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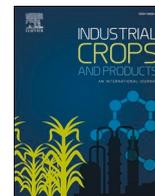
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# Optimising and scaling up hot water extraction of tannins from Norway spruce and Scots pine bark

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## ABSTRACT

Tannins from Norway spruce (*Picea abies* [L.] Karst.) and Scots pine (*Pinus sylvestris* L.) bark were extracted with water at different temperatures (60–140 °C) in an ASE-350 system in order to optimize yield. In addition, the effect of chemicals such as urea, sodium bisulfite (NaHSO<sub>3</sub>), sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), and sodium benzoate on the yield was also investigated. Bark from debarking processes at both a sawmill and a pulp mill were included. The highest overall yield expressed as total dissolved solids (TDS) was obtained with hot water extraction of spruce bark at 140 °C. The TDS was 117 mg/g and it contained 47 mg/g tannins. With an increase in extraction temperature over 100 °C, the proportion of tannins decreased, whereas the proportion of carbohydrates increased. The addition of sodium carbonate improved yield within a 60–90 °C temperature range compared with pure water. Other chemicals did not improve the yield. Pine bark showed similar extraction yields to spruce bark but the proportion of tannins was lower in spruce than in pine. Pure water at 110 °C was chosen to be used for piloting in larger scale 300-liter extraction vessel. Based on the results, a machine-learning approach was applied using seemingly unrelated regression models (SUR). The models were able to predict the extracted tannin yields of spruce and pine bark when extractions were scaled up to 2 liters and then to 300 liters.

## 1. Introduction

Softwood bark is under intensive research since it is a source of many valuable biochemicals and may provide a sustainable alternative to fossil sources of chemicals such as phenolic adhesives. Bark is widely available resource in Nordic countries. Debarking at pulp mills and at saw mills produces a side stream of 10 % of wood volume. Bark structure and chemistry is complex and it is a truly recalcitrant material for bio-refining. Thus, it is mainly used for combustion (Barjoveanu et al., 2020).

Industrial spruce and pine bark may contain inner and outer bark and sapwood in various proportions depending on the debarking process. The inner and outer bark of Scots pine has been reported to contain a variety of extractable compounds such as sugars, lignans, flavonoids, catechins, and procyanidins (Bianchi et al., 2014; Karonen et al., 2004, 2004; Matthews et al., 1997; Pan and Lundgren, 1996; Raitanen et al., 2020). Stilbene and stilbene glucosides can be extracted from Norway spruce bark and they are effective antioxidants (Jyske et al., 2020, 2014; Krogell et al., 2012; Mulat et al., 2014). Moreover, some hydroxystilbene structures are found to be incorporated in the lignin in Norway

spruce bark and can further create complex polyphenol structures (Rencoret et al., 2019). Hot water can extract different amounts of compounds from inner and outer bark of Scots pine and Norway spruce (Raitanen et al., 2020). The extraction yield of tannin was higher for whole spruce bark (81.2 mg/g) than that of pine bark (36.0 mg/g). In both cases, more tannins were extracted from inner bark than from outer bark.

Tannins can be defined by their leather tannin function and can be divided into condensed and hydrolysable tannins (Pizzi, 2008). These compounds have been extracted from different types of biomass using solvents such as hot water, supercritical fluids, ionic liquids, and methods such as pressurized or subcritical water, ultrasound, or microwave-assisted extraction (de Hoyos-Martínez et al., 2019). Water-based extractive techniques are an environmentally friendly way to extract biomass. Water and steam are available at pulp mills and in the food industry and methods to concentrate and recycle them after extraction processes are available. Hot water has been used to extract carbohydrates from spruce and pine bark for ethanol production (Kemppainen et al., 2012).

The extraction of tannins can be part of a cascading process (Rasi

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et al., 2019), where bark is comprehensively utilized by first drawing out tannins with hot water, then applying a pyrolysis process to produce biochar, and finally using the pyrolysis oil for anaerobic gasification in order to improve cost efficiency and feasibility. The extraction temperature and chemical additions all have an effect on tannin yield, which can vary considerably even within one tree species (Bianchi et al., 2016). The concentration and purification steps for producing tannins from spruce bark can have a large environmental footprint (Carlqvist et al., 2020; Ding et al., 2017) and thus, processes need to be chosen carefully depending on applications and end-use.

The applications of tannins include adhesives, foams (Feng et al., 2013), antioxidants, antivirals, anti-inflammatory agents (Fraga-Corral et al., 2020; Jablonsky et al., 2017), corrosion inhibitors, ingredients for beverages and food (Granato et al., 2022; Pap et al., 2021; Serrano et al., 2009), adsorbents for proteins and antibiotics, and medicinal applications (Shirmohammadi et al., 2018). Two of the most promising industrial applications for tannins are as cures in the traditional leather industry and as adhesives (Pizzi, 2019).

The aim of this study was to optimize tannin yield from spruce and pine bark by using water and added chemicals at different temperatures. Softwood bark was selected from both pulp mills and sawmills. Since our goal was to use tannins as adhesives, we hypothesized that the tannin portion of extracted dry matter has an effect on reactivity when used in formaldehyde-based resins. We also hypothesize that there is an optimum temperature for tannin yield and that with the help of machine learning, we can predict tannin extraction rates based on assessing results at laboratory scale (100 mL) and scaling them up to 2 L and 300 L.

## 2. Materials and methods

### 2.1. Spruce and pine bark

Bark material for the extraction of tannins was provided by Metsä Fibre, Finland. Samples of Norway spruce and Scots pine bark were obtained both from pulp mills and sawmills. They included altogether four different batches (Table 1).

The sample codes are: Norway spruce bark (Spruce S = bark from Metsä-Fibre Renko sawmill, spruce P = bark for Metsä-Fibre Joutseno pulp mill) or Scots pine bark samples (Pine S = bark from sawmill Metsä Fibre Kyrö sawmill, Pine P = bark from Metsä-Fibre Joutseno pulp mill) were included in the experiments. Samples were milled using a ATREX® mill by Megatrex (Lempäälä, Finland). An Atrex mill is based on two counter-rotating rotors, the design of which can be adjusted to various materials. During processing, the material is subjected to very strong shear, turbulence, and impact forces. Grinding results were controlled by adjusting the rotating speed of the rotors. One sample was ground with a Fritsch Pulverisette laboratory mill (Idar-Oberstein, Germany), which had crushing and cutting blades (Table 1). Fig. 1 shows the results of milling of the bark. In addition, one sample of spruce bark (Spruce M) was obtained for the 300 L pilot-scale extraction test from a pulp mill (Metsä Fibre, Rauma) and milled using a Megatrex Atrex mill (Megatrex, Lempäälä, Finland).

**Table 1**

Bark samples of Norway spruce (*Picea abies* [L.] Karst.) and Scots pine (*Pinus sylvestris* L.) provided for extraction either from a sawmill (S) or a pulp mill (P). Milling was done by an ATREX® mill based on two counter-rotating rotors (Megatrex) except for one pine sample.

Sample code	Provider	Source	Species	Mill	Dry weight %
Spruce S	Metsä Fibre	Sawmill	Spruce	Megatrex	45.5
Spruce P	Metsä Fibre	Pulp mill	Spruce	Megatrex	43.8
Spruce M	Metsä Fibre	Pulp mill	Spruce	Megatrex	74.2
Pine S	Metsä Fibre	Sawmill	Pine	Megatrex	47.9
Pine P	Metsä Fibre	Pulp mill	Pine	Fritsch	36.1

### 2.2. Bark extractions

#### 2.2.1. Extractions with ASE-350

Bark samples were extracted with pure water or water with chemical additions at different temperatures. Chemicals were added in order to improve the extraction yield of tannins. Extractions were performed at laboratory scale using an ASE-350 (Thermo Fisher, Waltham, USA) extraction system. This accelerated solvent extraction (ASE) system extracts compounds from solid and semi-solid samples quickly with a small solvent volume. A smaller solvent volume and less time is needed than with traditional techniques such as Soxhlet or sonication extractions that are commonly used in biomass extractions. Each extraction cell had a cellulose filter with 1.2 µm pore size and a stainless steel frit of 10 µm pore size to separate solids and liquids.

Four different chemicals including urea, sodium bisulfite (NaHSO<sub>3</sub>), sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), and sodium benzoate were applied prior to extraction process. Extraction conditions and chemical additions are shown in Table 2.

Extraction temperatures varied between 60 °C and 140 °C and extraction time was always 120 min. Before the extractions, samples were dried at 105 °C overnight in order to determine their dry matter content. Based on dry mass, the amount of fresh bark was adjusted to include 10 g of dry bark in a 100 mL extraction vessel. The amount of water in the extraction vessel was adjusted by reducing the volume that the bark occupied. The volume of the bark sample was calculated using 1.5 g/mL density for a solid bark fraction; thus, 10 g of dry bark would take up 6.7 mL of the extraction vessel's volume and the vessel was filled to 100 mL with water. Since the moisture content of fresh bark samples varied, the concentration of extraction chemicals needed to be adjusted to ensure they were the same for all samples. Fresh samples were chosen for each extraction since they represent actual samples that would be used in industrial processes.

To give an example, the contents of a spruce bark sample from a sawmill are extracted using water with the addition of Na<sub>2</sub>CO<sub>3</sub>. A total of 22 g of fresh bark is used, equivalent to 10 g dry bark and 12 g of initial water content. The bark occupies 6.7 mL of the volume of the extraction vessel. The amount of additional water required to fill a 100 mL extraction vessel is therefore:

100 mL (vessel's volume) - 6.7 mL (solid bark fraction) - 12 mL (water content of fresh bark) = 81.3 mL.

If the final concentration of Na<sub>2</sub>CO<sub>3</sub> in the extraction vessel is aimed to be 5 g/L, a total of 5.735 g/L Na<sub>2</sub>CO<sub>3</sub> is added into the 81.3 mL volume of water to achieve that. It is assumed that the concentration of chemicals is equal in the bark and in the extract. The amount of extract is measured after each procedure.

#### 2.2.2. Extractions with a 2-liter reactor

A sample of 276.5 g of fresh spruce bark (i.e. 159 g of dry Spruce P) was extracted with a 1/10 liquid-to-bark ratio in a 2 L reactor (Büchi, Switzerland), with and without stirring. Stirring speed was 600 rpm. Solids and liquids were separated with a 50 µm pore size frit. Extractions were conducted both with pure water at 110 °C and with 5 g/L of Na<sub>2</sub>CO<sub>3</sub> at 90 °C for two hours.

#### 2.2.3. Extractions in a 300-liter vessel

Extractions were further scaled up to obtain a large batch of tannins for adhesives experiments. A total of 60.64 kg of fresh bark (i.e. 45 kg of dry weight, Spruce M) was extracted with water at 110 °C in a 300 L reactor (Kilpeläinen et al., 2014). Solids and liquids were separated after each extraction by passing the extract through a series of stainless steel meshes of decreasing mesh size down to 0.3 mm. Extraction time was 120 min. Bark was placed in a reactor and the system was heated using 120 °C steam for 10 min with a 1.8 kg/min steam flow. When the heating phase was over, the reactor was filled with water at 115 °C. The temperature was measured inside the reactor with nine thermo pairs that were inserted with steel spikes inside. After two hours of extraction,



Fig. 1. Norway spruce bark grinded with an ATREX® mill showing particle size distribution with a large amount of fines and fibrous sticks containing wood.

Table 2

Different extraction temperatures and chemicals used for bark samples of Norway spruce bark and Scots pine. Bark was obtained either from a sawmill (S) or a pulp mill (P). For abbreviations, see Table 1.

Chemical addition	Bark samples	Temperatures (°C)	Concentration (g/l)
none	Spruce S, Spruce P, Pine S, Pine P	60, 75, 90, 110 and 140	none
Urea	Spruce S, Spruce P, Pine S	60, 75, 90	20
NaHSO <sub>3</sub>	Spruce S, Spruce P, Pine S	60, 75, 90	20
Na <sub>2</sub> CO <sub>3</sub>	Spruce S, Spruce P, Pine S	60, 75, 90	5
Sodium benzoate	Spruce S, Spruce P, Pine S	60, 75, 90	10

the extract was cooled down in a heat exchanger and collected into a 1000 L container.

### 2.3. Analytical methods

The amount of total dissolved solids (TDS) was measured by freeze-drying the extracts. Reactivity of the dried extracts was determined with a Stiasny number test (Yazaki and Hillis, 1980). A sample of 100 mg of extracted dried tannins was dissolved in 10 mL of distilled water. 1 mL of 10 M HCl and 2 mL of 37% formaldehyde were added. When the mixture was heated under reflux for 30 min, a tannin-formaldehyde condensation product was precipitated. The mixture was filtered while hot with a pre-weighted glass fiber filter and the precipitate was washed with water (2 × 10 mL). Filter and precipitate were dried at 105 °C. The Stiasny number is expressed as a percentage of the weight of the starting dried extracted material.

The amount of tannins present was determined by a UV-Spectrometer (Shimadzu, Kyoto, Japan). Tannin samples were dissolved in a 0.1 M NaOH solution and then measured at 280 nm. The results were calculated with a calibration curve of purified tannin Fintan QP (Silvateam S.p.A, San Michele Mondovì, Italy).

### 2.4. Stiasny number method related to tannin content

In order to validate how the Stiasny method is correlated with the tannin content, a set of samples with known content of tannins, stilbenes, and carbohydrates were analyzed. The compounds included purified tannin Tannino QS-SOL (Silvateam S.p.A, San Michele Mondovì,

Italy), piceid (polydatin Sigma-Aldrich, USA), softwood hemicellulose galactoglucomannan (GGM), which was ethanol-precipitated from a pressurized hot water extract from spruce sawdust (Kilpeläinen et al., 2014), gallic acid (Sigma-Aldrich, USA, catechin (Sigma-Aldrich, USA), and glucose (Sigma-Aldrich, USA) (Table 3).

The Stiasny numbers of pure compounds were used to calculate the Stiasny number of mixtures of purified compounds:

$$\text{Stiasny} = \text{Tannino} * \left(\frac{\text{wt}\%}{100}\right) + \text{Glucose} * \left(\frac{\text{wt}\%}{100}\right) + \text{GGM} * \left(\frac{\text{wt}\%}{100}\right) + \text{Piceid} * \left(\frac{\text{wt}\%}{100}\right)$$

Where Tannino is measured as the Stiasny number of Tannino QS-SOL, Glucose is measured as the Stiasny number of glucose, GGM is measured as the Stiasny number of GGM and Piceid is measured as the Stiasny number of piceid. Weight percent is the portion of compound in the whole sample.

### 2.5. Statistical methods

Statistical analyses were applied to the laboratory data to find out how extraction parameters could affect the extraction yield and whether the models could also predict scaled-up results. Models can work on a small laboratory scale but when the method is scaled up to larger reactors, new phenomena can emerge. For example, when scaling up flow-through extraction (Kilpeläinen et al., 2014), the channeling of sawdust

Table 3

Samples for the validation of Stiasny number determination.

Sample
Hot water extract 300 L extraction at 110 °C
Eluent from pressed from bark of 300 L extraction
Extract of 2 L sodium carbonate 5% extraction at 90 °C
Extract of 2 L sodium carbonate 5% extraction with stirring at 90 °C
Extract of 2 L hot water extraction at 110 °C
Extract of 2 L hot water extraction with stirring at 110 °C
Glucose
GGM
Piceid (polydatin)
Gallic acid
Catechin
Tannino QS-SOL
50% Tannino QS-SOL + 50% glucose
25 Tannino QS-SOL + 50% glucose + 25% GGM
40% Tannino QS-SOL + 40% glucose + 10% GGM + 10% piceid
75% Tannino QS-SOL + 20% glucose + 5% GGM

can be observed at the 300 L scale.

The aim was to model extracts with predictors such as wood species (*Species*), sample origin from either pulp mill or sawmill (*From*), used chemical (*Chemical*) and extraction temperature (*Extraction temperature*). Total dissolved solids (*TDS*), tannin yield (*Tannins*), reactivity by Stiasny number (*Stiasny*), and tannin portion of TDS (*Tannins vs. TDS*) were chosen as responses.

Seemingly unrelated regression (SUR) models with four responses (four equations) were fitted. The models can be written as:

$$y_1 = x_1\beta_1 + \varepsilon_1$$

$$y_2 = x_2\beta_2 + \varepsilon_2$$

$$y_3 = x_3\beta_3 + \varepsilon_3$$

$$y_4 = x_4\beta_4 + \varepsilon_4$$

where  $x_g$  ( $1 \times K_g$ ) and  $\beta_g$  ( $K_g \times 1$ ) denote the predictor and parameter vector, respectively, with  $g = 1, 2, 3, 4$  and dimension  $K_g$  (number of predictors).  $y_1, y_2, y_3$  and  $y_4$  denote *Tannins vs. TDS*, *Stiasny*, *TDS* and *Tannins*. The predictor set were fixed as the same for each response and thus the SUR is equivalent to four separate OLS regressions.

Our aim was to find the set with the best predictive performance simultaneously for each response using a machine learning approach. In supervised machine learning, the idea is to maximize predictive performance by dividing the data into three sets – training, validation and test set, Training and validation sets are used for model selection and the test set is used for final model evaluation. We had three separate test sets: 1) a 2 L reactor (non-stirred), 2) a 2 L reactor (stirred) and 3) a 300 L reactor. The model's prediction capability was separately applied to these test sets. Training and validation sets were combined and cross-validation was applied. *Chemical*, *Species*, *Extraction temperature* and *From* were considered as potential predictors. Every possible combination of these predictors was constructed as well as each two-way interaction. Ten-fold cross validation (10-fold CV) was performed and the CV coefficient of determination ( $R^2$ ) was used as a performance metric and for each separate model (combination of predictors). The set of predictors that maximized the mean of CV  $R^2$ -values from the four separate models was considered the best. This predictor set then specified the final four models.

The final model included the following predictors: *Chemical*, *Species*, *Extraction temperature*, *From*, *Chemical:Extraction.temperature*, *Chemical:Species*, *Chemical:From*, *Species:Extraction.temperature* and *Species:From*, where: denotes interaction between two predictors.

The relative importance of each predictor was assessed by the contribution of each predictor to  $R^2$  by using the lmg approach, which takes into account the correlation between predictors. Each predictor receives a value between 0 and 1 where a higher value indicates greater importance and the total values sum up to one (for further details, see (Grömping, 2006)). This approach takes into consideration only the main effects. Nevertheless, it can be seen as a useful approximation of relative importance.

The dataset consisted of 112 observations and contained no missing values. The mean CV  $R^2$ -value for the final models was 92.9 %. The individual CV  $R^2$  values for the Tannin portion of TDS, Stiasny, TDS and Tannins were 76.0%, 91.3%, 96.9% and 80.4 %, respectively.

### 3. Results and discussion

#### 3.1. Extraction results

Fig. 1 shows the amount of hot water-extracted tannins and other compounds from four different bark samples at a temperature range 60–140 degrees Celsius. An extraction vessel theoretically contains 93.3 mL of liquid if the volume of 10 g of dry bark is included. In

practice, an average of 70.1 wt% of liquid can be collected after extraction with the ASE-350 unit, and the standard deviation is 5.3 wt%. On average, bark residue retains 29.9% of extract which can be obtained by additionally pressing the extracted bark after the process.

The highest TDS values were obtained from water extractions at 140 °C. Spruce bark samples from a sawmill (167 mg/g) and a pulp mill (175 mg/g) had similar TDS amounts to pine bark from a sawmill (166 mg/g). In the case of pine bark from a pulp mill, the yield was significantly lower (104 mg/g). Yields at lower temperatures were at the same level except for pine bark from a pulp mill, which had a lower amount.

The yields were on average lower than those previously reported. For example, spruce bark has been reported to yield 196–209 mg/g of TDS when extraction is done at 90 °C using winter-collected bark (Kempainen et al., 2014). The TDS of hot water-extracted pine and spruce bark has been reported to be 112 mg/g and 152 mg/g, respectively (Raitanen et al., 2020). Lower amounts have been reported by Rasi et al. (2019): 65 mg/g and 88 mg/g for pine and spruce respectively. Different debarking and bark storage conditions can cause significant variation. Other compounds shown in Fig. 1 are mostly carbohydrates (Bianchi et al., 2016; Kempainen et al., 2014; Neiva et al., 2018; Raitanen et al., 2020).

The highest yields of tannins were found in spruce bark (47 mg/g; Fig. 1). Pine bark showed significantly lower values, varying between 15 and 16 mg/g. Tannin yields increased along with the extraction temperature. Variable tannin yields have been reported in literature. For spruce, inner and outer bark hot water extraction at 100 °C resulted in 27 mg/g and 55 mg/g of condensed tannins, respectively (Krogell et al., 2012). Hot water extraction at 90 °C has resulted in 81 mg/g of tannins from spruce whole bark and 36 mg/g from pine bark (Raitanen et al., 2020). Hot water extraction at 75 °C has reportedly yielded lower tannin amounts, 12 mg/g and 32 mg/g from pine and spruce bark, respectively (Rasi et al., 2019).

Part of the increased tannin yield may be accounted for by lignin subunit-derived phenols as the extraction temperature increases, since they have absorption in the same 280 nm wavelength than other phenols (Varila et al., 2020). In addition, hydroxystilbene structures can be incorporated into the lignin structure of Norway spruce (Rencoret et al., 2019), which also creates challenges in absorption measurements of extracts, since the absorption of lignin and other polyphenols such as tannins or stilbenes overlap.

The proportion of tannins in the TDS was higher in spruce bark (27–46% dry wt) than in pine depending on extraction temperature (75–110 °C). Pine bark samples from a sawmill had the lowest proportion of tannins (5–9% dry wt) compared with bark originating from a pulp mill (14–23% dry wt). In general, the extraction yield of tannins was higher with all the bark samples of spruce than from those of pine.

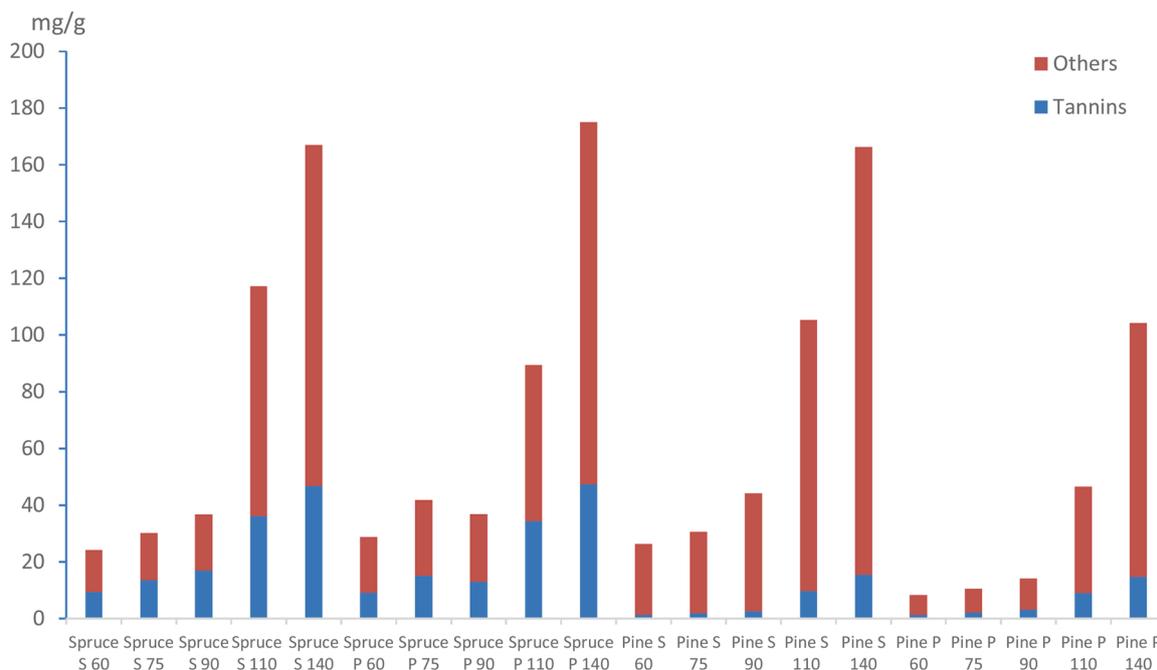
The lowest pH (3.6–4.1) was measured in spruce bark extract at 140 °C. The pH of pine bark extracts varied between 3.7 and 3.8. In most cases, the pH decreased as the extraction temperature increased.

Reactivity of the dried extracts was measured using the Stiasny number method. Spruce samples had higher Stiasny numbers than pine samples. These values decreased again at higher extraction temperatures (140 °C) possibly due to the lower proportion of tannins in the extract. The highest Stiasny numbers were obtained at a 60–90 °C extraction temperature range. There were differences in Stiasny values between extracts of samples from a sawmill (Spruce S and Pine S) and a pulp mill (Spruce P and Pine P). These values varied considerably between different bark samples, which indicates that composition of the extracted material varies depending on the bark source.

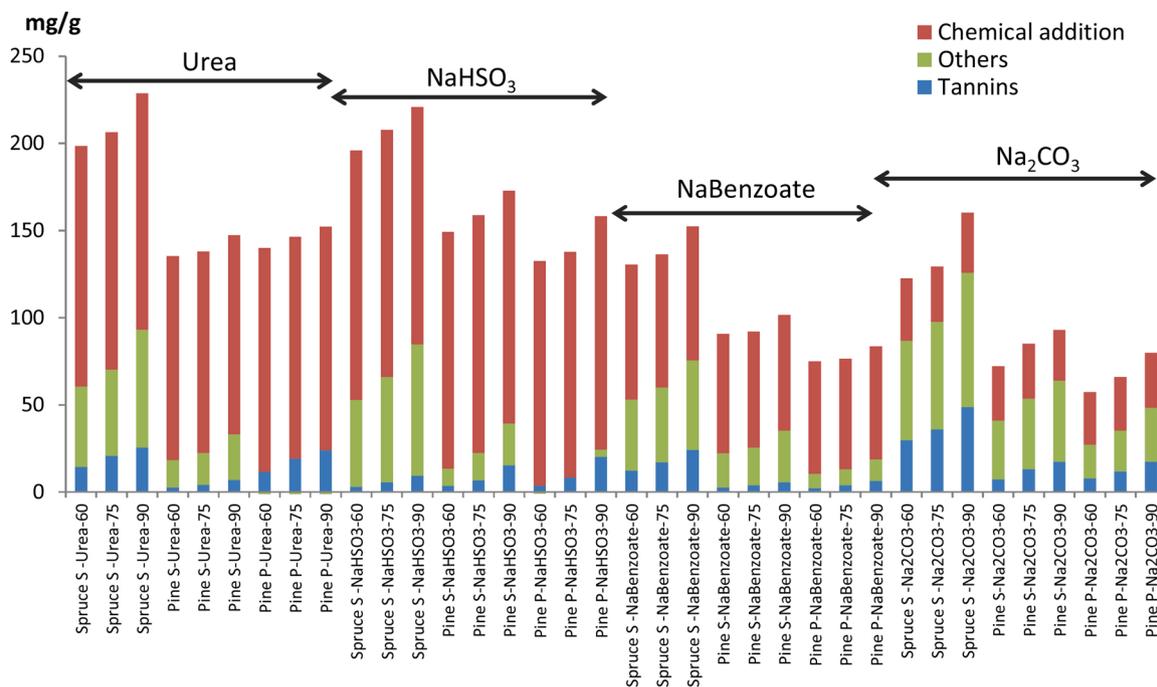
#### 3.1.1. Extractions with chemical additions

Extracts using added chemicals had higher TDS values than water extractions (Fig. 2).

The highest yield of tannins (49 mg/g at 90 °C, Fig. 2) was obtained from spruce bark using sodium carbonate. Pine bark extracted with pure



**Fig. 2.** The extraction yield of Norway spruce (Spruce) and Scots pine (Pine) bark. Bark was obtained either from a sawmill (S) or pulp mill (P). Results are based on dry weight. The amount of tannins was measured with the UV-280 method. The amount of other compounds is calculated by reducing the amount of tannins from the total dissolved solids of the extract. For extraction conditions, see Table 2.



**Fig. 3.** The extraction yield of Norway spruce (Spruce) and Scots pine (Pine) bark. Bark was obtained either from a sawmill (S) or pulp mill (P). Results are based on dry weight. The amount of tannins was measured with the UV-280 method. The proportion of each chemical addition (red bars) is calculated from the original chemical concentration. The amount of other compounds (green bars) is calculated by subtracting the amounts of tannins (blue bars) and chemical additions from the total dissolved solids of extract. For extraction conditions and chemical additions, see Table 2.

water showed only 17 mg/g tannins at 90 °C. The proportion of added chemicals in the TDS was over 50 wt% except for sodium carbonate. In the case of sodium bisulphite, most of the TDS comprised the added chemical.

In urea extraction, the pH increased as the extraction temperature increased. This may be due to degradation of urea into ammonia, which is more alkaline. Sodium bisulphite extracts had similar pH values to the

hot water extract (3.3–3.9). The highest pH values were obtained in sodium carbonate extracts (8.0–8.8).

The Stiasny number was highest for sodium carbonate extracts (data not shown). Spruce S had the highest Stiasny number and the values were at the same level as those from hot water extracts from Spruce P. The addition of urea, sodium bisulphite, and sodium benzoate decreased the values. The lowest values were measured for Pine S and Pine P.

Chemical additions represented a large part of the dried extract, especially with pine samples, which could explain the low Stiasny values.

### 3.1.2. Stiasny number results and the composition of extracts

The correlation between the Stiasny numbers and the TDS, tannin yield, chemical additions, and the tannin portion of the TDS was analyzed. The Stiasny number and the tannin proportion of the TDS showed a positive correlation (Fig. 4). The TDS, chemical additions, and yields of other compounds and tannins did not show any clear correlation with reactivity (Supplementary information). It has been assessed that high quality adhesive could be produced with Stiasny number 65 (Chupin et al., 2013). Still good quality adhesives have been produced with Stiasny number 46. A series of Stiasny measurements were further conducted for dried extracts, standard compounds, and samples containing tannins and carbohydrates in different mixtures (Table 4).

Carbohydrates such as glucose and softwood GGM had no Stiasny values. However, spruce stilbene glucoside piceid, gallic acid, catechin, and Tannino QS-SOL had high values of 97, 80, 106, and 85, respectively. The Stiasny numbers of these compounds were then used to calculate corresponding values for artificial samples using equation 1. The correlation between measured and predicted Stiasny numbers showed a linear correlation calculated with this equation ( $R^2=0.9887$ ).

The results from Stiasny number measurements indicate that the reactivity increased with a higher proportion of tannins in the TDS. If a really high reactivity is needed, any other compounds should be purified from samples and any additional chemicals used in the extraction process should be recycled. Such extracts can be used in technical applications where further purification steps are not needed. Purified tannins may be targeted for specialized products where enhanced properties are needed, such as food or medicinal applications. Purified condensed tannins can be also readily modified chemically to give desired functionalities (Arbenz and Avérous, 2015).

## 3.2. Scaling up

### 3.2.1. Extractions with a 2-liter reactor

The conditions which gave a high yield and tannin portions at a laboratory scale were selected to extract spruce bark in a 2 L reactor (Fig. 4). With sodium carbonate, the extraction at 90 °C resulted in a TDS of 143 mg/g for a stirred sample and 122 mg/g for a sample without stirring, while tannin yields were 69 mg/g and 70 mg/g with stirred and non-stirred samples, respectively. The amount of other compounds was 20 mg/g and 42 mg/g for stirred and non-stirred

**Table 4**

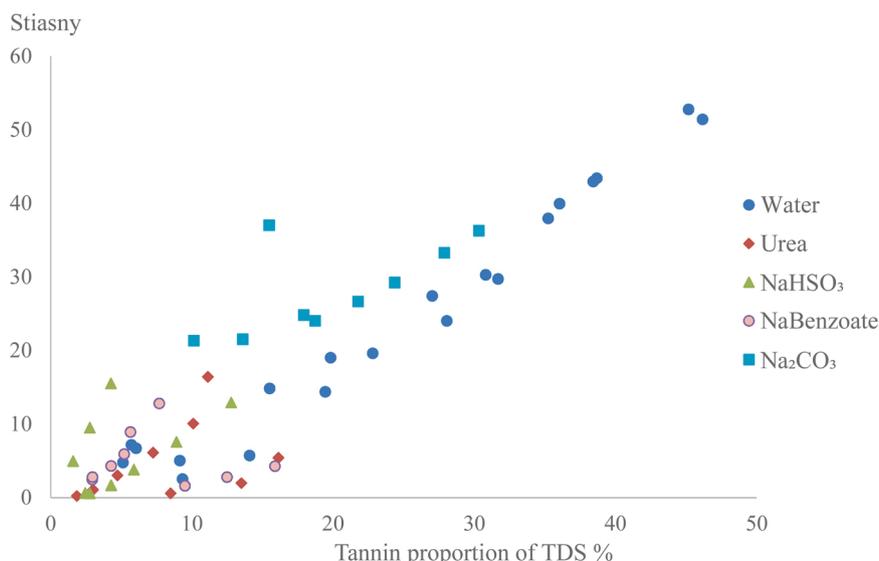
Stiasny numbers of dried extracts of Norway spruce bark, the standard compounds and samples with tannin and carbohydrate mixtures.

Sample	Stiasny number
Hot water extract 300 L extraction at 110 °C	35
Eluent pressed from bark of 300 L extraction	14
Extract of 2 L sodium carbonate 5% extraction at 90 °C	49
Extract of 2 L sodium carbonate 5% extraction with stirring at 90 °C	46
Extract of 2 L hot water extraction at 110 °C	45
Extract of 2 L hot water extraction with stirring at 110 °C	36
Glucose	0
GGM	0
Piceid (polydatin)	97
Gallic acid	80
Catechin	106
Tannino QS-SOL	85
50% Tannino QS-SOL + 50% glucose	42
25% Tannino QS-SOL + 50% glucose + 25% GGM	26
40% Tannino QS-SOL + 40% glucose + 10% GGM + 10% piceid	46
75% Tannino QS-SOL + 20% glucose + 5% GGM	65

sample, respectively. The rest of the TDS in both cases was sodium carbonate. Results indicate that stirring did not increase the efficiency of extracting tannins but did increase the amount of other compounds, which were mainly carbohydrates. The respective proportion of tannins were 56% and 49% of the TDS. The Stiasny numbers were 46 for stirred and 49 for non-stirred samples.

Extraction with  $\text{Na}_2\text{CO}_3$  increased the yields. The amount of tannins and TDS were 37 mg/g and 78 mg/g for stirred and 29 mg/g and 63 mg/g for non-stirred extraction, respectively. The proportion of tannins within the TDS was 47% in both extractions. The Stiasny number was 36 for stirred and 45 for non-stirred samples.

Extraction yields in this study were lower than in previously reported extraction yields using the same 2 L reactor (Raitanen et al., 2020) and when the same analytical methods were used. In previous extractions, the TDS of spruce bark with hot water extraction at 90 °C was 152 mg/g and tannin yield was 81 mg/g (53% proportion of tannins from TDS), which indicates that spruce bark samples (Spruce P) in these experiments contained lower amounts of extractable compounds. However, the samples used in the previous study were harvested straight from the forest during winter in early February, which may explain the higher extraction yield.



**Fig. 4.** Scatterplot between Stiasny numbers and the tannin proportion of TDS extracted either with pure water or with chemical addition.

### 3.2.2. Extractions with a 300-liter reactor

Hot water extraction at 110 °C was further scaled up to a 300 L reactor. At the beginning of the extraction process, the measured temperature inside the reactor was higher than the 110 °C target temperature. Also, temperature readings were unevenly distributed inside the reactor at the start of the process but stabilized to 106 °C during extraction. Temperature differences indicate that the water first formed channels through the bark bed inside the reactor but gradually became more uniformly distributed through the bed. Similar behavior was observed when sawdust was extracted using the same flow-through system (Kilpeläinen et al., 2014). Pre-steaming usually enables a more even flow through biomass in a reactor but bark, as a more water-resistant material, behaves differently. Bark tends to be more tightly packed inside the reactor after the extraction process and forms a uniform, tight plug-like mass. This behavior may be due to the fact that the bark was milled to a very fine particle size beforehand.

### 3.2.3. Scaling up

At pilot scale, 300 L hot water extractions at 110 °C yielded tannin and TDS at 29 mg/g and 72 mg/g, respectively. These quantities were lower than extractions performed in the same conditions with the ASE-350 (36 mg/g and 117 mg/g for Spruce S). However, they were at the same or similar levels as the 2 L non-stirred extractions (29 mg/g tannin and 63 mg/g TDS).

Different samples were used for extraction at 110 °C with 2 L and 300 L reactors (Spruce S and Spruce M, respectively). A very fine particle size seems to be preferable at the benchmark scale but at larger scales this decreases the yield. Extractions in the same 300 L system with bark screened using 20 × 20 mm sieves to remove fine particles yielded a higher TDS (Varila et al., 2020). Hot water extraction at 70 °C for 80 min yielded 111 mg/g of bark and a higher temperature extraction at 90 °C for 40 min gave 110 mg/g of TDS. Extraction yields were 53% and 54% higher, even at lower temperatures and when shorter extraction times were used. Based on these results, milling bark to a very fine particle size may not be preferable in pilot-scale extractions in a batch system without stirring. In addition, the portion of wood in bark should be minimized and controlled by screening the bark before extraction, since sapwood contain lower amount of extractable tannins.

Extractions with added sodium carbonate had lower tannin yields with the ASE-350 than with the 2 L reactor. Tannin yield was 49 mg/g with the ASE-350. The same extractions with the 2 L reactor yielded 70 mg/g tannins when non-stirred and 69 mg/g for stirred samples, which were 43% and 41% higher than with the ASE-350 extractions.

Yields which do not contain the added chemicals were 122 mg/g for the non-stirred extractions and 143 mg/g for the stirred extractions, which were 3% lower and 13% higher, respectively, than with the ASE-350. One explanation for the higher tannin yield in the 2 L reactor may be due to variation in solid and liquid separation after extraction, depending on the system. In the ASE-350, a 1.2 µm pore-size cellulose filter and 10 µm stainless steel frit were used. In the 2 L reactor, a 50 µm stainless steel filter was used. Tighter filters in the ASE-350 could partially block tannins aggregates, which would not then pass through the filter into the extract.

### 3.3. Industrial bark and extraction yields

Possible reasons for relatively low yields from our 2 L reactor extractions, compared with previous results, may be related to log storage time, debarking methods, and bark storage time prior to extraction. After a tree has been felled, weathering in a log yard can start to degrade compounds in the bark (Bianchi et al., 2016). The original amount of compounds in spruce logs and their degradation rate varies according to temperature and seasons (Halmemies et al., 2021). The amount of polyphenols, especially stilbenes, decreases during the storage of spruce (Jyske et al., 2020). After 24 weeks' storage time, only 5–7% of stilbenes and 44% of condensed tannins remain.

Debarking further breaks down the bark structure, allowing microbial activity during storage of bark. A decrease in the amount of extractives available from spruce bark in the pile occurs in the first two weeks of storage (Routa et al., 2020). The main deterioration pathways for wood chips are respiration reactions, microbial reactions, and chemical oxidation reactions (Hakkila, 1989). In the case of pellets, bark pellets are more susceptible to degradation than wood pellets since they contain more of the nitrogen that is needed for fungal growth (Lehtikangas, 2000).

Temperature and microbial activities have been shown to be the main reasons for chemical changes in piles of forest residue chips (Jirjis and Theander, 1990). Stilbenes are prone to degrade when bark is heated (Jylhä et al., 2021). In thermal drying at 50–70 °C, 36–43% of stilbenes are lost after 10 h. Debarking can yield a bark sample that also contains sapwood, which does not contain as much polyphenols as inner or outer bark. Ideally, bark could be obtained straight from forests during the wintertime and only the bark would be isolated, but in practice, industrial bark can have long storage times and may often contain sapwood. One way to separate bark from fine wood is to use sieves (20 mm × 20 mm) and a high portion (97.3 ± 1.1%) of bark can be obtained (Varila et al., 2020). For the industrial use of bark extracts, the logistical chain of bark and storage conditions should be optimized to prevent degradation of tannins before extraction to achieve as high a tannin yield as possible.

### 3.4. Modeling results

Results from the final models were visualized by obtaining a combination of predictors for each of the four models. Every possible combination was considered, from a range of possible values for categorical predictors, to a sequence of values from minimum to maximum for quantitative variables. These predictor values resulted in predicted responses for: *Tannin portion of TDS*; *Stiasny*; *TDS*; and *Tannins*. The predictions are shown in Figs. 5–7 for each response except for *TDS*.

Fig. 5 shows that the predicted proportion of tannins increases as the extraction temperature rises to 140 °C for pine bark. With the addition of sodium carbonate, the yield is highest at 90 °C, and is equivalent to the same level as extracted using pure water at higher temperatures.

Overall, the amount of tannins is lower in pine than in spruce bark. The model shows that the highest proportion of tannins can already be obtained at low temperatures (60–90 °C) for spruce bark (Fig. 5). Here, the portion of tannins decreases as the extraction temperature increases. Sodium carbonate improves the yield, compared with urea and sodium benzoate, which give lower yields than water alone. Chemical additions had the highest relative importance on the model (0.38), after wood species (0.16). Bark source and extraction temperatures were of only minor importance (0.05).

The results show that for extractions using only water, the *Stiasny* number decreases along with an increase in temperature for spruce bark (Fig. 6). Still water extractions had the highest predicted *Stiasny* numbers for spruce extracts, compared with those using chemical additions. Extractions with chemical additions have different *Stiasny* numbers but the values are at the same level at 60–90 °C temperature range for spruce. In the case of chemical additions, the model predicted that sodium carbonate addition would yield the extract with the highest *Stiasny* numbers for pine.

As with predicted results for the tannin portion of the TDS, relative importance was highest for chemical addition (0.42) and wood species (0.31). The relative importance of the wood source (0.01) and extraction temperature (0.01) were low.

The model predicts that pine bark extractions yield less tannins than for spruce bark (Fig. 7). Tannin yields increased as the extraction temperature rises. Unexpectedly, the model predicted higher tannin yields for pine than for spruce bark at 110 °C and 140 °C.

In the case of spruce extractions, predicted yields were highest at 60–90 °C with sodium carbonate. According to the model, urea and

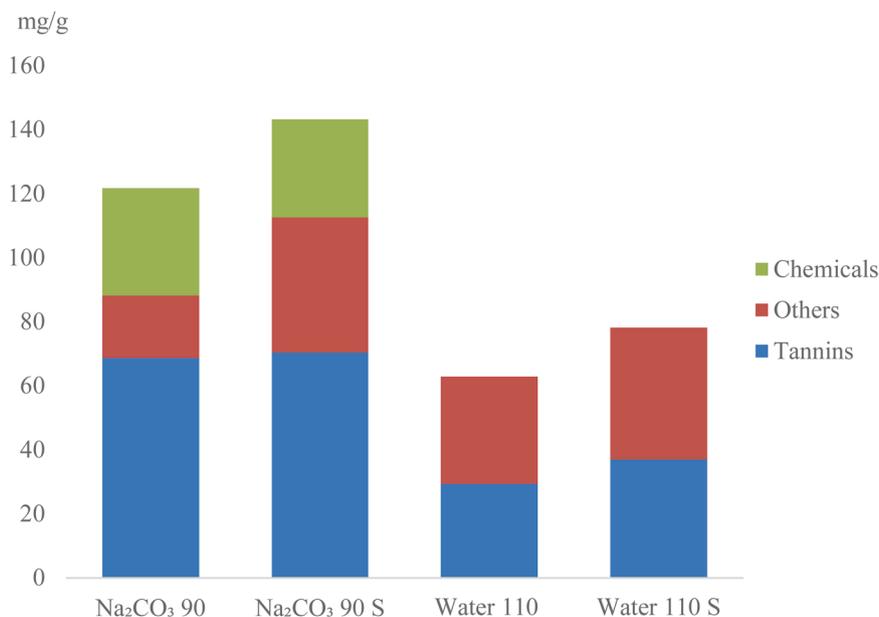


Fig. 5. Composition of extracts of Norway spruce bark in a 2-liter reactor. Extractions were carried out with addition of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) and with hot water at 90 °C or 110 °C and without or with stirring (S).

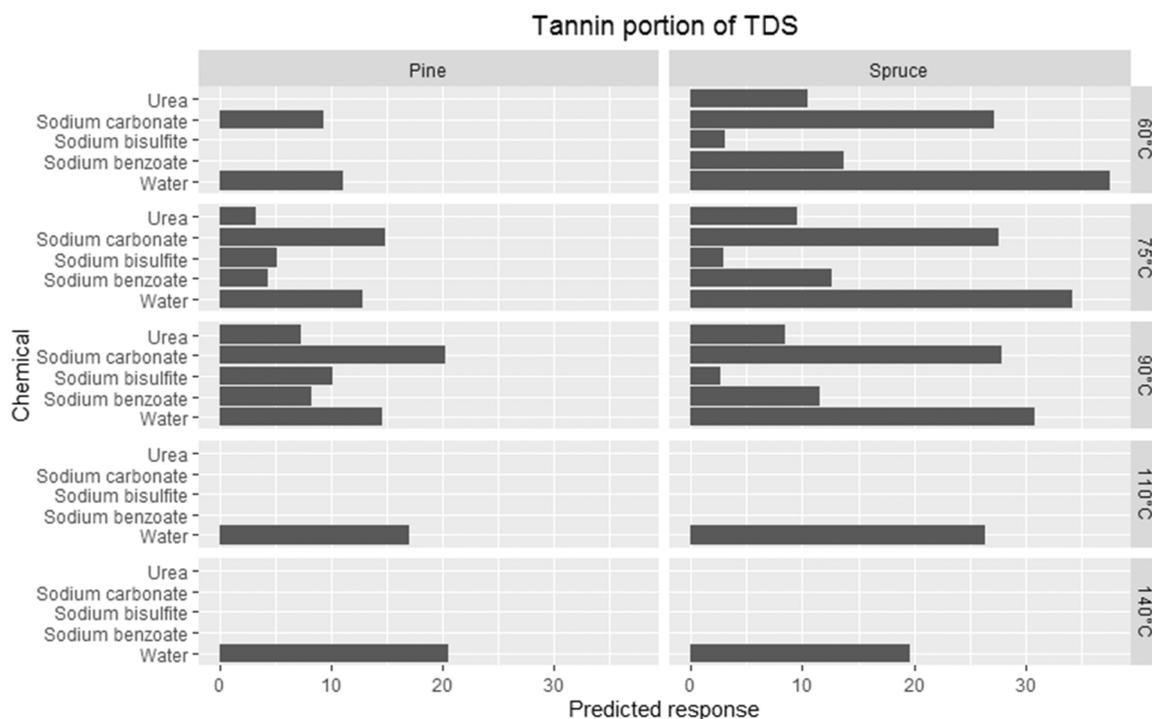


Fig. 6. The predicted proportion of tannins in the total dissolved solids (TDS) of Scots pine and Norway spruce bark from a sawmill at different temperatures (right axes) and with chemical additions or with pure hot water (left axis). For extraction conditions and chemical additions, see Table 2. Expressed as a percentage of dry weight.

sodium benzoate would give higher tannin yields than sodium bisulfite and water extractions. Water extraction tannin yields were predicted to be surprisingly low at 60–90 °C, especially for spruce bark extractions.

Unlike the predicted tannin portion of TDS (Fig. 5) or the Stiasny numbers (Fig. 6), extraction temperature had the highest relative importance for the Tannins model (0.28). Chemical additions and wood species had similar values (0.14 and 0.14, respectively). The wood source had a minimal effect (0.05) on results.

### 3.5. Modeling predictions and scaling up results

Chemical additions showed a strong impact on the tannin portion of TDS and the Stiasny number, and a moderate effect on tannin yield. This is reasonable, since chemical additions represented over 50% of the TDS content of other chemicals besides sodium carbonate. Predictions show that it is likely to be important to recycle chemicals before using tannins in adhesives. In terms of the tannin yield, the factor of highest importance was the extraction temperature, after the wood species. The source

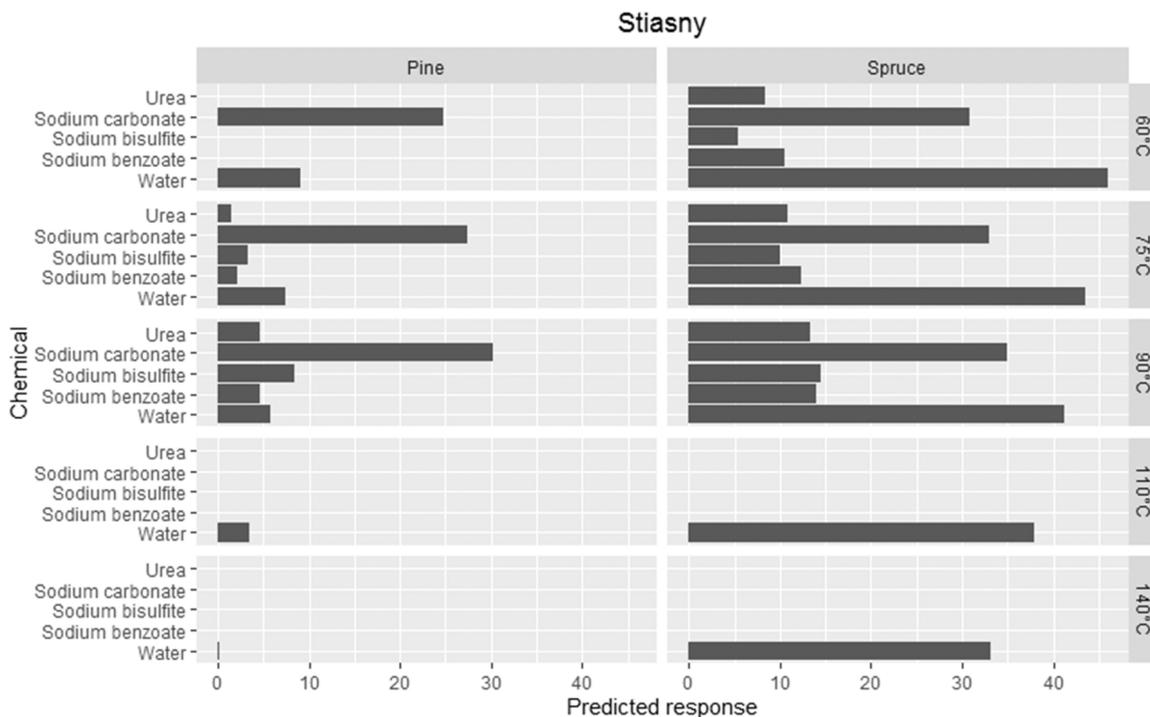


Fig. 7. The predicted Stiasny number of the extract of Scots pine and Norway spruce bark from a sawmill at different temperatures (right axes) and with chemical additions or with pure hot water (left axis). For extraction conditions and chemical additions, see Table 2. Expressed as a Stiasny number.

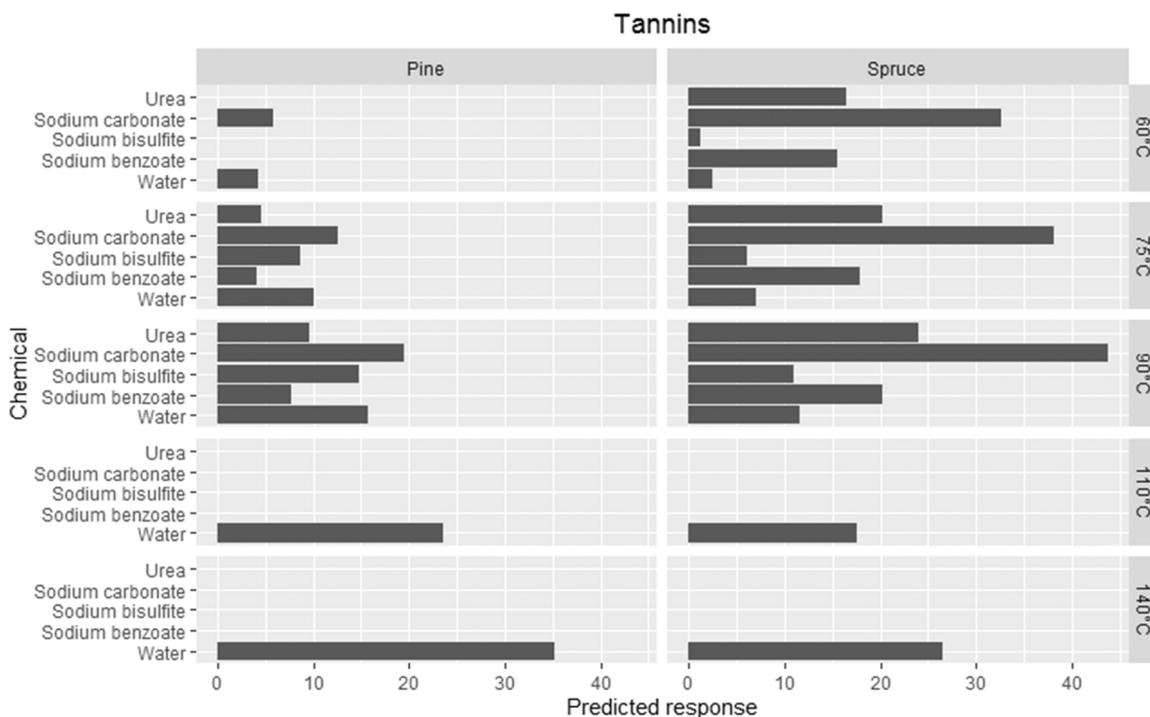


Fig. 8. The predicted amount of tannins extracted from Scots pine and Norway spruce bark from a sawmill at different temperatures (right axes) and with chemical additions or with pure hot water (left axis). For extraction conditions and chemical additions, see Table 2. Expressed as a mg/g of dry weight.

of the bark, either from a sawmill or a pulp mill, had relatively little importance within the model. Results indicate that the tannin content of pulp mill and sawmill bark reduces to similar values after storage.

For 110 °C hot water extraction for pulp mill spruce bark (Spruce P) the model predicted yields of 28 mg/g of tannin and 102 mg/g of TDS (Table 5). The model predictions were performed using bootstrapping

with 5000 samples, from which a median prediction (best point prediction) was obtained along with a 95% confidence interval. Tannin and TDS yields from 2 L reactor hot water extractions were 37 mg/g and 78 mg/g for stirred, and 29 mg/g and 63 mg/g for non-stirred techniques, respectively. The hot water extraction yield for pulp mill spruce bark (Spruce M) with the 300 L vessel were 29 mg/g of tannins and

**Table 5**

Comparison of tannin and TDS yields predicted by modelling (based on laboratory data) and extraction results at 2 L and 300 L scale (test data). The modelling results show the median bootstrap prediction along with the corresponding 95% confidence interval (in brackets).

Hot water extraction at 110 °C				
	Model prediction	2 L reactor (non-stirred)	2 L reactor (Stirred)	300 L reactor
Tannins	28 (20; 33)	29	37	29
TDS	102 (89; 114)	63	78	72
Na <sub>2</sub> CO <sub>3</sub> addition at 90 °C				
	Model prediction	2 L reactor	2 L reactor stirred	
Tannins	65 (51; 79)	70	69	
TDS	180 (153; 204)	122	143	

72 mg/g of TDS. The model predicted scaled-up yields of tannins even when different bark batches from pulp mills (Spruce M and Spruce P) were used in 2 L and 300 L extractions. However, the tannin yield from the stirred extraction technique was higher than the model predicted. By comparison, predicted TDS values were higher than what was actually obtained from 2 L and 300 L extractions. Both 2 L non-stirred and 300 L reactor tannin yields are included in the model's confidence interval. This indicates that the model predicts well for tannins. The same can't be said for TDS.

For 90 °C hot sodium carbonate extraction for pulp mill spruce bark the predicted tannin and TDS yields were 65 mg/g and 180 mg/g respectively. Tannin yields for stirred and non-stirred 2 L reactor extractions were 69 and 70 mg/g. TDS values were 143 mg/g and 122 mg/g for stirred and non-stirred extractions, respectively, which were lower than the models' predictions. The model predicts well for sodium carbonate extraction tannin yield, since the reactor yields are included in the model's confidence interval. For TDS yield, the model overestimates the yield.

### 3.6. Conclusions

When two types of bark were extracted with hot water and chemical additions, spruce bark showed higher tannin yields compared with pine bark. The Stiasny numbers decreased along with the tannin proportion of the TDS, indicating that carbohydrate removal or recycling of added chemicals increases reactivity. Optimal conditions for extracting tannins involve the use of hot water at 110 °C and the addition of 5 g/l sodium carbonate at 90 °C. The latter approach was used for the 2 L extraction technique, while hot water extraction at 110 °C was employed when using the 300 L reactor. The results confirm that the methods are scalable. A machine learning approach using SUR models was applied to predict yields from 2 L and 300 L extraction volumes. This method was found to be suitable to predict results when scaling up extraction methods.

### CRedit authorship contribution statement

**Petri Kilpeläinen:** Conceptualization, Methodology, Investigation, Writing – original draft. **Eero Liski:** Formal analysis, Methodology, Investigation, Writing – original draft. **Pekka Saranpää:** Conceptualization, Methodology, Supervision, Funding acquisition, Project administration, Writing – original draft.

### 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.

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### References

- Arbenz, A., Avérous, L., 2015. Chemical modification of tannins to elaborate aromatic biobased macromolecular architectures. *Green Chem.* 17, 2626–2646. <https://doi.org/10.1039/C5GC00282F>.
- Barjoveanu, G., Pătrăuțanu, O.-A., Teodosiu, C., Volf, I., 2020. Life cycle assessment of polyphenols extraction processes from waste biomass. *Sci. Rep.* 10, 13632. <https://doi.org/10.1038/s41598-020-70587-w>.
- Bianchi, S., Gloess, A.N., Krosiakova, I., Mayer, I., Pichelin, F., 2014. Analysis of the structure of condensed tannins in water extracts from bark tissues of Norway spruce (*Picea abies* [Karst.]) and Silver fir (*Abies alba* [Mill.]) using MALDI-TOF mass spectrometry. *Ind. Crop. Prod.* 61, 430–437. <https://doi.org/10.1016/j.indcrop.2014.07.038>.
- Bianchi, S., Koch, G., Janzon, R., Mayer, I., Saake, B., Pichelin, F., 2016. Hot water extraction of Norway spruce (*Picea abies* [Karst.]) bark: analyses of the influence of bark aging and process parameters on the extract composition. *Holzforchung* 70, 619–631. <https://doi.org/10.1515/hf-2015-0160>.
- Carlqvist, K., Arshadi, M., Mossing, T., Östman, U., Brännström, H., Halmemies, E., Nurmi, J., Lidén, G., Börjesson, P., 2020. Life-cycle assessment of the production of cationized tannins from Norway spruce bark as flocculants in wastewater treatment. *Biofuels Bioprod. Biorefin.* 14, 1270–1285. <https://doi.org/10.1002/bbb.21399>.
- Chupin, L., Motillon, C., Charrier-El Bouhtoury, F., Pizzi, A., Charrier, B., 2013. Characterisation of maritime pine (*Pinus pinaster*) bark tannins extracted under different conditions by spectroscopic methods, FTIR and HPLC. *Ind. Crop. Prod.* 49, 897–903. <https://doi.org/10.1016/j.indcrop.2013.06.045>.
- Ding, T., Bianchi, S., Ganne-Chédeville, C., Kilpeläinen, P., Haapala, A., Rätty, T., 2017. Life cycle assessment of tannin extraction from spruce bark. *iForest: Biogeosciences and Forestry* 10, 807–814.
- Feng, S., Cheng, S., Yuan, Z., Leitch, M., Xu, C., Charles, 2013. Valorization of bark for chemicals and materials: a review. *Renew. Sustain. Energy Rev.* 26, 560–578. <https://doi.org/10.1016/j.rser.2013.06.024>.
- Fraga-Corral, M., García-Oliveira, P., Pereira, A.G., Lourenço-Lopes, C., Jimenez-Lopez, C., Prieto, M.A., Simal-Gandara, J., 2020. Technological application of tannin-based extracts. *Molecules* 25, 614. <https://doi.org/10.3390/molecules25030614>.
- Granato, D., Reshamwala, D., Korpinen, R., Azevedo, L., Vieira do Carmo, M.A., Cruz, T. M., Marques, M.B., Wen, M., Zhang, L., Marjomäki, V., Kilpeläinen, P., 2022. From the forest to the plate – Hemicelluloses, galactoglucomannan, glucuronoxylan, and phenolic-rich extracts from unconventional sources as functional food ingredients. *Food Chem.* 381, 132284. <https://doi.org/10.1016/j.foodchem.2022.132284>.
- Grömping, U., 2006. Relative importance for linear regression in R: the package relaimpo. *J. Stat. Soft.* 17. <https://doi.org/10.18637/jss.v017.i01>.
- Hakkila, P., 1989. Utilization of Residual Forest Biomass, in: Utilization of Residual Forest Biomass. Springer, Berlin, Heidelberg, pp. 352–477. [https://doi.org/10.1007/978-3-642-74072-5\\_8](https://doi.org/10.1007/978-3-642-74072-5_8).
- Halmemies, E.S., Brännström, H.E., Nurmi, J., Läspä, O., Alén, R., 2021. Effect of seasonal storage on single-stem bark extractives of Norway spruce (*Picea abies*). *Forests* 12, 736. <https://doi.org/10.3390/f12060736>.
- de Hoyos-Martínez, P.L., Merle, J., Labidi, J., Charrier – El Bouhtoury, F., 2019. Tannins extraction: a key point for their valorization and cleaner production. *J. Clean. Prod.* 206, 1138–1155. <https://doi.org/10.1016/j.jclepro.2018.09.243>.
- Jablonsky, M., Nosalova, J., Sladkova, A., Haz, A., Kreps, F., Valka, J., Miertus, S., Frečer, V., Ondřejovic, M., Sima, J., Surina, I., 2017. Valorisation of softwood bark through extraction of utilizable chemicals. *Biotechnol. Adv.* 35, 726–750. <https://doi.org/10.1016/j.biotechadv.2017.07.007>.
- Jirjis, R., Theander, O., 1990. The effect of seasonal storage on the chemical composition of forest residue chips. *Scand. J. For. Res.* 5, 437–448. <https://doi.org/10.1080/02827589009382626>.
- Jylhä, P., Halmemies, E., Hellström, J., Hujala, M., Kilpeläinen, P., Brännström, H., 2021. The effect of thermal drying on the contents of condensed tannins and stilbenes in Norway spruce (*Picea abies* [L.] Karst.) sawmill bark. *Ind. Crop. Prod.* 173, 114090. <https://doi.org/10.1016/j.indcrop.2021.114090>.
- Jyske, T., Laakso, T., Latva-Mäenpää, H., Tapanila, T., Saranpää, P., 2014. Yield of stilbene glucosides from the bark of young and old Norway spruce stems. *Biomass Bioenergy* 71, 216–227. <https://doi.org/10.1016/j.biombioe.2014.10.005>.
- Jyske, T., Brännström, H., Sarjala, T., Hellström, J., Halmemies, E., Raitanen, J.-E., Kaseva, J., Lagerquist, L., Eklund, P., Nurmi, J., 2020. Fate of antioxidative compounds within bark during storage: a case of norway spruce logs. *Molecules* 25, 4228. <https://doi.org/10.3390/molecules25184228>.
- Karonen, M., Loponen, J., Ossipov, V., Pihlaja, K., 2004. Analysis of procyanidins in pine bark with reversed-phase and normal-phase high-performance liquid

- chromatography–electrospray ionization mass spectrometry. *Anal. Chim. Acta* 522, 105–112. <https://doi.org/10.1016/j.aca.2004.06.041>.
- Kempainen, K., Siika-aho, M., Pattathil, S., Giovando, S., Kruus, K., 2014. Spruce bark as an industrial source of condensed tannins and non-cellulosic sugars. *Ind. Crop. Prod.* 52, 158–168. <https://doi.org/10.1016/j.indcrop.2013.10.009>.
- Kilpeläinen, P.O., Hautala, S.S., Byman, O.O., Tanner, L.J., Korpinen, R.I., Lillandt, M.K.-J., Pranovich, A.V., Kitunen, V.H., Willför, S.M., Ilvesniemi, H.S., 2014. Pressurized hot water flow-through extraction system scale up from the laboratory to the pilot scale. *Green Chem.* 16, 3186–3194. <https://doi.org/10.1039/C4GC00274A>.
- Krogell, J., Holmbom, B., Pranovich, A., Hemming, J., Willför, S., 2012. Extraction and chemical characterization of Norway spruce inner and outer bark. *Nord. Pulp Pap. Res. J.* 27, 6–17. <https://doi.org/10.3183/npprj-2012-27-01-p006-017>.
- Lehtikangas, P., 2000. Storage effects on pelletised sawdust, logging residues and bark. *Biomass Bioenergy* 19, 287–293. [https://doi.org/10.1016/S0961-9534\(00\)00046-5](https://doi.org/10.1016/S0961-9534(00)00046-5).
- Matthews, S., Mila, I., Scalbert, A., Donnelly, D.M.X., 1997. Extractable and non-extractable proanthocyanidins in barks. *Phytochem.* 45, 405–410. [https://doi.org/10.1016/S0031-9422\(96\)00873-4](https://doi.org/10.1016/S0031-9422(96)00873-4).
- Mulat, D.G., Latva-Mäenpää, H., Koskela, H., Saranpää, P., Wähälä, K., 2014. Rapid chemical characterisation of stilbenes in the root bark of Norway spruce by off-line HPLC/DAD NMR: rapid chemical characterisation of stilbenes. *Phytochem. Anal.* 25, 529–536. <https://doi.org/10.1002/pca.2523>.
- Neiva, D.M., Araújo, S., Gominho, J., Carneiro, A., de, C., Pereira, H., 2018. An integrated characterization of *Picea abies* industrial bark regarding chemical composition, thermal properties and polar extracts activity. *PLoS ONE* 13, e0208270. <https://doi.org/10.1371/journal.pone.0208270>.
- Pan, H., Lundgren, L.N., 1996. Phenolics from inner bark of *Pinus sylvestris*. *Phytochemistry* 42, 1185–1189. [https://doi.org/10.1016/0031-9422\(96\)00122-7](https://doi.org/10.1016/0031-9422(96)00122-7).
- Pap, N., Reshamwala, D., Korpinen, R., Kilpeläinen, P., Fidelis, M., Furtado, M.M., Sant’Ana, A.S., Wen, M., Zhang, L., Hellström, J., 2021. Toxicological and bioactivity evaluation of blackcurrant press cake, sea buckthorn leaves and bark from Scots pine and Norway spruce extracts under a green integrated approach. *Food Chem. Toxicol.* 112284
- Pizzi, 2019. Tannins: perspectives and actual industrial applications. *Biomolecules* 9, 344. <https://doi.org/10.3390/biom9080344>.
- Pizzi, A., 2008. Tannins: Major Sources, Properties and Applications, in: *Monomers, Polymers and Composites from Renewable Resources*. Elsevier, New York, pp. 179–199. <https://doi.org/10.1016/B978-0-08-045316-3.00008-9>.
- Raitanen, J.-E., Järvenpää, E., Korpinen, R., Mäkinen, S., Hellström, J., Kilpeläinen, P., Liimatainen, J., Ora, A., Tupasela, T., Jyske, T., 2020. Tannins of conifer bark as nordic piqancy—sustainable preservative and aroma. *Molecules* 25, 567. <https://doi.org/10.3390/molecules25030567>.
- Rasi, S., Kilpeläinen, P., Rasa, K., Korpinen, R., Raitanen, J.-E., Vainio, M., Kitunen, V., Pulkkinen, H., Jyske, T., 2019. Cascade processing of softwood bark with hot water extraction, pyrolysis and anaerobic digestion. *Bioresour. Technol.* 292, 121893. <https://doi.org/10.1016/j.biortech.2019.121893>.
- Rencoret, J., Neiva, D., Marques, G., Gutiérrez, A., Kim, H., Gominho, J., Pereira, H., Ralph, J., del Río, J.C., 2019. Hydroxystilbene glucosides are incorporated into Norway spruce bark lignin. *Plant Physiol.* 180, 1310–1321. <https://doi.org/10.1104/pp.19.00344>.
- Routa, J., Brännström, H., Laitila, J., 2020. Effects of storage on dry matter, energy content and amount of extractives in Norway spruce bark. *Biomass Bioenergy* 143, 105821. <https://doi.org/10.1016/j.biombioe.2020.105821>.
- Serrano, J., Puupponen-Pimiä, R., Dauer, A., Aura, A.-M., Saura-Calixto, F., 2009. Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. *Mol. Nutr. Food Res.* 53, S310–S329. <https://doi.org/10.1002/mnfr.200900039>.
- Shirmohammadi, Y., Efhamisizi, D., Pizzi, A., 2018. Tannins as a sustainable raw material for green chemistry: A review. *Ind. Crop. Prod.* 126, 316–332. <https://doi.org/10.1016/j.indcrop.2018.10.034>.
- Varila, T., Brännström, H., Kilpeläinen, P., Hellström, J., Romar, H., Nurmi, J., Lassi, U., 2020. From Norway spruce bark to carbon foams: characterization, and applications. *Bioresources* 15, 3561–3666.
- Yazaki, Y., Hillis, W.E., 1980. Molecular size distribution of radiata pine bark extracts and its effect on properties. *Holzforschung* 34, 125–130. <https://doi.org/10.1515/hfsg.1980.34.4.125>.