Aminomethylation of spruce tannins and their application as coagulants for water clarification

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Abstract

This study explored the potential for using tannin extract from spruce bark (*Picea abies*) as a coagulant. Spruce tannins were extracted with hot water and pulverized through spray drying and freeze drying. The pulverized tannins were cationized via the Mannich reaction with formaldehyde and diethanolamine or ethanolamine, and the coagulation performances of the spruce tannin coagulants were compared with an industrially extracted quebracho tannin, which was cationized with the same method. Jar test experiments with kaolin/river water indicated that all of the tannin coagulants were able to enhance particle settling significantly, although the quebracho tannin coagulants were slightly more efficient than the spruce tannin coagulants. Since the phenolic groups play the major role in the modification, the higher coagulation efficiency was probably related to the higher amount of proanthocyanidin in the quebracho tannin, and subsequently the higher cationic charge density obtained for the quebracho tannin coagulants. Nevertheless, the spruce tannin coagulants could still be considered to be effective products because they possessed more stable

turbidity and a total surface charge close to zero over a wide dosage range. The ethanolamine was a better amine source, at least for the coagulation of river water with a positive charge demand. Moreover, the study established the importance of charge density as an essential indicator for coagulant performance.

Keywords: Spruce bark tannin extract, Bio-coagulants, Mannich reaction, Charge density, Spray drying, Freeze drying.

1. Introduction

One of the most prominent environmental footprints of rapid industrialization is the production of a large volume of industrial effluent. These effluents contain a high concentration of suspended solids and organic materials which need to be eliminated before they can be discharged into the environment. One of the essential processes during industrial wastewater treatment is coagulation-flocculation, owing to its efficiency in reducing turbidity, dissolved organic matter and colour [1, 2, 3]. It has been suggested that these aesthetic qualities are improved through two primary removal mechanisms: namely, charge neutralization and sweep coagulation. In charge neutralization, the positive charges from a positively charged coagulant are attracted to the negative charges of the colloids by electrostatic interaction. Precipitable flocs are then formed due to particle collision and agglomeration, induced by electrostatic charge neutralization. During sweep coagulation, organic matter/colloidal contaminants are entrapped on the surface of the insoluble hydroxide metal or organic polymer and are swept down with the precipitates as the suspension settles [4]. Besides charge neutralization and sweep coagulation, patch coagulation and bridging flocculation can also be utilized in the coagulation-flocculation process [5]. Patch coagulation mechanism refers to a mechanism in

which a polymer adsorbs on the particle surface and forms cationic patches. Subsequently, the oppositely charged patches of the particle are attracted to each other. When the polymer forms loops and tails, which are able to attach to other particles, the mechanism is called bridging flocculation.

The effectiveness in improving aesthetic water quality by conventional chemical-based coagulants is well documented in the literature. However, the applicability of these coagulants has been marred by some disadvantages which include high chemical cost, generation of a large volume of non-degradable sludge that requires further processing thus increasing operational cost, production of a toxic by-product with detrimental effects to human health and the environment [6]. Other setbacks are ineffectiveness in cold waters and an increase in the acidity of treated waters [7,8]. Metal coagulants are often effective over a limited pH range, partly due to the optimal region for the formation of the charged species [9]. Therefore, it is imperative that efforts are made to develop more eco-friendly coagulants that could replace these conventional coagulants.

Bio-based coagulants provide a viable alternative to chemical-based coagulants because they possess the ability to counteract the afore-mentioned concerns. Bio-coagulants are highly degradable and produce a non-toxic and lesser volume of sludge [10]. Another advantage of bio-based coagulants over chemical-based is that raw materials can be sourced locally. For example, the use of indigenous plants will help eliminate the cost of purchasing and importing chemicals, especially in developing countries [11]. One type of bio-coagulant that has attracted more commercial interest in recent years is tannin-based polymers. This has been mainly attributed to their low cost, as tannins are widely distributed in the bark of many tree species, and to their effectiveness over a wide range of pH. For example, Grenda et al. [12] showed that tannin-based coagulant recorded impressive color removal levels for some synthetic dye water samples over a pH range of 1-14, a feat that has been reported as impossible with most metal salts. They are also worthy of consideration owing to their simple modification procedures, which include polymerization and aminomethylation. The aminomethylation process is known as the Mannich reaction, and it involves the introduction of a

positive charge into the complex tannin structures [13]. During the Mannich reaction, an iminium ion is produced through the condensation of an aldehyde with an amino compound, which then substitutes hydrogen in the polyphenolic matrix of tannin [12].

The cationization of the anionic polyphenolic structure of tannins allows them to be able to destabilize anionic contaminants during water purification. Nonetheless, studies indicate that the efficiency of tannin-based polymers as a coagulant is dictated by several factors including the structure, origin and chemical modification procedure [12,14]. Grenda et al. [12] examined the influence of the method of synthesis on the physicochemical properties of tannin coagulants by comparing two cationization procedures (one step and dual step system) while other parameters and conditions remained constant. The studies revealed that the dual system recorded a higher reduction in turbidity, colour and COD, indicating the importance of the synthesis procedure on the overall effectiveness of the tannin coagulant. Another widely reported factor that determines the performance of these bio-coagulants during water purification is the effluent characteristics. Effluent properties such as pH, salinity and organic matter concentration are known to play a significant role in the viability of these coagulants. For example, the surface charge of colloids and the degree of ionization of polymers are both highly pH-dependent and, hence, the level of flocculation is considerably affected by the pH of the effluent [15], whereas salinity is believed to inhibit the chemical activity of polymers by masking their functional sites and altering their pristine chemical structure [16]. Finally, results from earlier research have also pointed out the importance of optimum dosage, as a higher coagulant dosage could lead to a charge reversal, resulting in the restabilization of the suspension [17].

This study aimed to investigate the possibility of using Mannich-modified tannin extract from the bark of spruce (*Picea abies*) as a bio-coagulant in water treatment applications. Although many scientific papers have been published on the coagulative performance of tannin-based coagulants from different tree species such as *Acacia mearnsii* (acacia) [12,18,19], *Schinopsis balansae* (quebracho) [12,18,19] and *Castanea sativa* (castanea) [19], no study was found to have explored the

coagulation performance of a bio-coagulant of any spruce species. Spruce bark is a major "side-stream" waste of many forestry industries, especially in northern European countries. Earlier studies have reported that the tannin content in spruce bark varies and has been estimated to range between 4% and 15% [20,21]. In this study, two types of spruce bark tannins (the main difference being in the pulverization method, i.e. spray drying and freeze drying) were chemically modified using two different amines (diethanolamine and ethanolamine) and compared to the reference material, quebracho tannin. The study with spruce tannin obtained from the two commonly used pulverization methods provides new information on the most suitable modification pathway for spruce tannin extract. The tannins were characterized by X-ray photoelectron spectroscopy (XPS) and ultra-high-performance liquid chromatography triple quadrupole mass spectrometry to obtain information about the phenolic compounds present [22,23]. Molecular weights and charge densities were measured for the obtained bio-coagulants. Previous research studies have rarely reported charge density for obtained bio-coagulants even though it is one of the most important parameters that determine the performance of coagulants. Finally, the ability of bio-coagulants to remove turbidity and organic matter from a kaolin/river water mixture was investigated, and the jar test residue was characterized by XPS.

2. Materials and Method

2.1 Materials

Fresh spruce bark for tannin extraction was obtained from Stora Enso Oyj Veitsiluoto mill in Kemi, Finland. The dry matter content of the bark was approx. 50%. Spray-dried spruce tannin (referred to hereinafter as SDS) was provided by VTT, the Technical Research Centre of Finland. Spruce bark was subjected to hot water extraction at 90℃. The obtained tannin extract was then spray-dried at 210-220 mbar with inlet and outlet temperatures of 175 ℃ and 75 ℃ respectively, to obtain a tannin powder with 6 % moisture content [24]. Quebracho tannin (QS-SOL) was kindly supplied by

Silvateam (Italy). The chemicals and reagents used for the study were of analytical grade. Ethanolamine (ETH) and diethanolamine (DEA) were supplied by Sigma Aldrich Chemicals, Germany. Milli-Q ultrapure water was used throughout this study except where indicated. Formaldehyde (37% v/w) was produced in France by VWR International while Merck KGaA Germany manufactured the HCl used for acidification of the synthesized coagulants. The sodium polyethylenesulphonate (PesNa) and poly-diallyl-dimethyl-ammonium-chloride (PolyDadmac) solutions used for the measurement of charge density of modified coagulants and total surface charge of water samples were supplied by BTG instruments AB, Sweden. The NaOH used to adjust the pH of the jar test water was supplied by VWR Chemicals.

Test water for the jar tests was produced by mixing a certain volume of river water and a weighed amount of kaolin (see section 2.3). The water sample was collected from the Oulu river (Oulu, Finland). The kaolin used was of sieved fraction size 0.063-0.5 mm and was collected from Pihlajavaara 64° 48´, located in the Kainuu region of Finland [25]. The nutrients and elemental content of the river water was measured according to standardized methods: Cl, F and SO_4^{2-} analysis was performed by ion chromatography (SFS-EN ISO 10304-1:2009); the NO_2-N and NO_3-N in the river water were analysed using a continuous flow analyser (SFS-EN ISO 13395:1997), the NH_4-N and PO_4-P were also analysed with a continuous flow analyser using the SFS-EN ISO 11732:2005 and SFS-EN ISO 15681-2:2005 methods, respectively. Inductively coupled plasma optimal emission spectrometry (ICP-OES) (SFS-EN ISO 17294-2:2016) was employed to measure the concentration of the elements.

2.2 Tannin extraction and pulverization

Tannins were extracted from the fresh spruce bark within 48 h of delivery to avoid degradation and immobilization of extractable tannin due to prolonged storage. Before tannin extraction, large chips of wood (pure wood over 5 cm and 0.3 cm in length and thickness, respectively) were removed

149 manually from the spruce bark sample. This was followed by weighing the bark/deionized water of
150 1:10 ratio (10% w/v) and heated at a temperature of 85 ℃, with mechanical stirring at 100 rpm. The
151 ratio and temperature were selected according to previous research by Kemppainen [24], which
152 reported a higher percentage of bound sugar in the tannin extract when the temperature was increased
153 to 90℃ and a minimal increase in tannin extract when the bark/water ratio was increased above 1:10
154 (10% w/v). The mixture was heated for 2 h, after which the tannin extract was decanted. Next, the
155 extracted tannins and residual bark were effectively separated by centrifuging at 3000 rpm for 10
156 minutes. Finally, the tannin extract was stored in a plastic capped bottle at -18 ℃ for further
157 processing.
158 Before pulverization of the spruce tannins, the tannin concentration in the extract was increased with
159 the aid of a rotary evaporator. During the tannin concentration process, 300 ml of water was removed
160 from every 500 ml of the spruce extract in the rotary evaporator at 65 to 70 °C. At the end of the
161 vacuum evaporation exercise, the tannin concentration of the extract was measured by acid butanol
162 assay for proanthocyanidins [26], which revealed that the tannin concentration in the extract had
163 increased from 1442 mg/l to 2650 mg/l. Pulverization of the tannin was performed through freeze
164 drying with a SCANVAC cool-safe freeze dryer and a completely freeze-dried tannin was obtained
165 after 16 days (referred to hereinafter as FDS (Freeze-dried spruce)).
166
167 **2.3 Kaolin/river water mixture**
168 The kaolin/river water was prepared by spiking 5 l of river water with 1 g of fine kaolin (200 mg/l).
169 In order to obtain an effective kaolin dispersion, it was important to adjust the pH of the suspension
170 slightly [13,27]. A stable kaolin suspension was achieved by adjusting the pH of the river water to
171 7.5 from its initial pH of ~6 with 0.5 M of NaOH before the addition of kaolin. The suspension was
172 blended by mixing vigorously with a magnetic stirrer at a rate of 800 rpm for 2 h. The characteristics
173 of the obtained kaolin/river water mixture are shown in Table 3.

2.4 Mannich modification of tannin-based coagulant

Modification was performed by adapting the Mannich modification process found in the literature [12]. The Mannich reaction was initiated by dissolving 2.5 g of powdered tannin in 5 ml of MQ water at room temperature. The tannin powder was added gradually in batches and stirred with a laboratory spoon to avoid tannin aggregation and to ensure its complete dissolution. It is important to note that physical observation of the tannins revealed that the QS-SOL and SDS tannins were composed of symmetric, fine powdered particles while the freeze-dried tannin was composed of flaky powdered particles. FDS tannin had the largest volume per gramme but was the most soluble when compared to the other tannins. The dissolution was preceded by raising the temperature of the tannin solution to 70 ℃ before adding 10.7 ml of diethanolamine or 4.9 ml of ethanolamine, and the pH was adjusted to 6.5 with concentrated HCl (37%) under mechanical agitation by a magnetic stirrer. The temperature was again raised to 80 ℃, and 1.38 ml of formaldehyde was added gradually with a peristaltic pump over 90 minutes. After the addition of formaldehyde, the temperature of the solution was increased to 85 ℃ and mixed continuously for 3 h. At the end of this time, the reaction was stopped by adding 5 ml of MQ water. The modified product was standardized by transferring it to a 50 ml volumetric flask and filling it up to the mark with MQ water. The solid content of the coagulants obtained before standardization was ~12% (w/v) and ~14% (w/v) for the DEA and ETH modification respectively and ~5% (w/v) for both amine modifications after standardization.

2.5. Characterization of tannin samples, tannin-based coagulants and jar test residue

The non-modified and modified tannin samples were analysed by ultra-performance liquid chromatography assisted by diode array (UPLC-DAD) and triple quadruple mass spectrometry (MS) detection. Specifically, we used the group-specific MS/MS analyses of procyanidin and prodelphinidin sub-units of proanthocyanidins together with the negative ion full scan analysis in the ion range of *m/z* 100-1200, as shown in detail in Engström et al. [22].

In addition, tannin samples and jar test residues were studied by XPS. The XPS characterization was performed with a Thermo Fisher Scientific ESCALAB 250xi using a rotating monochromatic Al anode generating an X-ray beam at 1486.6 eV. The XPS data were analysed with Avantage software, and the charge correction was performed by setting the binding energy (BE) of adventitious carbon to 284.8 eV while the Shirley function was used to subtract the background. The charge density measurement of the coagulants was performed with a Mütek particle charge detector PCD 03 pH (Hersching, Germany) by titrating with PesNa. The molecular weight (MW) of the coagulants was determined through high-pressure size exclusion chromatography (HP-SEC, Aligent 1100 Series Liquid Chromatography, Hewlett-Packard, USA) equipped with a Shimadzu RID-10A detector. 0.1 M of $NaNO_3$ was used as eluent with a flow rate of 0.5 ml/min in Ultrahydrogel (500 + 125) columns.

2.6. Water analyses

The turbidity of the water samples was measured with a HACH 2100Q portable turbidimeter while the UV absorbance of the water samples was measured with a Shimadzu ultraviolet spectrometer at 254 nm. The total surface charge of the water samples was determined with a Mütek particle charge detector by titrating 10 ml of the supernatants with PesNa or Poly-Dadmac titrant. The pH of the solutions was determined with a VWR phenomenal pH 1000 L. For river water and kaolin/river water, the SUVA value (specific ultraviolet absorbance) was calculated based on the measurement of the ultraviolet absorbance (UVA) of the water at an optical wavelength of 254 nm and the concentrations of dissolved organic carbon (DOC) according to EPA methods [28]. DOC measurement was carried out with a SIEVERS 900 portable TOC analyser. Before the UVA and DOC measurements, the samples were filtered through a 0.45 µm filter membrane (VWR, polyethersulphone membrane).

2.7. Coagulation experiments

For the coagulation experiments, Kemira flocculator 2000 jar test apparatus with conventional 1.0 l glass beakers was used. During the tests, the beakers were filled with the kaolin/river water to 800 ml. The suspension was then dosed with a different tannin coagulant and the solutions were subjected to rapid mixing at 150 rpm for 1 minute, slow mixing at 40 rpm for 20 minutes and sedimentation for 30 minutes. Turbidity samples were taken from 5 cm below the surface after the sedimentation period and were measured immediately. This was then followed by the extraction of 200 ml of the supernatant from a point 3 cm below the surface of the test water sample for other water analyses. Jar test residues were obtained by carefully decanting the supernatant from the sedimented flocs in the glass beakers. The recovered flocs were centrifuged at 2500 rpm for 10 minutes and the supernatants were again decanted. The flocs were oven-dried at 40 ℃ for 8 hours to obtain dried residues.

3. Results and Discussions

3.1. Characterization of tannin extracts

The UPLC-DAD-MS/MS analyses did indeed reveal that the polyphenol composition of the FDS and SDS spruce samples and that of QS-SOL tannin were quite different. The UPLC chromatograms show that the FDS sample contained numerous simple phenolic compounds, represented by sharp chromatographic peaks on top of the chromatographic hump of proanthocyanidins (PA) (Fig. 1A). On the other hand, the SDS sample was dominated by the proanthocyanidin hump and had only low levels of simple phenolics (Fig. 2A). The QS-SOL tannin sample contained the proanthocyanidin hump, together with some simple phenolics (Fig. 3A) such as gallic acid at 1.2 mins.

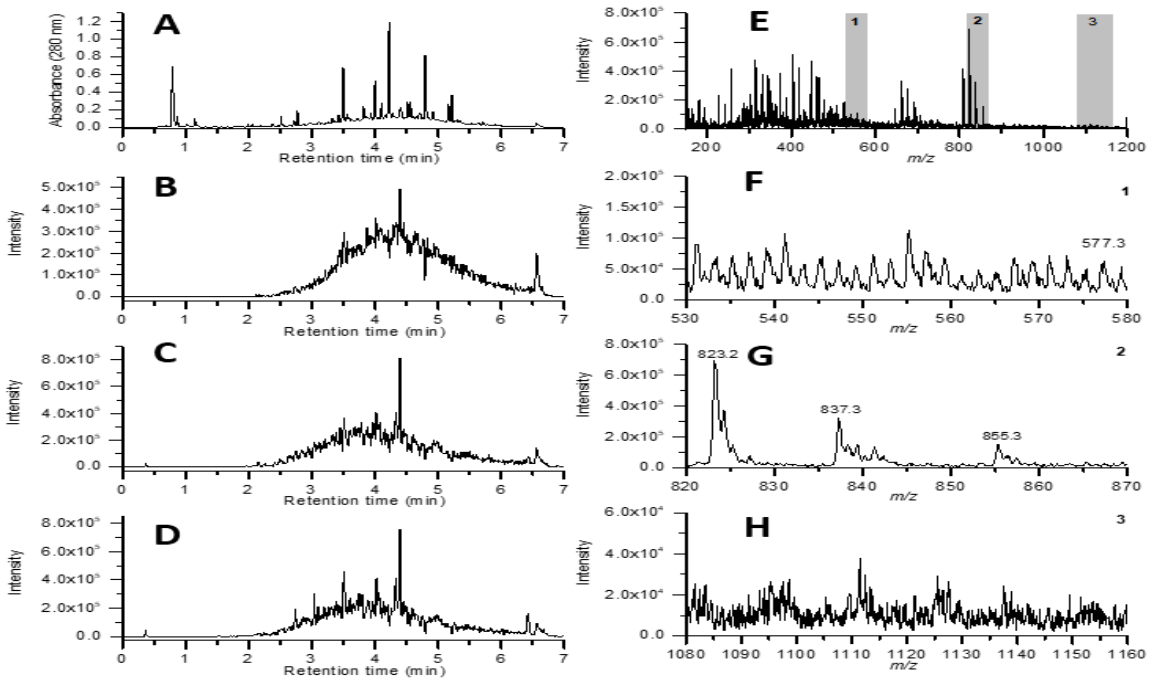


Figure 1. The UPLC-DAD-MS characteristics of the FDS sample: (A) the UPLC chromatogram at 280 nm, (B-D) procyanidin MS/MS fingerprints at different cone voltages, (E) full scan spectra, (F-H) zoomed mass spectra for the m/z regions for dimeric, trimeric and tetrameric procyanidins.

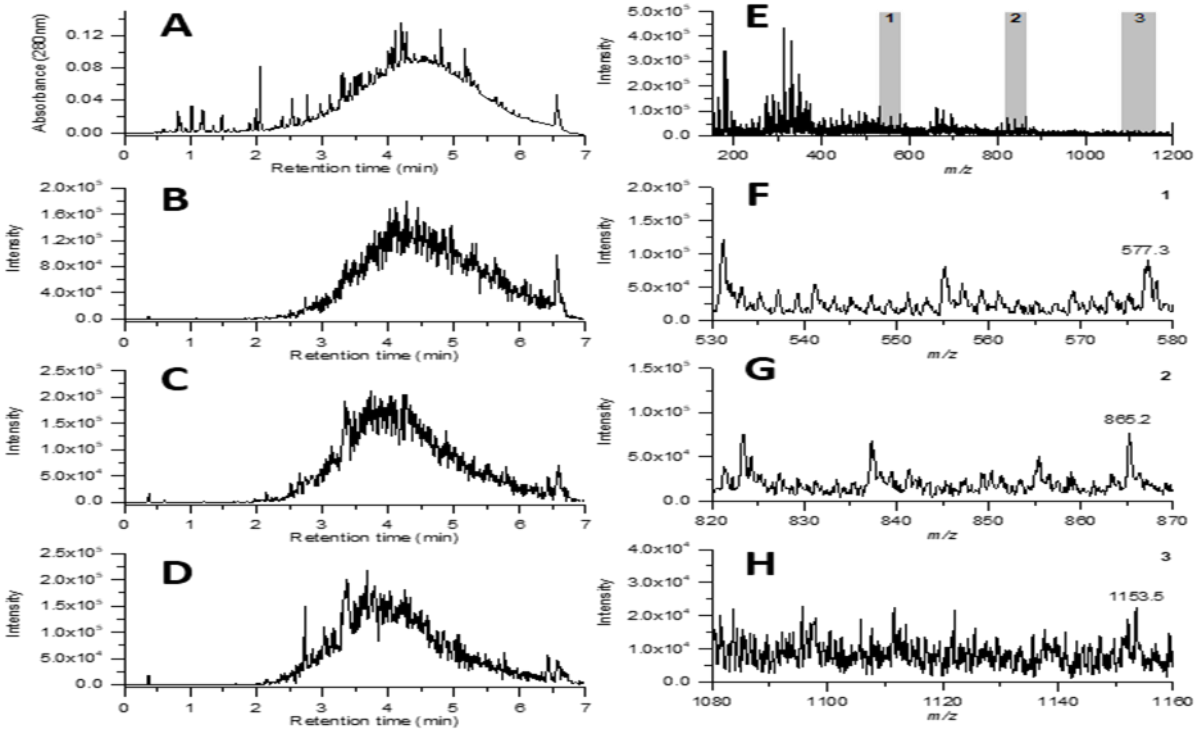


Figure 2. The UPLC-DAD-MS characteristics of the SDS sample: (A) the UPLC chromatogram at 280 nm, (B-D) procyanidin MS/MS fingerprints at different cone voltages, (E) full scan spectra, (F-H) zoomed mass spectra for the m/z regions for dimeric, trimeric and tetrameric procyanidins.

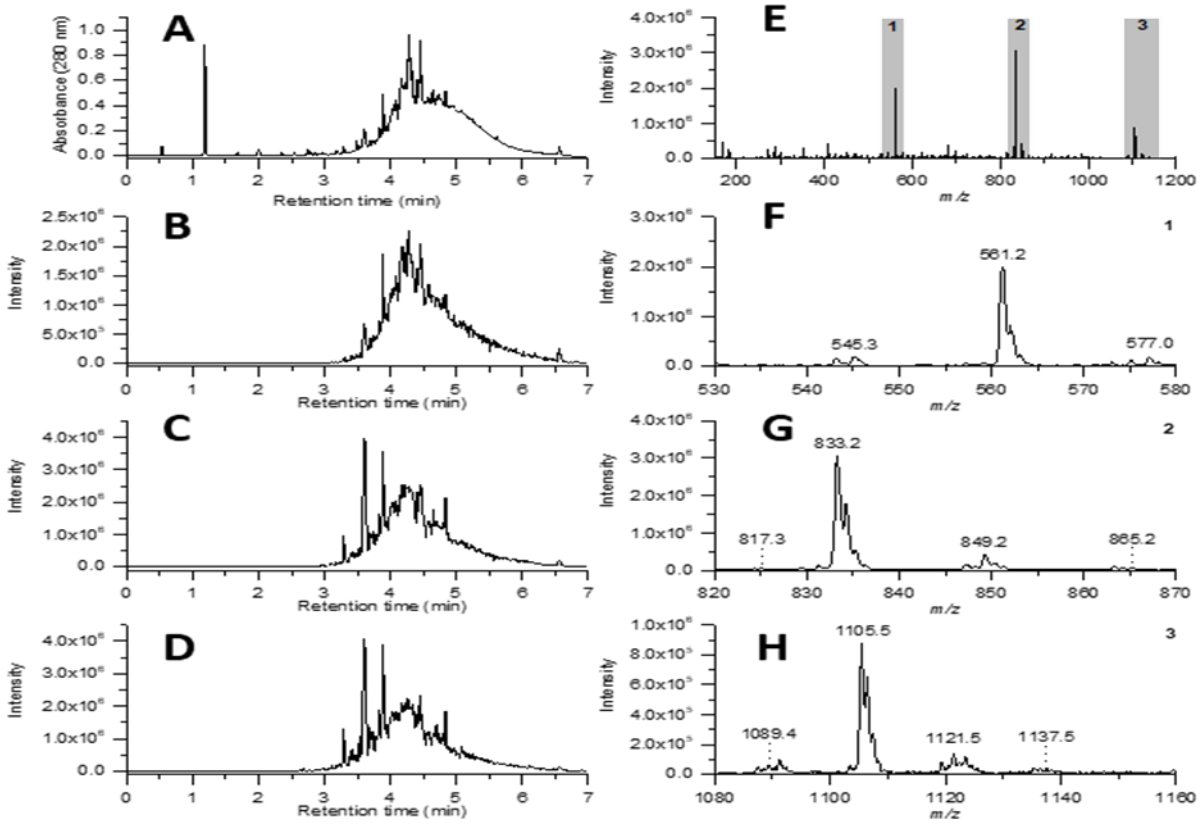


Figure 3. The UPLC-DAD-MS characteristics of the QS-SOL sample: (A) the UPLC chromatogram at 280 nm, (B-D) procyanidin MS/MS fingerprints at different cone voltages, (E) full scan spectra, (F-H) zoomed mass spectra for the m/z regions for dimeric, trimeric and tetrameric procyanidins.

The MS/MS method produced clear procyanidin fingerprints for all three samples (Figs. 1B-D, 2B-D, 3B-D), while the prodelphinidin fingerprints were much smaller in intensity (data not shown). The more numerous ions in the full scan ESI-MS spectra (panels E in Figs. 1-3) of the FDS tannin sample further confirmed that it contained simpler phenolics than the SDS tannin. The ion regions corresponding to the procyanidin dimers (m/z 577), trimers (m/z 865) and tetramers (m/z 1153) are

shown in panel E and zoomed in panels F-H, highlighting the fact that only the SDS tannin sample produced clear signals for these PA oligomers. For the QS-SOL tannin, these ions were scarce, whereas the ions at m/z 561, 833 and 1105 dominated the full scan mass spectra. These correspond to 5-deoxy PA dimers, trimers and tetramers showing that the quebracho proanthocyanidins differ a lot from the spruce proanthocyanidins. These data allowed us to calculate the PA concentration, the mean degree of polymerization (mDP) and the PC/PD ratio for the spruce (Table 1), but not for the quebracho PAs, since the Engström method [22] is not suited for detecting 5-deoxy PAs. Nevertheless, the QS-SOL tannin sample clearly had the highest PA content, although not all of its PA sub-units were included in the quantification.

Table 1. The proanthocyanidin (PA) content, the procyanidin (PC) content and the mean degree of polymerization (mDP) of the proanthocyanidins in the FDS, SDS and QS-SOL samples.

Sample	PA (mg/g)	PC-%	mDP
FDS	17.3	95	4.4
SDS	11.7	81	2.8
QS-SOL	64.6	-	-

The XPS wide spectra for the tannin samples (Supplementary Fig. S1) indicate that carbon (C1s 284.8 eV) and oxygen (O1s 532 eV) were the only detectable elements. Although the presence of a significant amount of hydrogen in the tannin matrix is generally known, XPS is unable to detect hydrogen. Moreover, with that in mind, it can be said that the XPS spectra are in confirmatory agreement with the generally represented chemical composition (C, H and O) of tannin. The C1s spectrum for the tannin samples presented in Fig. 4 clearly revealed two main peaks at 284.8 eV and at 286.5 eV, which can be assigned to C–C, C=C and C–H (C1 component) and C–OH and C–O (C2 component), respectively [29]. A minor peak was observed at ~288.0 eV with the spruce tannin

samples (Fig. 4, Supplementary Fig. S2A and 2B) and can be assigned to C=O (C3 component) [30]. The presence of the C3 carbon component was attributed to the presence of non-stilbene glycosidic and polymeric sugars in spruce tannin extracts [31].

Furthermore, a shake-up satellite, namely the π–π* component, was observed in the spectra of the FDS and QS-SOL tannins at BE ~293 eV and ~292 eV, respectively, but was featureless in the SDS tannin. The π bond is a characteristic feature of the sp^2 aromatic carbon constituent, and its intensity is dictated by the contribution of C=C bonding to the overall C1s spectrum [32]. However, it is essential to note that the shake-up satellite was more pronounced in the QS-SOL, which reflects a higher amount of sp^2 aromatic carbon.

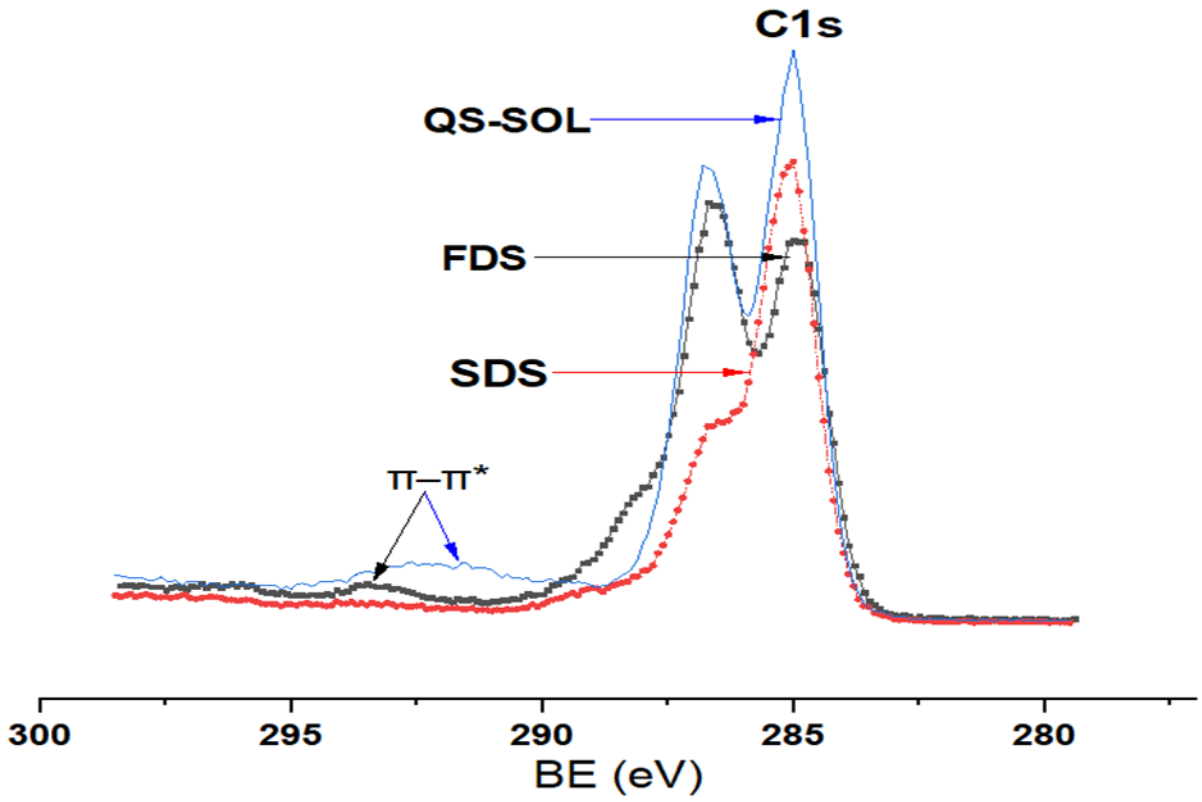


Figure 4. Overlaying comparison of C1s spectra for tannin samples.

The percentage amount of the C2 component (C–OH and C–O) was 42.2% for FDS, 43.9% for QS-SOL and 27.5% for SDS tannins (Supplementary Fig. S2). For QS-SOL tannin, the higher amount of phenolic groups could have contributed to the prominence of the C2 component, which is also in

agreement with the proanthocyanidin content (Table 1). Kemppainen [24] had earlier reported that quebracho tannin contained almost seven times more phenolic groups than spruce tannin. The higher amount of the C2 component in the FDS tannin sample as compared to SDS tannin could be attributed to the greater amount of proanthocyanidins (Table 1) and the presence of simple phenolics (Fig. 1).

3.2. Characterization of tannin coagulants

The modified bio-coagulants did not contain detectable levels of the original PAs, but their UPLC-chromatograms showed a retention time shift for the "tannin hump" (Fig. S3 and S4). The MS/MS fingerprints did not show any traces of PC or PD building blocks (data not shown), neither did the full scan mass spectra witness any ions specific to the natural PAs found in native tannin samples. This suggested that the modification reactions were successful.

The average molecular weights of all the coagulants were low, at approximately 390 g/mol (Fig. S5, ~37.5 min). However, a similar value has been reported for a commercial tannin coagulant [33]. Table 2 shows the charge density of the modified coagulants with two different amine sources. The quebracho tannin coagulant had the highest charge density with both amine sources. It could be deduced that the charge densities of the ethanolamine-modified coagulants were higher than their diethanolamine counterparts. Comparison of charge densities with other studies is difficult since most of the studies do not report these values for tannin-based coagulants and different aminomethylation pathways were commonly used [12,18,19]. However, at least Gang [13] reported a similar charge density for a tannin-based coagulant (3.1 meq/g) prepared through the Mannich modification of tannin (from an unknown source) with ammonium chloride and formaldehyde.

Table 2. Charge densities of the tannin coagulants. Range of charge densities represents the deviation in two repeats.

Bio-coagulant	Charge density (meq/g)
FDS-DEA	0.83 ± 0.10
SDS-DEA	0.85 ± 0.04
QS-SOL-DEA	2.79 ± 0.30
FDS-ETH	1.65 ± 0.31
SDS-ETH	1.88 ± 0.17
QS-SOL-ETH	3.84 ± 0.41

According to the charge density results in Table 2, the FDS was discovered to have the lowest charge density after both amine modifications. Aside from the variation in pulverization methods, one recorded difference between the Mannich-modified spruce tannins is that the FDS tannin came from a tree harvested towards the end of the winter season, while the SDS tannin was extracted from summer-harvested trees. Earlier researchers [24,34] have reported a higher amount of glucose units in the tannins of winter-felled spruce trees while a higher tannin content was recorded in the bark of summer-harvested trees.

3.3 Coagulative performance of tannin coagulants

Although the lower efficiency of commercial tannin coagulants in removing humics as compared to iron coagulants has been reported earlier [33], river water was selected for the test water, but only after spiking with kaolin. The characteristics of the river water sample before and after spiking with kaolin are shown in Table 3. Results from these analyses indicate that the river water had a low initial particle and colloid amount (low turbidity) but the turbidity was increased by spiking with fine kaolin. The high UV_{254} and SUVA values of the river water indicate the presence of high aromatic and humic

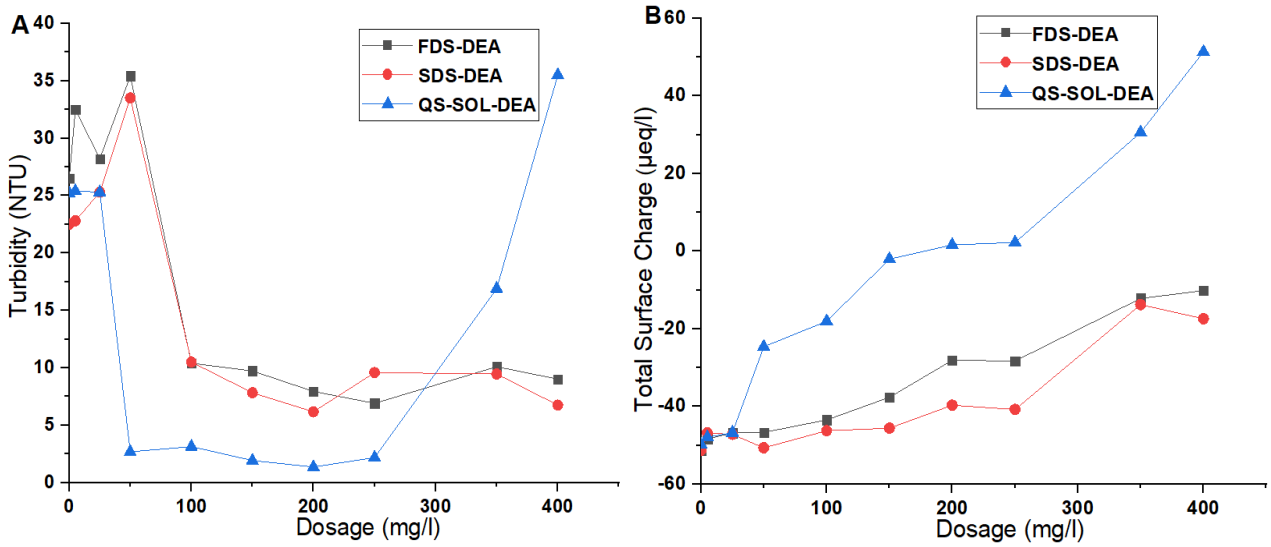
type organic substances. The analysed parameters also showed that spiking with kaolin had a minimal effect on the pristine characteristics of the river water.

Table 3. Characteristics of river water and kaolin/river water.

	Turbidity (NTU)	**pH**	**UV_{254} 1/cm**	**DOC (mg/l)**	**TSC (µeq/l)**	**SUVA (l/mg-M)**
River water	1.3	6.1	0.328	11.7	-45.41	2.803
Kaolin/river water	25.2	7.5	0.363	11.2	-50.65	3.135

The Mannich-modified tannins were able to reduce the turbidity of the kaolin/river water well. Fig. 5A and Fig. 5C show the relationship between various coagulant dosages and turbidity removal. A detailed comparison of their performance showed that, during the clarification test, the quebracho tannin coagulant slightly outperformed the spruce tannin coagulants in both amine modifications by achieving >90% turbidity reduction at a dosage of 50 mg/l for the DEA modification (Fig. 5A) and 25 mg/l for the ETH modification (Fig. 5C). However, these results also indicate that the modified spruce tannin coagulants recorded an impressive turbidity reduction at 100 mg/l dosages for the DEA modifications and 50 mg/l for the ETH modifications. This trend was observed to be in contrast with earlier publications where it was claimed that DEA Mannich-modified quebracho tannin exhibited better performance over the ETH modification for removal of dyes and surfactants from synthetic effluents [18,19]. However, the performance of bio-based coagulants has been reported to vary in different effluents due to the distinctive characteristics of the effluent concerned [15,16]. In addition, the turbidity values was noticed to be stable over a wide range of dosage with spruce tannin coagulants in both amine modifications. This could be of immense benefit when used in applications where the coagulant dose is constant, and dosing is not based on fluctuations in water quality.

Fig 5B and D show the total surface charge of water samples after the jar test for tannin coagulants using DEA and ETH as amines, respectively. The results indicate that all the water samples tested with both classes of coagulant modification exhibited a decrease in surface charge (i.e. the TSC became less negative) as the coagulant dosage increased. When ETH was used as an amine, a lower dose of coagulant was required to achieve an optimal dosage in comparison with DEA. The results for the total surface charge of the water samples after jar testing also revealed that, when overdosed with a higher amount of coagulant, the use of QS-SOL tannin coagulants resulted in a high cationic charge whereas the water treated with spruce tannin coagulants had a TSC of close to zero. The trends portray a strong interrelationship between the charge density (Table 3), total surface charge and turbidity removal. Thus, it is reasonable to conclude that the higher the charge density of coagulant, the lower the dose required to neutralize negatively charged colloids. This trend is supported by Gang [13], who also tested Mannich-modified tannin (unknown source) in kaolin-spiked river water and concluded that the optimal dosage of tannin-based coagulants was highly dependent on its charge density for effluents with a charge demand.



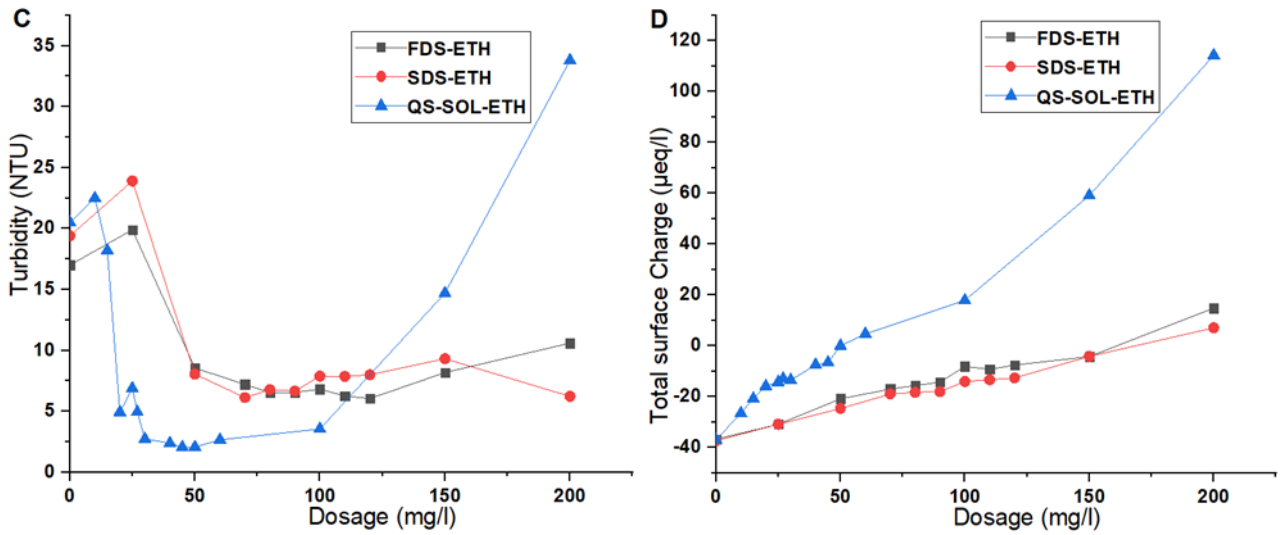


Figure 5. (A) Turbidity clarification performances of bio-coagulants with DEA as amine source, (B) total surface charge of water sample with DEA as amine source, (C) Turbidity clarification performance of bio-coagulants with ETH as amine source, (D) total surface charge of water sample with ETH as amine source.

Figs. 6A and B plot the UV_{254} as a function of the dosage of DEA and ETH modified coagulants, respectively. For DEA modification, the results showed that UV_{254} absorbance decreased with QS-SOL and SDS as the coagulant dosage increased, but the absorbance value of SDS-DEA-treated samples was observed to increase after exceeding a dosage of 100 mg/l. In contrast, the UV_{254} absorbance value of the FDS-DEA treated water samples increased as the dosage increased, which indicates an increase in organic compounds [35,36]. The results of the UV_{254} absorbance trends obtained from the ETH tannin modifications were similar to those of the DEA modifications. The only exception was that the UV_{254} of the QS-SOL-ETH-treated water samples increased after reaching the optimal dosage of 25 mg/l, which signifies that optimal dosage is a combination of turbidity and UV_{254}.

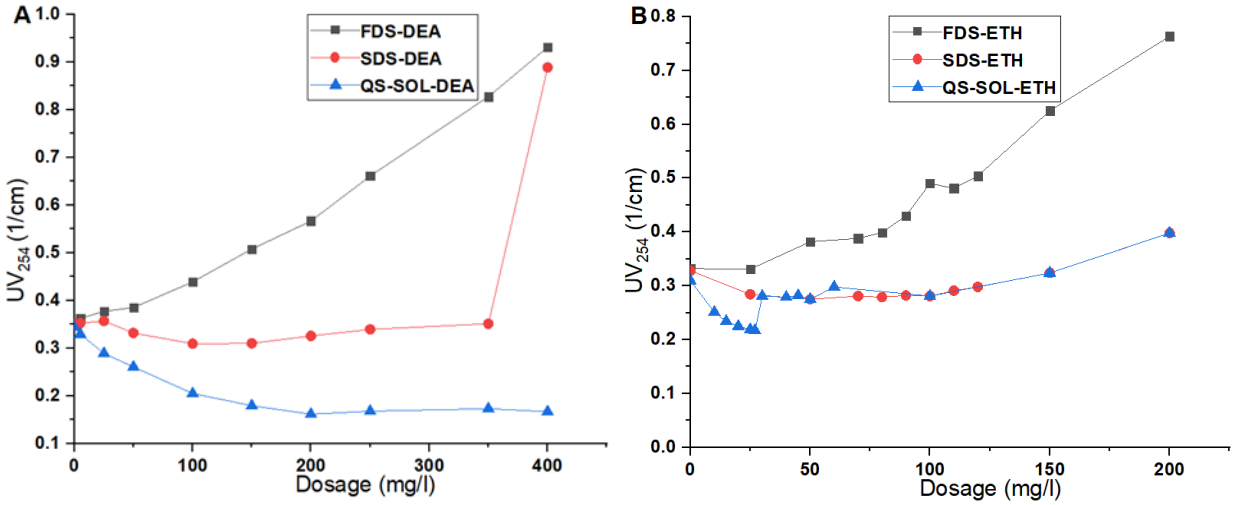


Figure 6. UV absorbance of the treated water with (A) DEA as amine source, (B) with ETH as amine source.

In general, the results revealed that quebracho tannin coagulants possess better coagulative potential in both amine modifications in comparison with the spruce tannin coagulants. The variation in performance could be best attributed to two factors. Since the primary mechanism behind the aminomethylation process is the addition of an iminium ion to the phenolic ring of tannin, the availability of more phenolic groups in the quebarcho tannin provides more active sites for the iminium ion to substitute hydrogen in the aromatic tannin structure. Another factor that might have contributed to the poorer performance of the spruce tannin coagulants is the presence of impurities. Tannin extracts from spruce bark mostly consist of ash and carbohydrates [24,37] and lower levels of tannins, which leads to the formation of undesired by-products during aminomethylation [19]. Zhang and Gellerstedt [37] showed that one in two of the flavonoid monomers in the condensed tannin of spruce was covalently bonded to glucose; here we showed that FDS spruce tannin contains more flavonoid glycosides than SDS tannin (peaks at 4-6 mins shown in Figs. 1-2). Kemppainen [24] also corroborated this claim but further observed that the portion of monosaccharides such as glucose in spruce tannin extract was higher in winter-felled trees than in those harvested during the summer season. These by-products and impurities with different chemical

structures and properties have a high probability of interfering with the Mannich reaction, and it appears that the aminomethylation might not have proceeded so efficiently with the spruce tannin extract and consequently, reducing the regioselectivity of the Mannich modification [19]. However, purification of the extract from impurities could be performed to overcome this drawback. Covalently bonded carbohydrates such as polysaccharides and disaccharides can be hydrolyzed to monosaccharides with hydrolytic enzymes, which could be dissociated from tannin through size-based separation techniques such as ultrafiltration [24]. It should be stated that purification of the extract would result in high cost, and thus should be avoided. Nevertheless, this study has proved that spruce-based tannin coagulants also performed efficiently, and further optimization of both extraction and Mannich modification might improve their properties.

3.4 Characterization of jar test residues

XPS analysis of the jar test residues revealed some changes induced by the Mannich reaction on the pristine structure of the tannins. The XPS spectra for the residues confirmed the presence of Al, Si and N as additional elements to the tannin structure for all bio-coagulants. Al and Si derived mainly from the kaolin while N originated from the amine used in the Mannich reaction. Furthermore, the spectra for the residues revealed the presence of barium in some samples. In order to ascertain the origin of the barium, a detailed characterization of nutrient and elemental content was performed on the river water. The results from the analysis (Supplementary Table S1) showed that Ba was present in low concentration (7.2 µg/l), which indicates that the bio-coagulants might possess a high affinity for Ba. The reason behind this trend was not investigated in this study.

The N1s spectra for the spruce tannin coagulant residues in the DEA modifications had two visible peak components, intense peaks at ~399.9 eV and less intense peaks at ~401.9 eV, while that of quebracho is the reverse (Supplementary Fig. S6 (A-C)). The peak at binding energy ~399.9 eV indicates the presence of non-protonanted amine (N1 component) and the second peak at ~401.9 eV

444 is assigned to protonated amine (N2 component) [38]. On the other hand, the N1s spectra of the jar
445 test residues of the ETH modifications for spruce tannins were observed to have a stronger peak at
446 ~399.9 eV, assigned to non-protonated amine. However, peak fitting (Supplementary Fig. S6(D-F))
447 clearly revealed the presence of protonated amine. Fitting of the C1s peaks of the coagulant residues
448 showed the presence of three components at ~284.8 eV (C–C, C–H, C=C), ~286 eV (C–O, C–N) and
449 ~288 eV (O–C–O, C=O) (Supplementary Fig. S7) [28,30]. A visual comparison of the C1s spectra of
450 the tannin samples (Fig. S2) and jar test residues (Fig. S7) shows a decrease in the proportion of the
451 component at lower BE (C–C, C–H, C=C), consistent with the disappearance of the π–π* shake-up
452 peak in the tannin samples associated with the aromatic conjugation [32,39].
453
454 **4. Conclusions**
455 The existence of companies producing tannin coagulants and many industrial applications of tannin
456 coagulants are clear indicators that tannin-based coagulants are a viable option for real application in
457 water treatment. This present study has demonstrated that tannins extracted from spruce bark can be
458 cationized to produce efficient bio-based coagulants. For application in surface water with a positive
459 charge demand, ethanolamine seems to be a better amine source to combine with formaldehyde in
460 the Mannich reaction. Bio-coagulants made from spruce tannins, in comparison with
461 quebracho, showed some lower charge densities and lower turbidity removal, which were attributed
462 to two factors: a reduction in the regioselectivity of the Mannich reaction for spruce tannins due to
463 the presence of impurities and a smaller amount of phenolic groups to accept the iminium ions
464 generated during the Mannich reaction. Nonetheless, the spruce tannin coagulants could still be
465 considered a good product because it possessed more stable residual turbidity and a TSC close to zero
466 over a wide range of dosage; more importantly, it could be further optimized to improve its
467 applicability as a bio-based coagulant since only two amines were tested during this study.

From the two spruce tannin pulverization methods considered in this study, the water treatment results obtained indicate that spray drying is the more suitable method. Freeze drying is a much slower process and a prolonged pulverization process might have negatively affected the quality of the tannin. Furthermore, variation in the season of tree harvesting was also considered to have affected the performance of the freeze-dried tannin, as the bark of trees harvested in the winter contains a smaller amount of tannin and a greater amount of glucose. However, the PA amount was higher in the freeze-dried tannin sample and the freeze-dried tannin also had higher solubility in the modification. Thus, more studies are required to optimize the extraction and modification process of spruce tannins.

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