleaner USC 600 TH (Avantor, USA) at 20 °C before further use. Filtration under the Büchner funnel was performed using the rotavapor unit at room temperature. The filtrates were further concentrated using the rotavapor unit at room temperature.

2.2. Experimental flow

The Wiley-milled (<1 mm mesh) spruce (inner) bark was extracted under the Soxhlet unit (Electromantle: ColeParmer Extractors: Lenz) with n-hexane for 2–3 h to remove the lipophilic extracts (Supplementary Fig. 6). Then n-hexane treated (inner) bark underwent a second extraction using the ultrasonic cleaner USC 600 TH (Avantor, Pennsylvania, USA) at the solid-solvent ratio of 1:30 under optimized conditions (60 °C for 20 min). [13] The extracts were first filtered through the membrane (diameter 15–20 µm), and the filtrates were further concentrated using the rotavapor unit at room temperature. The freeze-dried 60% ethanol crude extracts were preserved in their solid form to prevent any further degradation. Then the extract-free solid residue was removed with aqueous citric acid (1:30, w/v, pH 2) at 90 °C for 60 min for chelating pectic polysaccharides. The slurry was again filtered through a membrane (diameter size of 15–20 µm), and the

![Fig. 1. Valorisation of industrial spruce bark and its bark-press effluents for co-production of stilbenoids and pectin. Schematic demonstrating the use of spruce bark between the traditional combustion for energy and a future circular bioeconomy concept.](image-url)
citric acid treated spruce bark was preserved for further analyses. Pure ethanol was added to the liquid (final ethanol concentration 75% (v/v)), and the mixture was kept cold (-5 °C) for pectin precipitation. In the case of S5, the 75% (v/v) ethanol was introduced to chelate the pectin from the lyophilized S5 (l:s 60:1) in the cold room (-5 °C) overnight. Alternatively, the pectin precipitation can be achieved by introducing pure ethanol to adjust the initial liquid form of filtered S5 to a final ethanol concentration of 75% (v/v) (Supplementary Fig. 5). Centrifugation (8000 rpm, Eppendorf 5804R) and freeze drying were implemented to obtain freeze-dried crude citric acid pectin (CAP) from S1-S5. The crude pectin was further purified from dialysis membranes (Spectra/Por, MWCO 6–8 kDa, 96 h). Finally, the collected pectin precipitates were further centrifuged and freeze dried to obtain dialyzed citric acid pectin (DCAP).

Ultrafiltration. Ultrafiltration of the bark-press effluents S5-May was conducted using an ultrafiltration unit (Alfalaval Labstar M20-0.72, Sweden) with 2 kDa (Alfalaval GR 90PP) and 5 kDa (Alfalaval GR 95PP) filters (Supplementary Fig. 5). 700 ml of filtered S5-May was first diluted six times with water to a final volume of 4L. The sample was placed into an ultrafiltration tank, and the filtration was conducted for 2 h. These steps were performed separately for both 2 kDa and 5 kDa filters. After the filtration, the sample filtrates were characterized.

### 2.3. Characterization techniques

Nuclear magnetic resonance (NMR) spectroscopy has been applied for structural feature analysis of n-hexane extracts, stilbenoids, and pectin. Spectra images were processed using a Topspin 4.0 (Bruker). The image contours were colorized using Adobe Illustrator. The detailed experimental parameters are summarized here.

1H, 13C, and HSQC for n-hexane extracts. Both 1D (1H and 13C) and 2D 1H–13C heteronuclear single quantum coherence (HSQC) measurements were conducted using an Avance NEO 600 (Bruker) spectrometer operating at 298 K. [13] DMSO-d_6 / pyridine-d_5 (v/v 4/1) was adopted as the deuterated solvent for chemical shift calibration. The following parameters were applied for 1H NMR (a relaxation delay of 1 s, number scans of 32, and spectra width of 16.0 ppm) and 13C NMR (spectral width of 236 ppm, acquisition time of 0.36 s, and a relaxation delay of 4 s), 2D HSQC NMR spectrum was conducted over the frequency axis at F1 (spectra width of 220 ppm and acquisition time of 1.9 ms), and F2 (spectra width of 13.0 ppm and acquisition time of 65 ms), respectively. A relaxation delay of 2 ns and an acquisition of 16 scans were applied.

1H and HSQC for pectin. 1H NMR spectroscopy was applied to calculate the degree of methylation (DM) and degree of acetylation (DA) of the pectin according to the literature. [24] 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt (TSP-d_4) (5C/6H, 0/0 ppm) was used as the reference both for the chemical shift calibration and 1H NMR quantitation. The following parameters were applied: a relaxation delay of 5 s, number scans of 170, and spectra width of 16.0 ppm, 2D 1H–13C HSQC measurements were conducted using a 400 MHz Bruker Avance III spectrometer with relaxation delay of 2 s, data scans of 1024 data points, an F2 acquisition time of 198.5 ms and 13C (spectral width of 220 ppm and a F1 acquisition time of 2.9 ms) with 150 scans using solvent of DMSO-d_6 / pyridine-d_5 (v/v 4/1) containing internal standard (1,3,5 trioxane). The internal standard was only introduced for samples of S1–S4, and S5-November.

Chemical compositional analysis. The chemical composition of the spruce (inner) bark, the stilbenoid extracts, and pectin followed NREL/TP-510-4261831. The quantitation of the hydrolysed monosaccharide was determined using the HPAEC-PAD, the detailed experimental parameters are summarized in a previous study. [13] The GalA determination by acid hydrolysis was expected to bring a degradation of GalA, so a recovery coefficient of 59.2% was applied for quantification of GalA using an HPLC ( Dionex Ultimate 3000) equipped with the refractive index detector and column module of phenomenex Rezex ROA-Organic Acid H (8 μm, 300 × 7.8 mm, Thermo Scientific, USA). The eluent (0.0025 M H_2SO_4) was set at the flow rate of 0.5 ml/min at temperature of 55 °C.

UV–vis spectroscopy. An UV–vis spectrophotometer ( Shimadzu UV-2550) was employed to test the UV absorption (190–400 nm) for both stilbenoid extracts, authentic standards, and bark-press effluents S5-May, and its ultrafiltrates. Trans-polydatin was included as an external standard for quantitation of the stilbene glucosides using a UV–vis spectrometer. The stilbenoids and phenols were determined based on the linear calibration line that was built at absorption maximum of 320 nm and 280 nm, respectively. [13,25]

GC–MS. 10 mg of n-hexane extracts and crude stilbenoids were solubilized in 500 μl pyridine with tetracosane (C24) included as the internal standard (1 mg ml⁻¹). N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) (300 μl) was added into the mixture at room temperature for 12 h. The quantitative and qualitative measurement was conducted using the Shimadzu GCMS-QP2020SE (QP2010SE with Optic 4) using the column of HP5 (length 30 m; diameter 0.32 mm; film thickness 0.25 μm, Agilent technology). The temperature program was ramped from 80 °C (hold of 5 min) to 285 °C (hold of 10 min) at a rate of 4 °C per min.

GPC. The pectin samples were dissolved in 0.1 M NaCl. A high-performance gel permeation chromatography (GPC) system (Agilent, infinity 1260 quaternary pump) including a refractive index detector. The detailed experimental parameters are the same as reported previously. [26] Briefly: three Waters 7.8 mm × 300 mm Ultrahydrogel columns (500 Å, 250 Å, and 120 Å) with a 6 mm × 40 mm Ultrahydrogel guard column were used with the flow rate of 0.5 ml/min for separation of pectin using 0.1 M NaCl as eluent. The injection volume was 100 μl. The narrow dispersity pullulan standards were used to calibrate the columns and determine the absolute molar masses for pectin.

### 2.4. Techno-economic analysis (TEA)

Techno-economic analysis was performed for the baseline case and all scenarios using annual cash flow analysis, [27] accounting for incomes, capital cost (CAPEX) and operational cost (OPEX) (Supplementary Excel Sheet_B). Due to the existence of bark press, combustion and WWT processes in the baseline case, their capital costs were not considered in the calculation. The annual cash flow of the baseline includes incomes from the sale of electricity, and cost of wastewater treatment as well as the labour. For other scenarios, extra equipment cost was either estimated via scaling up and down in terms of NREL’s technical report [28] or from external sources and vendor. A 30-year depreciation was used for CAPEX evaluation. The equipment and labour costs were indexed with a Chemical Engineering Plant cost index (CEPCI) in their reference year to 596.2 in 2020. Costing information and their sources were documented in Supplementary Excel Sheet_C. In particular, the estimated price of stilbenoids (12.1 Euro/kg) is based on the average marketing values of the existing fossil-based UV blockers (2-ethylhexyl-4-methoxy cinnamate; benzophenones; phenolic
benzotriazoles) [29]. Processing cost that is associated with stability enhancement of stilbene is out of scope of this present study. Furthermore, diafiltration purification of pectin is not considered at our TEA assessment, however, the yield of pectin is after diafiltration to be conservative.

2.5. Life cycle assessment (LCA)

Following ISO standard 14,040 series, [30] LCAs for the bolt-on processes were performed using Simapro® (Version 9). Their system boundaries were illustrated in Fig. 4a and set as “cradle-to-gate”, including bark received at the plant, extraction, solvent recovery, and spray-drying of products. Chemicals and energy inputs as well as emissions during these processes were included in the system boundary. The functional unit was treating 1 ton of bark from a pulp and paper mill. Inventory data were provided in Supplementary and Supplementary Excel Sheet. The system expansion was applied as the allocation method to treat the main product, stilbenoids, which is intended to replace fossil-based UV filters (e.g., octabenzone). To be conservative, credits from other by-products (e.g., resin acids and pectin) replacing the conventional ones were not claimed. During the environmental impact assessment, CML 2001 was applied, and indicators were: abiotic depletion potential elements (ADP elements, kg Sb eq.), global warming potential (GWP, kg CO₂ eq.), ozone depletion potential (ODP, kg R11 eq.), eutrophication potential fossil (ADP fossil, MJ), global warming potential (GWP, kg CO₂ eq.), ozone depletion potential (ODP, kg R11 eq.), acidification potential (AP, kg SO₂ eq.), photochemical ozone creation potential (POCP, kg Ethene eq.), marine ecotoxicity potential (MEEP, kg DBP eq.), terrestrial ecotoxicity potential (TEEP, kg DBP eq.), and human toxicity (HT, kg DBP eq.).

3. Results

3.1. Composition of industrial spruce bark and bark-press effluents

To acquire an understanding about chemical profiles of materials (Supplementary Fig. 1-6), the overall chemical and carbohydrate composition were studied (Supplementary Fig. 7). Generally, the differences in the carbohydrate composition between the spruce inner bark (S1) and other industrially available spruce barks (S2-S4) were minor, while their overall chemical composition (i.e., lignin and extractives) exhibited significant variations from S2 to S4. The Klasson lignin content of S1 is almost less than half amount compared to that of the industrial barks (S2-S4), which indicates an over-contribution of lignin from other acid-insoluble components (i.e., suberin, protein, ash, fat) that are more abundantly present in highly heterogeneous outer bark compared to the inner bark. This is also supported by a significant reduction of Klasson lignin in the samples after extraction (S1_E to S4_E) (Supplementary Fig. 8-11). Furthermore the content of acetone extracts reduced almost two-fold from S2 to S3 (and S4), indicating that the debarking process dissolved part of the hydrophilic extractives. Pectin-characteristic monosaccharides (arabinose, rhamnose, GalA) indicated a presence of pectin in S1-S5. The significant presence of glucose and xylose indicated that cellulose and hemicellulose are other underutilized components of the industrial bark, but this subject requires further investigation that is not within scope of the present study.

As expected, the chemical compositions based on gas chromatography-mass spectrometry (GC-MS) of the hexane extracts from the spruce bark contained most of the resin acids found from spruce (pimaric, isopimaric, palustric, sandaracopimaric, dehydroabiatic, and neoabiatic acids), fatty acids (linoleic, oleic, linoleic, and palmitic acids), diterpenes (13-epimanoxy oxide and 15-hydroxydehydroabiatic acid), and sterols (25-hydroxycholesterol, beta-sitosterol acetate, campesterol, and beta-sitosterol) (Supplementary Fig. 12 and Supplementary Table 1). Similar identifications of the extracts have been reported previously for spruce bark using solvents of n-hexane/dichloromethane mixture (1:1; v/v) [31] or n-hexane alone [32]. The assignment of the mass spectra was referenced to the literature [33-35] and publicly available databases. In the HSQC NMR spectra (Supplementary Fig. 13-17), three well-resolved signals at 8c/6H of 123.87/ 7.114 ppm (C8/H8), 123.66/ 6.939 ppm (C14/H14), and 126.35/ 6.804 ppm (C12/H12) indicated the strong presence of dehydroabiatic acid. A clear cluster at the 8c/6H ranges (10-50 /0-3 ppm) highlights the aliphatic chains [36] of the fatty acids, indicating that the n-hexane extracts contain oleoresin and physiological resin-based extractives [37], which are located at parenchymal cell and resin canals of the spruce bark. Overall the subsequent extraction of stilbenoids and pectin was maximized due to the enhanced solvent penetration and solute diffusion by the prior removal of n-hexane extracts (at a yield of 3.4 to 8.6 w/w%, Table 1).

3.2. Mapping and ultrafiltration of stilbenoids

Full characterization and quantitation of the stilbenoids was carried
out by NMR (Fig. 2, Supplementary Fig. 18-23) and GC-MS (Supplementary Fig. 24 and Table 1), the presence of stilbenoids were confirmed in 60 % (v/v) ethanol extracts from S1-S5. 8.8 w/w% stilbenoids were quantified in the spruce inner bark (S1), which was consistent with the literature. [13,38] As expected, the stilbenoids were reduced from 5.1 w/w% (S2) to 1.4 w/w% (S3), and 1.2 w/w% (S4) due to the additional process steps of debarking and pressing, respectively. Another hypothesis would be that some of the stilbenoids are lost due to their poor stability since they are sensitive to light and temperature. [39] For example, trans-isomers of stilbene glucosides can be isomerized into cis-isomers under light and can be further photodegraded into phenanthrene under UV irradiation. [40] Encapsulation stilbene in carriers (e.g., liposomes, cyclodextrins, polymeric nanoparticles) and structural modification of stilbenes (e.g., hydroxylation, methoxylation, glycosylation, halogenation) are considered to be the promising strategies to improve their stability. [14,41] A systematic study will be implemented to suppress the undesired isomerization by building ultrastable UV-filters in sunscreen.

The GC chromatogram and the mass spectra of the trimethylsilyl derivatives of astringin, isorhapontin, and polydatin are plotted (Supplementary Fig. 24). The characteristic m/z 361 fragment indicates the glycosidic part of the stilbene glucosides. For the first time, the stilbenoids were also identified from the solid form of the bark-press effluents (S5), which were previously observed in the debarking wastewater of spruce bark [42]. Generally, the bark-press effluents (S5) and the debarking effluents are currently collectively recycled to the wastewater treatment (WWT) plant for biological treatment at the pulp and paper mill. If the stilbenoids could be extracted prior to WWT, it could not only reduce the chemical oxygen demand (COD) burden of the subsequent WWT but could also provide added value because of the novel biochemicals that could be produced. The presence of the monosaccharides in spruce inner bark and spruce bark was confirmed by both GC-MS and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Table 1), and the increased monosaccharide content after the acid hydrolysis was attributed to the cleavage of the glycosidic bond that is present in the stilbene glucosides (Supplementary Fig. 25). Furthermore, the presence of sucrose in S1-S4 was attributed to be a known cryoprotectant compound for responding to cold weather, which was also reported previously for willow bark. [13] The abundant presence of the monosaccharides (glucose and fructose) and disaccharide (sucrose) may be explained by the fact that these saccharides were easily solubilized at the debarking and bark-press (S5) stages (Fig. 4a).

Catechin and quinic acid (as an oxidation precursor of gallic acid) present in the extracts are typically considered to be the building blocks of polyflavonoid tannins, [43] which corresponds with the not-yet-identified components of the spruce bark 60 % (v/v) ethanol extracts (Table 1). The average molecular weight (Mw) and polydispersity index (PDI) of spruce bark tannins were reported to be 3.20–3.40 kDa and 1.68–1.84, respectively, which was close to the bark tannins from Douglas fir (Mw 5.71 kDa, PDI 2.66) and loblolly pine (Mw 11.6 kDa, PDI 3.54). [7] Furthermore ultrafiltration has been previously demonstrated for its effectiveness in tannin extraction from spruce bark. [44] Thus the possibility of ultrafiltration to reduce the tannin concentrations with 2 kDa and 5 kDa filters was studied for the bark-press effluents S5. The molecular weight cut-off of the filters was selected to be close to the reported Mw of the spruce bark tannins. Both 2 kDa and 5 kDa filters were effective (Supplementary Table 2) in removal of approximately 90% of high molecular weight polyphenols (e.g., polyflavonoid tannins) and polysaccharides based on the calculations from the UV–vis spectra.

![Fig. 2. 2D $^1$H–$^{13}$C heteronuclear single quantum coherence (HSQC) NMR spectrum (6C/8H, 29–131/2.0–8.0 ppm) of the 60 % (v/v) ethanol extracts from S1–S4 and the lyophilized bark-press effluents (Supplementary Fig. 5) (S5-November; S5-May; and ultrafiltrates S5-May-5 kDa; S5-May-2 kDa). Assignment of the main signals from stilbene glucosides: astringin (A, dark blue); isorhapontin (I, light blue) and polydatin (P, red), see S1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image-url)
Supplementary Fig. 26), which could be also noticed from the significant brown color reduction of the lyophilized and redissolved samples from the dark brown color (S5-May) to lemon yellow 2 kDa filtrate (S5-May-2 kDa) (Supplementary Fig. 5). Both selected filter sizes managed to preserve the stilbenoids from the feed at the laboratory scale, and this was also confirmed qualitatively by HSQC NMR in Fig. 2. Overall the ultrafiltration (both with 2 kDa and 5 kDa Mw cut-off) proved to be a viable technique for enrichment and purifying stilbenoids from the high molecular weight polyflavonoid tannins for the industrial spruce bark extracts.

3.3. Structural characteristics of pectin

Citric acid aqueous solutions were used to chelate pectic polysaccharides from the spruce bark. Compared with other strong mineral acids, extraction with citric acid is known for retaining pectin’s native structure to its maximum extent. [22,23] The pectin yield ranged from 5 to 8 w/w% for all samples, which agreed with the literature [45]. The dialysis treatment removed almost two-thirds of the small molecular weight fractions from the crude pectin. However the recovered pectin was only a small fraction of the original total amount of pectin present in the spruce bark (Supplementary Fig. 8–11). Although dialysis of the citric acid pectin (CA-P) had minimal effect on the relative monosaccharide composition (Table 2), the treatment resulted in an almost two-fold increase of the GalA in all pectin samples from S1-S5. The relative ratio between the rhamnose and GalA was roughly 10 times higher for pectin from S5 than from S1-S4, indicating that there was a much lower presence of HG fragments at SS-CA-P. Furthermore, the presence of GalA in pectin (SS-CA-P) was significantly reduced (Table 2), indicating that the HG domains could be more easily hydrolysed during the pressing stage. However both the HG and RG-I domains were abundant in the pectin that was recovered from spruce bark (S1-S4). The high ratio of (Gal + Ara)/Rha indicated that the RG-I domains were highly branched in pectin that was recovered from both the spruce bark (S1-S4) and the bark-press effluents S5. The glucose in the industrial-bark-derived pectin (S1-CA-P to S4-CA-P) likely originated from starch since a similar occurrence has been reported for willow bark [13].

The solution-state 2D HSQC NMR spectra (Fig. 3) revealed the typical interunit linkages of pectin. All spectra were assigned based on the literature. [46–48] Three clear signals at δC/δH of 56.0/3.80, 23.8/2.15 and 19.5/1.25 ppm indicated the presence of methyl groups of 1,4-α-d-GalpA(OMe), 1,4-α-d-GalpA(OAc) and rhamnose from S1-S4, respectively. The non-anomeric methylene (δC/δH of 69.0/3.75 ppm, 69.6/3.8 ppm, 74.0/4.72 and 83.0/4.25 ppm) was confirmed for the presence of GalA at S1-S4, however not in S5. Specific non-anomeric and anomeric methine signals revealed the presence of arabinofuranosyl groups (terminal and 1,3-, 1,5-, 2,5-, 1,3,5- and 1,2,3,5-linked) and galactopyranosyl groups (terminal and 1,4-linked) from the pectin that was recovered from S1-S5. Similar types of arabinofuranosyl and galactopyranosyl linkages have been reported from a pectin fraction (recovered by pressurised hot water extraction) of spruce bark through GC–MS. [45] Strong starch (1,4-α-d-GlcP) [49] signals were present in S1-S4, which suggests that starch could be covalently linked with the pectin in spruce bark; however this phenomenon was not observed in S5. Spruce bark pectin characteristics are: highly methylated and acetylated, a high proportion of HG domains and low proportion of the highly branched RG-I regions. However the main chemical characteristics of pectin from S5 was its richness in highly branched RG-I domains. Overall, this information could provide insights into the chemical composition and structure of spruce bark pectin.
possible design of the customized pectinase consortium for carrying out enzymatic degumming of spruce bark, similar to the microbial consortium that was tailored for willow bark [48]. However this is not within scope of this present study.

3.4. The techno-economic analysis and life cycle assessment

According to the above experimental results, Aspen Plus® v12 was applied to conduct process simulation prior to the techno-economic analysis (TEA) and life cycle assessment (LCA). The energy required...
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value-added products. However diverting the bark (S4) to produce pectin and stilbenoids during the debarking process. To improve the debarking technology was proposed, which would avoid the loss of pectin, which was precipitated by adding ethanol and spray dried. The ethanol via distillation. The crude stilbenoids were further purified by yield, weight-average molecular weight (Mw), polydispersity (Mw/Mn) and chemical composition of ethanol precipitated pectin before (CA-P) and after dialysis (DCA-P) from S1 to S5-November. HG and RG-I contents (mol %) were calculated from their monosaccharide composition. Degree of methylation (DM) and degree of acetylation (DA) were calculated from quantitative 1H NMR. Standard deviations are shown in parentheses based on two independent measurements. For clarification of the abbreviations, see Supplementary Fig. 6. For monosaccharide contents of pectin before and after the dialysis treatment, see Supplementary Fig. 27.

Table 2

<table>
<thead>
<tr>
<th>S1-CA-P</th>
<th>S1-DCA-P</th>
<th>S2-CA-P</th>
<th>S2-DCA-P</th>
<th>S3-CA-P</th>
<th>S3-DCA-P</th>
<th>S4-CA-P</th>
<th>S4-DCA-P</th>
<th>S5-CA-P</th>
<th>S5-DCA-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pectin Yield % (w/w bark)</td>
<td>6.3</td>
<td>2.1</td>
<td>4.9</td>
<td>1.8</td>
<td>7.2</td>
<td>2.2</td>
<td>8.4</td>
<td>2.7</td>
<td>6.2</td>
</tr>
<tr>
<td>Mw (kDa)</td>
<td>800 (76)</td>
<td>–</td>
<td>512 (34)</td>
<td>–</td>
<td>572 (3)</td>
<td>–</td>
<td>467 (3)</td>
<td>–</td>
<td>467 (75)</td>
</tr>
<tr>
<td>Mw/ Mm</td>
<td>2.5 (0.9)</td>
<td>2.4 (0.03)</td>
<td>–</td>
<td>2.9 (0.1)</td>
<td>–</td>
<td>2.5 (0.1)</td>
<td>–</td>
<td>2.5 (0)</td>
<td></td>
</tr>
<tr>
<td>Monosaccharides mg g⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ara</td>
<td>77 (0.1)</td>
<td>141 (3.1)</td>
<td>62 (1.8)</td>
<td>125 (0.3)</td>
<td>70 (0.2)</td>
<td>144 (0.3)</td>
<td>59 (0)</td>
<td>109 (0)</td>
<td>72 (0.1)</td>
</tr>
<tr>
<td>Rha</td>
<td>20 (0.0)</td>
<td>37 (0.4)</td>
<td>16 (0.4)</td>
<td>27 (0.2)</td>
<td>15 (0)</td>
<td>36 (0.1)</td>
<td>17 (0.1)</td>
<td>34 (0.2)</td>
<td>31 (0)</td>
</tr>
<tr>
<td>Gal</td>
<td>35 (0.1)</td>
<td>77 (1.8)</td>
<td>30 (0.4)</td>
<td>64 (0)</td>
<td>40 (0.1)</td>
<td>97 (0.2)</td>
<td>33 (0)</td>
<td>73 (0.1)</td>
<td>115 (0.1)</td>
</tr>
<tr>
<td>Glc</td>
<td>96 (0.1)</td>
<td>138 (3.2)</td>
<td>75 (0.2)</td>
<td>129 (0.9)</td>
<td>125 (0.6)</td>
<td>239 (0.7)</td>
<td>87 (0.2)</td>
<td>171 (1.6)</td>
<td>64 (0)</td>
</tr>
<tr>
<td>Xyl</td>
<td>6 (0.2)</td>
<td>9 (0.2)</td>
<td>7 (4.2)</td>
<td>8 (0)</td>
<td>7 (0.1)</td>
<td>14 (0.1)</td>
<td>5 (0)</td>
<td>9 (0)</td>
<td>19 (0)</td>
</tr>
<tr>
<td>Man</td>
<td>0</td>
<td>4 (0.7)</td>
<td>4 (1.7)</td>
<td>10 (0.1)</td>
<td>14 (0)</td>
<td>28 (0.2)</td>
<td>9 (0.1)</td>
<td>0 (0)</td>
<td>34 (0.2)</td>
</tr>
<tr>
<td>GalA</td>
<td>224 (13)</td>
<td>474 (52)</td>
<td>290 (1.1)</td>
<td>569 (32)</td>
<td>277 (0.7)</td>
<td>323 (5.6)</td>
<td>288 (2.1)</td>
<td>428 (4.8)</td>
<td>23 (0.1)</td>
</tr>
<tr>
<td>Overall</td>
<td>457 (13)</td>
<td>880 (59)</td>
<td>485 (6.1)</td>
<td>932 (31)</td>
<td>547 (0.5)</td>
<td>881 (6.5)</td>
<td>498 (2.3)</td>
<td>825 (3.0)</td>
<td>358 (0.4)</td>
</tr>
<tr>
<td>Molar composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rha/GalA</td>
<td>0.1 (0)</td>
<td>0.09 (0)</td>
<td>0.1 (0)</td>
<td>0.1 (0)</td>
<td>0.1 (0)</td>
<td>0.1 (0)</td>
<td>0.1 (0)</td>
<td>0.1 (0)</td>
<td>1.6 (0)</td>
</tr>
<tr>
<td>(Gal + Ara)/Rha</td>
<td>6 (0)</td>
<td>6 (0.1)</td>
<td>6 (0)</td>
<td>7 (0.1)</td>
<td>8 (0)</td>
<td>7 (0)</td>
<td>5 (0)</td>
<td>5 (0)</td>
<td>6 (0)</td>
</tr>
<tr>
<td>HG (mol %)</td>
<td>41 (1.5)</td>
<td>45 (2.5)</td>
<td>52 (0.7)</td>
<td>54 (1.6)</td>
<td>44 (0.1)</td>
<td>29 (0.4)</td>
<td>50 (0.2)</td>
<td>44 (0.4)</td>
<td>n.d</td>
</tr>
<tr>
<td>RG-I (mol %)</td>
<td>37 (1.0)</td>
<td>37 (1.7)</td>
<td>29 (0.3)</td>
<td>30 (0.9)</td>
<td>29 (0)</td>
<td>39 (0.2)</td>
<td>29 (0.2)</td>
<td>34 (0.1)</td>
<td>72 (0.1)</td>
</tr>
<tr>
<td>DM (%)</td>
<td>61 (3.2)</td>
<td>57 (3.3)</td>
<td>60 (2.7)</td>
<td>54 (1.7)</td>
<td>43 (0.3)</td>
<td>62 (2.3)</td>
<td>62 (3.3)</td>
<td>62 (1.0)</td>
<td>n.d</td>
</tr>
<tr>
<td>DA (%)</td>
<td>13 (0.1)</td>
<td>13 (0.1)</td>
<td>9 (0.2)</td>
<td>9 (0.3)</td>
<td>12 (0.2)</td>
<td>20 (0.1)</td>
<td>13 (0.5)</td>
<td>15 (0.1)</td>
<td>n.d</td>
</tr>
</tbody>
</table>

for evaporation of hexane and distillation of ethanol, as well as electricity for centrifuge and pumps were estimated and included to that of both analyses. The capacity of a Finnish pulp and paper mill is assumed to produce 219,000 tons of air-dried pulp per year, equivalent to that of a typical pulp and paper mill in the country. The amount of bark obtained was estimated based on the ratio of 0.47 t pulp/t wood and 1/9 t bark/t wood. [50] The baseline case is illustrated in Fig. 4. After pressing of the bark, bark-press effluents (S5) were treated in a WWT plant whilst the solids (S4) were combusted to produce electricity. As a bolt-on process before WWT, S5 went through centrifuge to remove solids and mixed with ethanol solution until 75 % (v/v) to precipitate at a cool temperature, yielding pectin. The resultant stream went through ultrafiltration where stilbenoids were obtained and pelleted via spray dryer (S5 W/EtOH). For S4 with moisture content around 70%, sequential extraction where stilbenoids were obtained and pelleted via spray dryer (S5 W/EtOH). While the obtained solid stream was extracted by citric acid to yield pectin, which was precipitated by adding ethanol and spray dried. The current debarking technology is based on the rotary debarker, involve usage of warm water for the debarking purposes in a pulp and paper mill. As a scenario of the future (S2), the debarking with the improved debarking technology was proposed, which would avoid the loss of pectin and stilbenoids during the debarking process. To improve the economic feasibility for all scenarios, the analysis of prospective scenarios was conducted by (1) removing ethanol extraction from S5 with stilbenoids as the only product (S5 W/EtOH) and (2) changing citric acid (S4, CA; S2, CA) to HCl (S4-HCl; S2-HCl). These assumptions were evaluated to have technological feasibilities based on the communication with our industrial partners. Information about scenario setups, their mass and energy flows as well as economic results are detailed in Supplementary Table 3 – Table 6 and Supplementary Excel Sheet A. Compared to the baseline, the bark-press effluents stream S5 (without ethanol pre-extraction) could yield profit with stilbenoids as value-added products. However diverting the bark (S4) to produce pectin and stilbenoids is not preferable from an economic perspective due to the high energy demand to recycle the extraction solvent ethanol and the additional capital costs associated with the bolt-on process. In the future scenarios (S2) where the improved debarking technology is applied, its economic performance could be significantly enhanced because of the higher yields of value-added stilbenoids, pectin, and resin acids. Furthermore the switch of acid from citric acid to HCl would improve its economic feasibility. [23] From a sustainability perspective (Fig. 4c.), some environmental savings indicated by negative values in comparison to the baseline were observed for S5. This is because the credits from stilbenoids replacing fossil-based UV filters overwhelmed environmental burdens induced by chemicals and energy inputs (Supplementary Table 7-Table 8, Supplementary Excel Sheet D). Overall, the proposed bolt-on processes using bark-press effluent side streams provides an economically feasible upgrade to improve profit of the existing pulp and paper mills and promote their environmental sustainability. The proposed innovative process of the future with the improved debarking technology could potentially deliver an annual profit of €20 million, if implemented in new greenfield projects of the pulp and paper industry in the future.

4. Discussion

We designed a holistic approach to improve the economic feasibility of the pulp and pulp mills by integrating bark valorization through recovery of stilbenoids and pectin from underutilized spruce bark, a side stream of the pulp and paper industry. For the first time, the stilbenoids were quantitatively characterized and their presence was qualitatively confirmed in spruce inner bark (8.8 w/w%), industrial bark (1.2–5.1 w/w%), and bark-press effluents (9.6 w/w% solid form) based on NMR and GC–MS analysis. Furthermore, ultrafiltration with both 2 kDa and 5 kDa filters was effective in removing roughly 90 w/w % of high molecular weight polyphenols from the 60 % (v/v) extracts, which has a high potential to be an industrially applicable process step for stilbenoids purification. Pectin chemistry from spruce bark was also elucidated by HSQC NMR for the first time. Characteristics of spruce bark pectin included highly methylated and acetylated HG regions, a low proportion of RG-I domains, and low branched hairy regions. The pectin characteristics were different between the industrial bark and bark-press effluents. By rough calculation, over one-hundred-thousand tons of stilbenoids and pectin produced by the spruce bark of the Finnish pulp and paper mills every year are currently not utilized, instead part of these compounds (or in their chlorinated form) [51] are a wastewater
five times, increased the bark-press effluent stream. In addition, the improved debarking technology that could be implemented in the future would enable the yields of stilbenoids, pectin, and resin acids, which could further improve the profitability up to £20 million per year for a medium sized pulp mill (Supplementary Excel Sheet A1). Overall we believe that the strategies presented in this work would aid in the decision-making process for designing greener, more advanced modern pulp and paper mills that utilize the wood bark to produce novel value-added products that are more environmentally friendly, industrially valuable, and socially more acceptable and beneficial processes directly addressing the challenges of circular bioeconomy. If the spruce bark’s pure stilbene were to be used in sunscreen instead of the millions of tons of synthesized UV-filters used in these products today, this would contribute positively to the mitigation of climate change and reduce the impact on marine ecosystems.

5. Data availability

The data supporting the findings of this study are available from the corresponding authors upon reasonable request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

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References


