ISOLATION OF PECTIC ACIDS FROM BLEACHED TMP WATER AND AGGREGATION OF MODEL AND TMP PECTIC ACIDS BY CALCIUM

Ville J. Saarimaa,* Andrey V. Pranovich, Anna C. Sundberg, and Bjarne R. Holmbom

Pectins are important structural elements in spruce fibres. Alkaline peroxide bleaching of spruce thermomechanical pulp (TMP) causes degradation and demethylation of pectins, yielding high-charge-density pectic acids. The pectic acids in fibres contribute strongly to the negative fibre charge, and the dissolved pectic acids increase the cationic demand of bleached TMP water. In this study, a method to isolate pectic acids from peroxide-bleached TMP pulp water is presented. The pectic acids were isolated and purified in good yield using a polyacrylate resin to remove lignin, a cellulose filter to remove galactoglucomannans (GGM), and an anion exchange resin to separate pectic acids from neutral carbohydrates. Salts and residual low-molar-mass carbohydrates were further removed from the isolated pectic acids by dialysis. The isolated pectic acids (>80% purity) had a low molar mass and a wide polydispersity (5.9 kDa, M_W/M_N 3.3). The aggregation and precipitation of the isolated pectic acids, as well as citrus fruit pectic acids with well-defined molar masses, by Ca^{2+}-ions were studied. The molar mass of pectic acids was a key factor determining the precipitation of Ca^{2+}-pectates. Pectic acids below 6 kDa were not precipitated by Ca^{2+}, while higher molar masses led first to partial and then to complete precipitation. The precipitated Ca^{2+}-pectates may impair paper machine runnability and paper quality.

Keywords: Spruce pectic acids, Calcium, Aggregation, TMP, Peroxide bleaching

*Contact information: Åbo Akademi University, Process Chemistry Centre, FI-20500 Turku/Åbo, Finland; *Corresponding author: vsaarima@abo.fi*

INTRODUCTION

Pectins are traditional gelling agents, thickeners, and water binders. Pectin-containing fruits, such as apple and currant, have been well known for centuries for their unique properties, which give gels and jellies their specific consistency (International pectin producers association 2007). Today, pectins are widely used as additives in many foods, such as yoghurt and ketchup, and also in pharmaceutical applications (Schols and Voragen 2002). The presence of pectins as fibre wall constituents in wood was demonstrated for the first time in the beginning of the 20th century (Hägglund 1928). Pectins in wood are largely composed of a backbone of methylated D-galacturonic acid units occasionally interrupted by L-rhamnose units. Later on, clarification of the morphological distribution of pectins in pine and spruce fibres led to increased attention to potential effects of pectins in papermaking from softwoods (Sjöström 1989; Rättö et al.

1993; Thornton et al. 1993; Westermark and Vennigerholz 1995; Sundberg K. et al. 1998). Pectins are concentrated in the primary wall and middle lamella (Westermark et al. 1986). Norway spruce (*Picea abies*) is a commonly used softwood species in production of mechanical pulp and paper in northern Europe. Although the concentration of pectins in spruce is relatively low (1-2%), pectins, together with xylans, are generally accepted to be the main contributors to anionic fibre charge of spruce pulp (Sjöström 1989; Sundberg A. et al. 2000). The anionic fibre charge is essential for adsorption of cationic papermaking polymers, such as retention aids, on fibres, and for fibre swelling. Fibre swelling in turn positively influences fibre bonding area, higher bonding area being beneficial for pulp and paper strength properties (Engstrand et al. 1991). Mechanical pulping introduces new fibre surfaces, exposing pectin-rich fibre middle lamellas and primary walls. Also cellulosic fine material, which is particularly rich in those fibre regions, is released during mechanical defibration (Hardell et al. 1980). Mechanical pulps are commonly bleached using hydrogen peroxide under alkaline conditions. Mainly due to high pH, during peroxide bleaching pectins are degraded to low-molar-mass fractions and demethylated, which causes 1/3 of the original pectins in fibres to dissolve into process water as high-charge-density pectic acids (Thornton et al. 1993; Pranovich et al. 2003). The residual pectic acids in fibres and fines contribute strongly to increased charge properties of TMP after peroxide bleaching, and the dissolved pectic acids constitute the major part of anionic charge (cationic demand) of bleached TMP water (Thornton et al. 1993). Due to their capacity to consume cationic retention chemicals, pectic acids are commonly regarded as detrimental substances, or so-called anionic trash, in papermaking (Thornton et al. 1993; Kekkonen et al. 2002; Ricard et al. 2005a,b). Degradation of the polymeric pectic acids into monomeric galacturonic acid by enzymatic pectinase treatment has been suggested and actually already used in some paper mills in order to eliminate their detrimental effects (Thornton 1994; Thornton et al. 1996; Reid and Ricard 2000; Peng et al. 2003; Ricard et al. 2005a,b).

Apart from forming complexes with cationic polymers, pectins are known to interact with cations such as Na^{+}, Mg^{2+}, Ca^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, and Sr^{2+} (Kohn 1987; Dronnet et al. 1994). Complexes with Na^{+} and Mg^{2+} are water soluble, while aggregation with other cations brings about precipitation of corresponding pectates (Rolin 1993; Wellner et al. 1998). In mechanical pulping, especially in peroxide-bleached TMP, Na^{+}, Ca^{2+}, and Mg^{2+} are some of the most abundant metal ions (Sundberg A. et al. 2000). Calcium concentrations in mechanical pulping and bleaching can be really high, especially if calcium carbonate or gypsum are used to fill or coat papers and the circulation waters from paper machine are run in countercurrent flow to pulp and paper production (Bräuer et al. 2001). The aggregation of pectins and pectic acids by Ca^{2+}-ions has been well established in literature (Grant et al. 1973; Dronnet et al. 1994; Garnier et al. 1994; Bracchini and Pérez 2001; Capel et al. 2006). Ca^{2+}-complexation is a two-stage process with an initial, strong dimerization of pectins by cross-linking, followed by formation of weak inter-chain associations governed by electrostatic interactions (Fig. 1). Recently, we have investigated how the formation of Ca^{2+}-pectates affects the release and retention of pectic acids during peroxide bleaching of TMP, drainage of mechanical pulp, deposition tendency of colloidal pitch, and the removal of pectic acids by dissolved air flotation (Saarimaa et al. 2006a-c 2007).

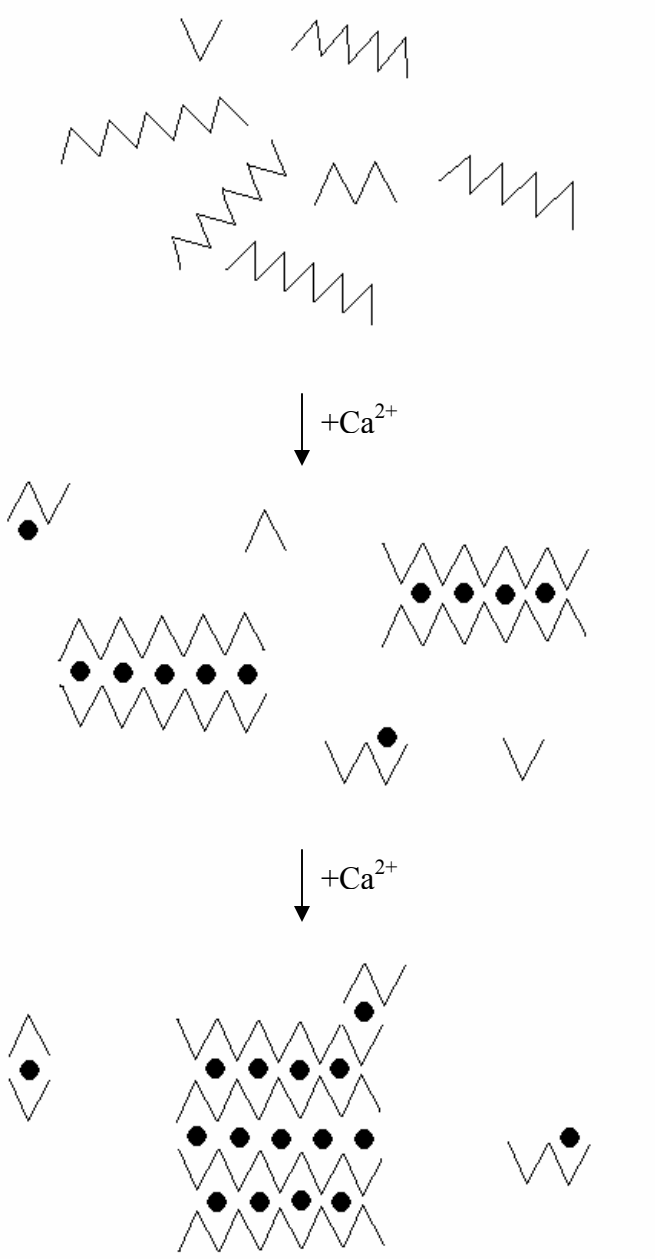

Fig. 1. Schematic picture of two-stage aggregation of pectins (/\/\/\) by calcium (•) (modified from Jarvis 2002).

However, although the effects of pectic acids in mechanical pulping and papermaking have received wide interest among paper researchers during the past 20 years, there is still limited information available of the structure and characteristics of the pectic acids released during mechanical pulping and bleaching. Therefore, in this study we introduce a novel method to isolate pectic acids from mechanical pulping process waters. The aggregation of the isolated pectic acids, as well as the aggregation of model pectic acids with well defined molar masses, by Ca^{2+}-ions were investigated.

EXPERIMENTAL

Materials

A typical press filtrate from peroxide-bleached thermomechanical pulp (TMP) was obtained from a mill in southern Finland using Norway spruce. The water was stored at -17°C and centrifuged prior to use in order to remove suspended solids. $CaCl_2$ of analytical grade was used. An Amberlite XAD-8 macroporous polyacrylate resin and an IRA-93 styrene divinylbenzene resin were from Fluka Chemie (AG, Switzerland). A dialysis membrane with a cut-off of 12-14 kDa was obtained from Medicell International LTD, UK. Tesorb cotton linters were used (Tervakoski Oy, Finland). The polyvinylidene difluoride (PVDF) membranes with pore size of 0.2 μm and cut-off of 180 000 g/mol were from Microdyn-Nadir Gmbh (Wiesbaden, Germany).

Measurements and Analyses

Carbohydrates were determined using acid methanolysis to obtain methyl glycosides of neutral sugar units and methyl ester methyl glycosides of uronic acids, followed by gas chromatography to determine monomer concentrations (Sundberg A. et al. 1996). Calcium in the water samples was measured using inductively coupled plasma optical emission spectroscopy (Optima 5300 DV, PerkinElmer, USA). Total organic carbon (TOC) was measured on a TOC-5050 Analyzer (Shimatzu Corp., Japan).

Preparation of Model Pectic Acids

Pectic acids with a narrow molar mass distribution (Table 1) were prepared from commercial citrus fruit pectin (Sigma-Aldrich Chemie GmbH, Germany) by alkaline hydrolysis with carefully controlled reaction time, pH and temperature (Patent pending). The reaction mixtures were cooled to room temperature, acidified to pH 4.0, concentrated in a vacuum rotor-evaporator, and then freeze-dried. The molar mass of the prepared pectic acids was determined by SEC-HPLC on an Agilent 1100 Series chromatograph equipped with Linear Ultrahydrogel column (Waters Corp., MA, USA). A miniDAWN Tristar MALLS detector by Wyatt Technologies (CA, USA) and a Shimadzu RID-10A refractive index detector (Japan) were used. The eluent was 100 mM NaCl, and the flow rate 0.5 mL/min. Previously published values of specific refractive index increments (dn/dc) were used for M_W determinations (Michielsen 1999; Fishman et al. 2000).

Table 1. Pectic Acids Used in Experiments.

Molar mass, M_w kDa	Degree of Polymerization DP	Polydispersity M_W/M_n
3.7	21	1.7
4.3	24	1.6
6.0	34	1.8
6.6	38	1.6
7.8	44	1.5
11.6	66	1.5

The prepared pectic acids contained less than 20% of neutral sugars. The dominating neutral sugars were arabinose (about 5%) and galactose (about 10%), which is typical for citrus fruit pectin (Renard and Thibault 1996). The model pectic acids contained about 20 w-% ash, mostly Na^+.

Isolation of Pectic Acids From Bleached TMP Water

The isolation of pectic acids was carried out on a typical press filtrate from peroxide-bleached thermomechanical pulp from a mill in southern Finland using Norway spruce. Extractives and other colloidal material were removed by polyvinylidene difluoride (PVDF) membranes with pore size of 0.2 μm and cut-off of 180 000 g/mol. A schematic overview of the isolation procedure is shown in Figure 2.

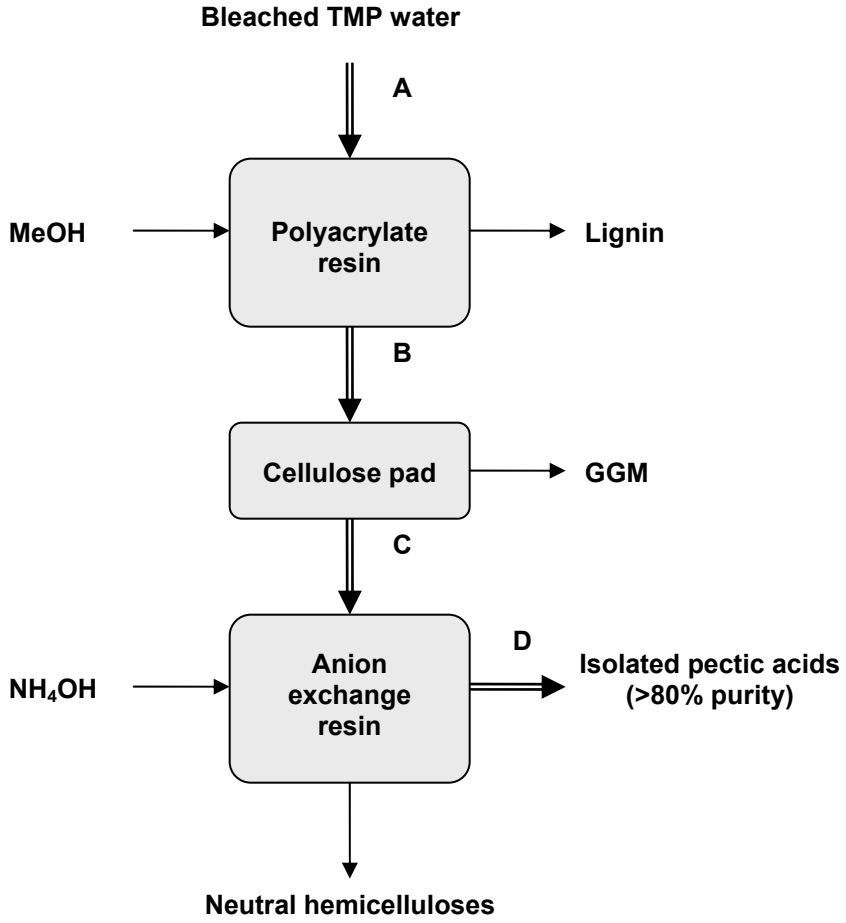

Fig. 2. Isolation of pectic acids from peroxide-bleached TMP water. Different steps in the isolation procedure are referred to as "A"-"D" in the text and in Figure 3.

The removal of lignin and other aromatic substances from the filtrate was carried out with a macroporous polyacrylate resin (Sjöström 1990, Sundberg, K. and Holmbom 1997, Pranovich et al. 2005). The resin was first cleaned by washing with 0.1 M NaOH, with distilled H_2O and with 0.01 M HCl. The XAD-8 resin was packed in a column (3 cm in diameter, 35 cm in height) and the column was rinsed with 0.01 M HCl until the total

organic carbon (TOC) of the effluent was <2 mg/L. About 0.9 L of the bleached TMP filtrate was acidified to pH 2.0 with 0.1 M HCl and passed through the column. The last void volume of influent was displaced with 0.01 M HCl. The hydrophilic effluent was collected, the pH was adjusted to 5.0 with 0.1 and 0.01 M HCl and the effluent was concentrated to approximately 1 L with a vacuum rotor-evaporator. The aromatic substances were desorbed from the resin with methanol.

After the lignin removal, the next step was to remove galactoglucomannans, filtering the sample through a pad of disintegrated cotton linters. The cotton linters (10x10 cm) were first cut into small pieces, put into hot water (70°C), and disintegrated with a household mixer. A uniform layer (1-2 cm) of cellulose was packed in a filter funnel with a diameter of 10 cm, and the lignin-free TMP filtrate was passed through the filter three times under suction in order to retain the GGM.

The final step of the isolation procedure was carried out with an anion exchange resin, a weak base of styrene divinylbenzene having an exchange capacity of 1.2-1.4 meq/mL (Sjöström 1990, Sundberg, K. and Holmbom 1997). The resin was first stirred for 1 h in 0.1 M NaOH, followed by decanting the solution. After that, the resin was washed with distilled water and with 0.1 M HCl. A glass column (3 cm in diameter, 35 cm in height) was packed with resin and rinsed with 0.5 M NaOH. The column was then rinsed with distilled water until the pH was stable, followed by rinsing with 1 M HCl and again with distilled water. The filtrate was passed through the column, followed by rinsing with distilled water. The resin was regenerated with 100 mL of 3 M NH_4OH, and the anionic hemicellulose fraction was collected.

Precipitation of Pectic Acids by Ca^{2+}

Solutions of pectic acids (about 30 mg/L) were prepared in distilled water, and the pH was carefully adjusted to 5.0, 6.5 or 8.0 with 0.01-0.1 M HCl and 0.01-0.1 M NaOH. The prepared solutions were pipetted into test tubes, 10 mL in each, and Ca^{2+} was added to obtain concentrations between 0 and 10 mmol/L. The test tubes were vigorously shaken and left to stand overnight. The next morning they were centrifuged (500 g, 30 min), and the concentration of pectic acids in the supernatant was determined by GC (Sundberg A. et al. 1996).

RESULTS AND DISCUSSION

Isolation of Pectic Acids from Bleached TMP Filtrate

The bleached TMP filtrate contained 0.47 mmol/L (19 mg/L) of Ca^{2+} and only traces of lipophilic extractives (<30 mg/L). The carbohydrate composition of the filtrate was: galactoglucomannans, 40.5%, arabinogalactans, 8.8%, arabinoglucuronoxylans, 5.6%, pectic acids, 35.5% and starch and glucans, 9.6%. The total polysaccharide concentration was 930 mg/L ("A" in Fig. 3). Monomer concentrations are presented instead of polysaccharide concentrations, since some of the sugars were most probably present in very low-molar-mass carbohydrates, especially in the last steps of the isolation procedure, which made the conversion to polysaccharides difficult. For polygalacturonic acids, for instance, it has been shown that a decrease in molar mass by acid de-

esterification yields a lower amount of rhamnose in the polymer backbone (Garnier et al. 1993).

The removal of lignin and other aromatic substances from the filtrate with a polyacrylate resin resulted in a slight decrease in total yield, but no specific removal of galacturonic acids or hemicelluloses was detected. The composition of the effluent is shown as column "B" in Fig. 3. The aromatic substances were desorbed from the resin with methanol. This fraction (about 1.1 g) contained only traces of hemicelluloses (15 mg/g) with no change in the original hemicellulose composition.

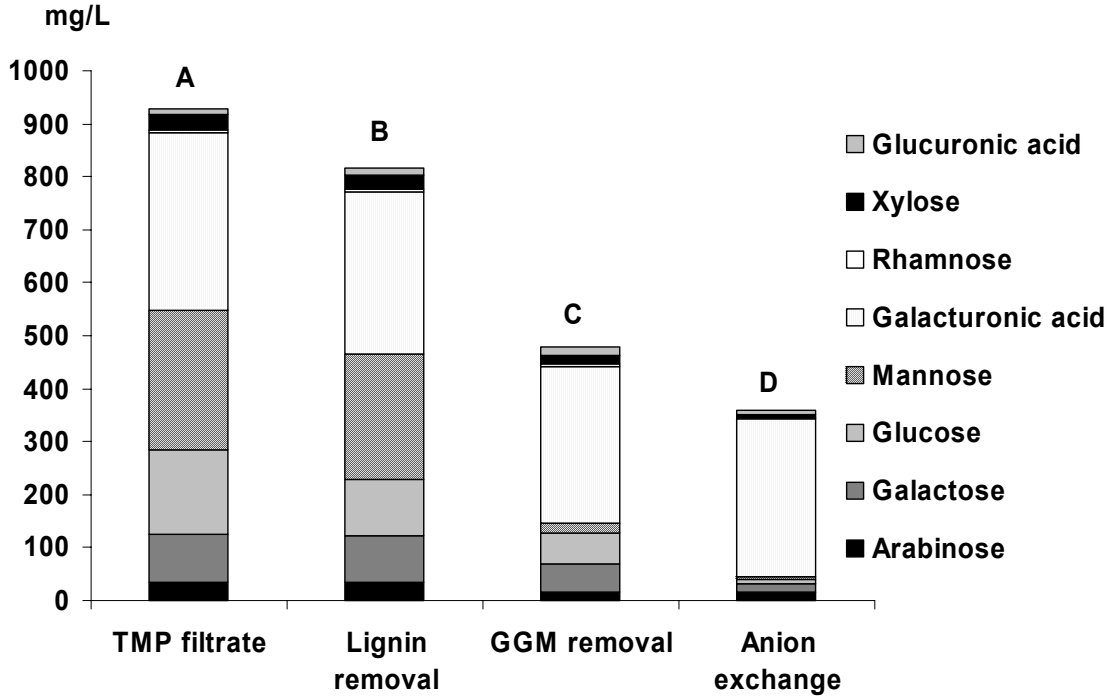

Fig. 3. Hemicelluloses in the TMP filtrate in the different steps of the pectic acid isolation procedure. Different steps in the isolation procedure (Fig. 2) are referred to as "A"-"D" in the text.

The sorption of galactoglucomannans (GGMs) onto different pulp fibres, especially onto chemical pulps, has been well demonstrated by Hannuksela (2004). The degree of glucomannan sorption increases after the glucomannans are deacetylated during peroxide bleaching of TMP. Galactoglucomannans were indeed efficiently removed by filtration through a cellulose pad ("C" in Fig. 3). Further purification of the filtrate with an anion exchange resin caused selective retention of acidic components. Neutral hemicelluloses passed through the column. The resin was regenerated with NH_4OH, revealing that galacturonic acids (>80%) were the main carbohydrates in the isolated product. Minor, but significant amounts of arabinose and galactose were also detected. Arabinose and galactose are known to exist as side chains of pectins, also in wood (Simson and Timell 1978; Schols and Voragen 2002). Traces of arabinose and galactose could also indicate the presence of acidic arabinogalactans (Willför et al. 2002). The anionic, pectic acid-rich fraction was finally purified by dialysis in order to remove salts and low molar-mass hemicelluloses. No galacturonic acids were removed by dialysis.

Some (about 10%) of the other carbohydrates were removed. The TMP pectic acids had a molar mass of about 5.9 kDa (DP 34) with a polydispersity of 3.3. Pectic acids in bleached TMP waters generally contain much more galacturonic acid (GalA) units and significantly less rhamnose (Rha) than pectins in fibres (Pranovich et al. 2003, Sundberg A et al. 2002). In the studied TMP water only a small amount of rhamnose was detected, and thus the pectic acids were practically completely composed of galacturonic acid units.

Aggregation of Pectic Acids with Different Molar Masses by Ca^{2+}

The aggregation and precipitation of pectic acids by Ca^{2+} was studied with model pectic acids from citrus fruit pectin and pectic acids isolated from the bleached TMP filtrate. The molar mass of pectic acids largely determined the degree to which they were precipitated and also influenced the required Ca^{2+}-concentration for complete precipitation (Figs. 4 and 5). The model pectic acids having molar mass less than 6 kDa (DP 34) were not precipitated, neither at pH 5 nor 6.5, while the pectic acids of higher molar mass were strongly precipitated. Precipitation of pectic acids occurred drastically at a certain Ca^{2+}-concentration, provided that the molar mass of pectic acids was high enough. Depending on the molar mass, there were still some pectic acids left after complete precipitation. The concentration of the residual pectic acids is most probably an indication of the molar mass distribution of the pectic acids: the lower the molar mass, the larger the low-molar-mass fraction that was not aggregated by Ca^{2}. Generally, some fluctuation in the percentage of residual GalA was seen before precipitation. The fluctuation can be attributed to inaccuracy in sampling and analysis.

It has been shown that the Ca^{2+}-binding of oligomeric pectic acid fragments is dependent on the degree of polymerization of pectic acids (Kohn and Larsen 1972; Kohn 1987). Aggregation of pectic acids by calcium is a two-stage process with initial dimerization followed by aggregation of the formed dimers (Grant et al. 1973). Ca^{2+}-pectates with DP lower than 7 (1.2 kDa) have been shown to be water-soluble, while higher DP (>10) leads to partial precipitation (Kohn 1987). It is also known that oligomeric pectic acids below DP 5 do not form complexes with cationic polymers (Thornton et al. 1993). A threshold of DP ~ 20 (3.5 kDa) has been proposed for strong cooperative effect in calcium binding (Kohn 1974). This was explained by presence of an array of specific binding sites regularly distributed along the pectic acid chain that present an ordered conformation (Bracchini and Pérez 2001). The difference between the molar masses required for precipitation in this study (6 kDa) and in the literature (3.5 kDa) could be explained by centrifugation forces. Kohn and Larsen (1972), Kohn (1974) and Kohn (1987) used centrifugation at 13.000-20.000 g to separate the pectates from the solution, while in this study the centrifugation was carried out at 500 g. The milder centrifugation force is justified by the relatively mild shear forces in papermaking wet-end, and the results in this study probably correlate well with the behaviour of pectic acids in real papermaking processes.

The rate of precipitation of the isolated pectic acids was in good accordance with the precipitation of the model pectic acids (Fig. 4), taking into account that the polydispersity of the isolated pectic acids was much wider than the polydispersity of

model pectic acids. Based on earlier studies and on our preparation procedure, both the model and the TMP pectic acids are assumed to be practically completely demethylated with a theoretical charge density of about 5-6 meq/g.

The MALLS detector used for determination of molar masses of pectic acids in this work is highly selective for high-molar-mass polymers. Since the prepared model citrus fruit pectic acids had extremely narrow molar mass distributions, the chromatogram peaks were clear and narrow. However, the isolated TMP pectic acid fraction resulted in a flat "peak" due to high polydispersity. Since the concentration of the polymer to be analyzed by MALLS has to be high in order to get a peak that does not disappear in the background noise, determination of the molar mass of the residual pectic acids left in the supernatant after precipitation was not carried out. Furthermore, those fractions probably also would have had extremely low molar mass, which causes difficulties in determination.

Increasing the pH from 5.0 to 6.5 did not affect the aggregation of pectic acids by Ca^{2+} (Figs. 4 and 5). Some experiments were also carried out at pH 8.0, giving similar results as at lower pH. The pKa-value of pectic acids is about 3.7. It has been reported that pectic acids are totally dissociated at pH 4.5 (Capel et al. 2006), and that pH does not influence pectin gels formed by Ca^{2+}-ions at a pH > 3.5 (Lootens et al. 2003). Therefore, it can be concluded that pectic acids behave in a similar manner in practically the whole papermaking pH range, from pH 5 to 8.

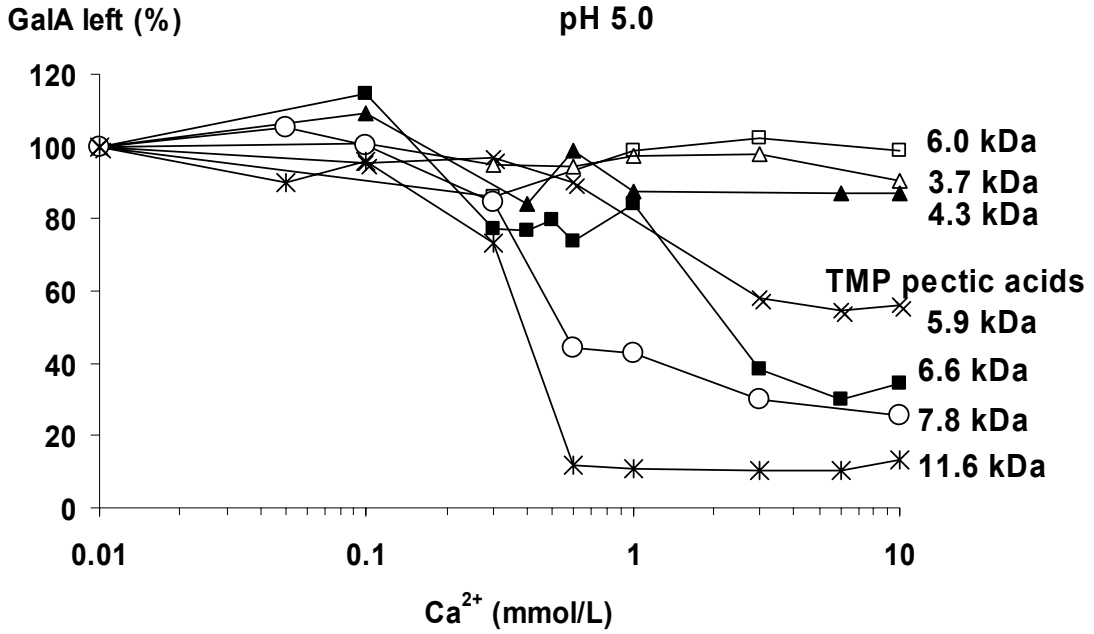

Fig. 4. Precipitation of pectic acids (about 30 mg/L) was determined by Ca^{2+}-concentration and molar mass at pH 5.0.

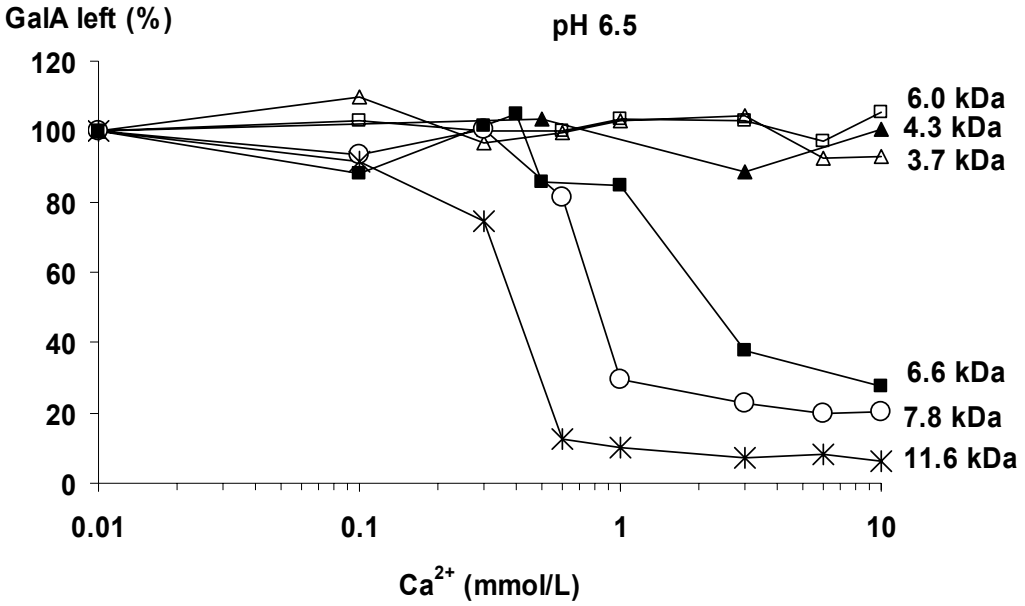

Fig. 5. Precipitation of pectic acids by Ca^{2+} at pH 6.5 was similar to pH 5.0.

A clear molar mass threshold for precipitation of pectic acids by Ca^{2+} was thus observed. Pectic acids below 6 kDa formed water-soluble complexes and are therefore probably not precipitated onto fibres in papermaking processes. However, at least some of the TMP process water pectic acids exceeded the critical molar mass for precipitation. Since the higher-molar-mass pectic acids were to a large extent aggregated and precipitated already at quite low Ca^{2+}-concentrations, it can be assumed that some of the pectic acids released during peroxide bleaching of TMP were instantly precipitated onto fibres and were not present in the bleached TMP filtrate that was used as a starting point in the isolation procedure.

Precipitated pectic acids can give manifold effects in mechanical papermaking. Detrimental dissolved and colloidal material has been claimed to interfere with wet-end additives such as retention polymers and wet/dry strength additives, and retard press dewatering and reduce dryer efficiency (Chung et al. 1993). Recently it has been shown that Ca^{2+}-pectates can impair dewatering during sheet formation, probably due to blocking of the voids and cavities in the porous fibre matrix (Saarimaa et al. 2007). The retained pectic acid aggregates also slightly decreased brightness of the formed paper sheets. Brightness is determined by light scattering (s) and absorption (k) coefficients. A decrease in light scattering coefficient was attributed to less scattering sites due to blocking of the porous sheet structure by Ca^{2+}-pectates. An increased adsorption coefficient in turn reflected higher retention of slightly coloured pectic acids in the pulp. Pectic acids are also precursors to various furan derivatives that are formed via hydrolysis, dehydration and condensation reactions under heat and humidity (Theander and Nelson 1988; Fischer and Beyer 2000; Jääskeläinen et al. 2007). Furan compounds are one of the key substances causing yellowing in pulps.

An enzymatic pectinase treatment could be used to depolymerize pectic acids to low-molar-mass fractions that are not retained in the pulp and thus do not show detrimental effects in papermaking (Thornton et al. 1996). In the literature, a 2% higher ISO brightness of TMP after pretreatment of wood chips with pectinase was obtained (Peng et al. 2003). After peroxide bleaching, a major part (2/3) of pectic acids is still associated with fibres. Based on the results of this study, the pectic acids remaining within the fibres might play greater role in brightness stability and pulp dewatering properties than the dissolved fraction, since the largest part (50-60%) of the dissolved pectic acids were low-molar-mass, non-detrimental pectic acid fragments.

CONCLUSIONS

1. Pectic acids can be selectively isolated from bleached mechanical pulping waters using a polyacrylate resin column to remove lignin, a cellulose pad to remove galactoglucomannans, an anion exchange resin to separate neutral and acidic carbohydrates, and finally a dialysis membrane to remove salts and residual low molar-mass carbohydrates. In this study, pectic acids with >80% purity were obtained.
2. The dissolved pectic acid fraction after peroxide bleaching of TMP is a polydisperse mixture of low-molar-mass galacturonic acids fragments.
3. The molar mass of pectic acids is the key factor, besides calcium concentration, determining the precipitation of dissolved pectic acids by Ca^{2+}. Pectic acids of molar masses <6 kDa are not precipitated by Ca^{2+}, but higher-molar-mass pectic acids are efficiently precipitated.

ACKNOWLEDGMENTS

This work is part of the activities of the Åbo Akademi Process Chemistry Centre within the Finnish Centre of Excellence Programme. Financial support from the international PhD programme in Pulp and Paper Science and Technology in Finland (PaPSaT) and from the Finnish Cultural Foundation is acknowledged. Kemira Oyj is acknowledged for providing the TMP filtrate.

REFERENCES CITED

Bracchini, I., and Pérez, S. (2001). "Molecular basis of Ca^{2+}-induced gelation in alginates and pectins: the egg-box model revisited," *Biomacromolecules* 2(4), 1089-1096.

Bräuer, P., Kappel, J., and Holler, M. (2001). "Anionic trash in mechanical pulping systems," *Pulp Pap. Can.* 102(4), 44-48.

Capel, F., Nicolai, T., Durand, D., Boulenguer, P., and Langendorff, V. (2006). "Calcium and acid induced gelation of (amidated) low methoxyl pectin," *Food Hydrocol.* 20(6), 901-907.

Chung, D. K., Cantrell, J., and Lefevre, G. (1993). "Control of detrimental substances," *Pulp Pap. Can.* 94(12), T403-T408.

Dronnet, V. M., Renard, C. M. G. C., Axelos, M. A. V., and Thibault, J.-F. (1994). "Characterisation and selectivity of divalent metal ions binding by citrus and sugar-beet pectins," *Carbohydr. Polym.* 30(4), 253-263.

Engstrand, P. Sjögren, B, Ölander, K., and Htun, M. (1991). "The significance of carboxylic groups for the physical properties of mechanical pulp fibres," *6th Intl. Symp. Wood Pulping Chem.*, Proceedings Vol. 1, Melbourne, Australia, pp. 75-79.

Fischer, K., and Beyer, M. (2000). "Comparison of light-induced and heat-induced yellowing of pulp," *Lenzinger Berichte* 79, 25-31.

Fishman, M. L., Chau, H. K., Hoagland, P., and Ayyad, K. (2000). "Characterization of pectin, flash-extracted from orange albedo by microwave heating, under pressure," *Carbohydr. Res.* 323(1-4), 126-138.

Garnier, C., Axelos, M. A. V., and Thibault, J.-F. (1993). "Phase diagrams of pectin-calcium systems: Influence of pH, ionic strength, and temperature on the gelation of pectins with different degrees of methylation," *Carbohydr. Res.* 240, 219-232.

Garnier, C., Axelos, M. A. V., and Thibault, J.-F. (1994). "Selectivity and cooperativity in the binding of calcium ions by pectins," *Carbohydr. Res.* 256, 71-81.

Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J. C., and Thom, D. (1973). "Biological interactions between polysaccharides and divalent cations: The egg-box model," *FEBS Lett.* 32(1), 195-198.

Hannuksela, T. (2004). *Mannans in mechanical pulping and papermaking. Naturally existing aids and promising wet end additives,* Doctoral thesis, Åbo Akademi University, Turku, Finland.

Hardell, H.-L., Leary, G. J., Stoll, M., and Westermark, U. (1980). "Variations in lignin structure in defined morphological parts of spruce," *Svensk Papperstidn.* 83(2), 44-49.

Hägglund, E. (1928). *Holzchemie*, Akademische Verlagsgesellschaft M.B.H., Leipzig, Germany, pp. 82-84.

International pectin producers association (2007). (http://www.ippa.info/history_of_pectin.htm).

Jarvis, M. C. (2002). *Pectins and their Manipulation*, ed., Seymour, G. and Knox, P., Blackwell Publishing Ltd, UK, pp. 99-130.

Jääskeläinen, A.-S., Adorjan, I., and Vuorinen, T. (2007). "Carbohydrate structures causing brightness reversion – a model study," *14th Intl. Symp. Wood, Fibre Pulping Chem*., Proceedings, Durban, South Africa.

Kekkonen, J., Lattu, H., and Stenius, P. (2002). "Formation, adsorption and interactions of poly-DADMAC/pectic acid complexes," *J. Pulp Pap. Sci.* 28(1), 6-12.

Kohn, R. (1974). "Ion binding of polyuronates-alginate and pectin", *Int. Carbohydr. Symp.*, Bratislava, Czechoslovakia, pp. 371-397.

Kohn, R. (1987). "Binding of divalent cations to oligomeric fragments of pectin," *Carbohydr. Res.* 160, 343-353.

Kohn, R., and Larsen, B. (1972). "Preparation of water-soluble polyuronic acids and their calcium salts, and the determination of calcium ion activity in relation to the degree of polymerization," Acta Chem. Scand. 26(6), 2455-2468.

Lootens, D., Capel, F., Durand, D., Nicolai, T., Boulenguer, P., and Langendorff, V. (2003). "Influence of pH, Ca concentration, temperature and amidation on the gelation of low methoxyl pectin," *Food Hydrocol.* 17(3), 237-244.

Michielsen, S. (1999). *Polymer Handbook*, 4th Ed., Brandrup, J., Immergut, E. H., and Grulke, E. A. (eds.), Wiley, NY, USA, pp. 547-627.

Peng, F., Ferritsius, R., and Ängsas, U. (2003). "Mechanical pulping with pectinase pretreatment of wood chips," *International Mechanical Pulping Conference*, QC, Canada, 335-340.

Pranovich, A. V., Sundberg, K. E., and Holmbom, B. R. (2003). "Chemical changes in thermomechanical pulp at alkaline conditions," *J. Wood Chem. Technol.* 23(1), 89-112.

Pranovich, A., Reunanen, M., Sjöholm, R., and Holmbom, B. (2005). "Dissolved lignin and other aromatic substances in thermomechanical pulp waters," *J. Wood Chem. Technol.* 25(3), 109-132.

Reid, I. and Ricard, M. (2000). "Pectinase in papermaking: solving retention problems in mechanical pulps bleached with hydrogen peroxide," *Enzyme Microb. Technol.* 26(2-4), 115-123.

Renard, C., and Thibault, J.-F. (1996). "Degradation of pectins in alkaline conditions: kinetics of demethylation," *Carbohydr. Res.* 286, 139-150.

Ricard, M., Orccotoma, J.-A., Ling, J., and Watson, R. (2005a). "Pectinase reduces cationic chemical costs in peroxide-bleached mechanical grades," *Pulp Pap. Can.* 106(12), T264-T268.

Ricard, M., Reid, I., and Orccotoma, J.-A. (2005b). "Pectinase reduces the cationic demand of peroxide-bleached TMP: a paper machine trial," *Pulp Pap. Can.* 106(12), T258-T263.

Rolin, C. (1993). *Industrial Gums. Polysaccharides and their Derivatives*, 3rd Ed., ed., Whistler, R. and BeMiller, J., Academic Press, Inc., San Diego, CA, USA, pp. 257-295.

Rättö, M., Kantelinen, A., Bailey, M., and Viikari, L. (1993). "Potential of enzymes for wood debarking," *Tappi J.* 76(2), 125-128.

Saarimaa, V., Sundberg, A., Holmbom, B., and Svedman, M. (2006a). "Effects of calcium on peroxide bleaching and washing of thermomechanical pulp," *Tappi J.* 5(10), 21-26.

Saarimaa, V., Vähäsalo, L., Sundberg, A., Pranovich, A., Holmbom, B., Svedman, M., and Örså, F. (2006b). "Influence of pectic acids on aggregation and deposition of colloidal pitch," *Nord. Pulp Pap. Res. J.* 21(5), 613-619.

Saarimaa, V., Sundberg, A., Holmbom, B., Blanco, A., Negro, C., and Fuente, E. (2006c). "Purification of peroxide-bleached TMP water by dissolved air flotation**",** Tappi J. 5(5), 15-21.

Saarimaa, V., Sundberg, A., and Holmbom, B. (2007). "Effect of Ca^{2+}-pectates on drainage of mechanical pulp," submitted to *J. Pulp Pap. Sci.*

Schols, H., and Voragen, A. (2002). *Pectins and their Manipulation*, ed., Seymour, G. and Knox, P., Blackwell Publishing Ltd, UK, pp. 1-29.

Simson, B. W., and Timell, T. E. (1978). "Polysaccharides in cambial tissues of *Populus tremuloides* and *Tilia Americana*. IV 4-*O*-methylglucuronoxylan and pectin," *Cellul. Chem. Technol.* 12, 79-84.

Sjöström, E. (1989). "The origin of charge on cellulosic fibres", *Nord. Pulp Pap. Res. J.* 4(2), 90-93.

Sjöström, J. (1990). "Fractionation and characterization of organic substances dissolved in water during groundwood pulping of spruce," *Nord. Pulp Pap. Res. J.* 5(1), 9-15.

Sundberg, A., Sundberg, K., Lilliandt, C., and Holmbom, B. (1996). "Determination of hemicelluloses and pectins in wood and pulp fibres by acid methanolysis and gas chromatography," *Nord. Pulp Pap. Res. J.* 11(4), 216-219.

Sundberg, A., Pranovich, A., and Holmbom, B. (2000). "Distribution of anionic groups in TMP suspensions," *J. Wood Chem. Technol.* 20(1), 71-92.

Sundberg, A., Konn, M., and Holmbom, B. (2002). *Unbleached and bleached fines-chemical characterization, sorption and interactions in papermaking*, Fines in closed circuits-Sub project report 3, SCAN FORSK REPORT 741, Åbo Akademi University.

Sundberg, K., and Holmbom, B. (1997). "Destabilization of colloidal wood resin caused by cellulosic fibers in thermomechanical pulp suspensions," *Pap. Puu* 79(1), 50-54.

Sundberg, K. E., Sundberg, A. C., Thornton, J. W., and Holmbom, B. R. (1998). "Pectic acids in the production of wood-containing paper," *Tappi J.* 81(7), 131-136.

Theander, O., and Nelson, D. A. (1988). *Advances in Carbohydrate Chemistry and Biochemistry Vol. 46*, ed., Tipson, R. S. and Horton, D., Academic Press, Inc., San Diego, CA, USA, pp. 273-326.

Thornton, J., Ekman, R., Holmbom, B., and Eckerman, C. (1993). "Release of potential "anionic trash" in peroxide bleaching of mechanical pulp," *Pap. Puu* 75(6), 426-431.

Thornton, J. W. (1994). "Enzymatic degradation of polygalacturonic acids released from mechanical pulp during peroxide bleaching," *Tappi J.* 77(3), 161-167.

Thornton, J. W., Eckerman, C. S., Ekman, R. O., and Holmbom, B. R. (1996). "Treatment of alkaline bleached mechanical wood pulp with pectinase," *US Patent 5,487,812*.

Wellner, N., Kacuráková, M., Malovíková, A., Wilson, R. H., and Belton, P. S. (1998). "FT-IR study of pectate and pectinate gels formed by divalent cations," *Carbohydr. Res.* 308(1-2), 123-131.

Westermark, U., Hardell, H.-L., and Iversen, T. (1986). "The content of protein and pectin in the lignified middle lamella/primary wall from spruce fibers," *Holzforschung* 40(2), 65-68.

Westermark, U., and Vennigerholz, F. (1995). "Morphological distribution of acidic and methylesterified pectin in the wood cell wall," *8th Intl. Symp. Wood Pulping Chem.*, Proceedings, Helsinki, Finland, pp. 101-106.

Willför, S., Sjöholm, R., Laine, C., and Holmbom, B. (2002). "Structural features of water-soluble arabinogalactans from Norway spruce and Scots pine heartwood," *Wood Sci. Technol.* 36(2), 101-110.

Article submitted: Sept. 10, 2007; First round of peer-review completed: Oct. 15, 2007; Revision received: Oct. 17, 2007; Article accepted and published: Oct. 19, 2007