

Improvement of the binding capacity of metal cations by sugar-beet pulp. 1. Impact of cross-linking treatments on composition, hydration and binding properties

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Saponification and cross-linking by formaldehyde and epichlorohydrin were performed on beet pulp in order to improve its potentialities as an ion-exchanger, either by increasing its cation-exchange capacity and/or by decreasing its hydration capacities. Effects of the treatments on sugar composition, on swelling and water-retention capacities, and on the binding of two metal cations (Zn^{2+} and Pb^{2+}) are reported. Saponification doubled the cation-exchange capacity without modifying the sugar composition or decreasing the hydration properties. The swelling capacity was thus identical to that of the initial pulp ($\sim 32 \text{ ml g}^{-1}$ at $\text{pH} \sim 7$ in water). Treatment with formaldehyde had little effects on the hydration properties. The best results in terms of hydration properties were obtained with epichlorohydrin, which lowered the swelling and water-retention capacities to 7 ml g^{-1} and 2 g g^{-1} , respectively, independently of pH and ionic strength. Binding properties were not altered by this treatment. However, the conditions used led to major changes in sugar composition, with severe loss of pectins at the lower epichlorohydrin levels. This loss was reduced at higher levels of epichlorohydrin, suggesting that pectins were involved in the cross-linking. Binding properties were not altered by such a treatment. © 1998 Published by Elsevier Science Limited. All rights reserved.

INTRODUCTION

In Europe, sucrose mainly comes from sugar-beet. Its extraction leads to the production of beet pulp which is one of the main by-products of the food industry. Its production reached 14×10^6 tonnes of dry matter in 1996 in the European Union. Its main use is incorporation in animal feeds, for which it is sold very cheaply. Many studies have been carried out to find alternative uses for sugar-beet pulp in order to increase its value (Hallanoro and Berghäll, 1994; Broughton *et al.*, 1995).

Pectic substances account for more than 40% of the dry matter of the beet pulp and are known to bind metal cations in solution (Jellinek and Sangal, 1972; Dronnet *et al.*, 1996) because of the carboxyl function of their main component, galacturonic acid (GalA). In spite of that, few studies have been carried out on the potential use of sugar-beet pulp to entrap metals in aqueous solutions. Langenhorst *et al.* (1961) undertook the decontamination of radioactive waste-water by raw beet pulp with a binding capacity of 0.62 meq g^{-1} . Sharma and Forster (1994) studied the sorption of Cr(VI) by low-cost adsorbents including beet pulp which was able to retain up to 4.4 meq g^{-1} at pH 2.

Recently, we studied the binding of some divalent metal cations by beet pulp (Dronnet *et al.*, 1997). One gram of

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pulp could bind up to ~ 0.6 meq of Cu^{2+} and Pb^{2+} . In the same conditions of pH and of beet pulp concentration, lower quantities of Zn^{2+} , Cd^{2+} (~ 0.5 meq g^{-1} each) and Ni^{2+} (~ 0.4 meq g^{-1}) were retained due to the lower affinity of pectins for these cations. The sorption of these metal cations was well described by the Langmuir isotherm.

The use of beet pulp as natural sorbent for the decontamination of industrial effluents contaminated with toxic metals may be a way to enhance its value. However, although the charge parameter in terms of dry matter may be similar to that of some synthetic resins or other natural sorbents, the hydration of beet pulp is too great to allow its direct use. Indeed, synthetic resins have much higher ionic retention capacities when expressed in meq per unit of hydrated volume. Langenhorst *et al.* (1961) reduced the swelling of the pulp by creating cross-links between the cell-wall polysaccharides using formaldehyde, and thus obtained a marked increase of the ionic-binding capacity per unit of exchanger volume. Laszlo and Dintzis (1994) found also a high cation-exchange capacity (approximately 1.3 meq g^{-1}) for epichlorohydrin-treated sugar-beet fibers.

Cross-linking has been extensively investigated to decrease the hydration properties independently of the external physico-chemical parameters (pH, ionic strength, etc.) while improving the mechanical properties of various biosorbents. The most used cross-linking reagents are: formaldehyde (Kumar and Dara, 1982; Holan *et al.*, 1993; Holan and Volesky, 1994; Ting and Teo, 1994; Vazquez *et al.*, 1994; Ting *et al.*, 1995), divinylsulfone (Holan *et al.*, 1993), glutaric dialdehyde (Holan and Volesky, 1994), phosphorus(V) oxychloride (Maranon and Sastre, 1992), citric acid (Wing, 1996) and epichlorohydrin (Holan and Volesky, 1994; Laszlo and Dintzis, 1994; Simkovic *et al.*, 1996). Epichlorohydrin was also studied for the cross-linking of soluble polysaccharides such as starch and pectins, especially for their use as chromatographic media for the separation and purification of enzymes (Kuniak and Marchessault, 1972; Rombouts *et al.*, 1979). Pectic acid was cross-linked with epichlorohydrin for use as an ion-exchanger in analytical chemistry or to remove traces of metal cations from waste-waters (Kohn *et al.*, 1976; Hatanaka *et al.*, 1990). Other methods of improving the mechanical properties and decreasing the hydration capacities are grafting onto synthetic polymers and entrapment into an organic or inorganic matrix (Darnall *et al.*, 1986; Bedell and Darnall, 1990).

In the present study, the binding capacity per volume of hydrated beet pulp was tentatively improved either by increasing the cation-exchange capacity by saponification or by reducing the bed volume by cross-linking with formaldehyde and epichlorohydrin. The impact of these treatments on the chemical composition, hydration properties, and binding capacity of Pb^{2+} and Zn^{2+} was studied.

MATERIALS AND METHODS

Beet pulp

Sugar-beet pulp (kindly supplied by Générale Sucrière, France) was prepared as previously described (Dronnet *et al.*, 1997). It was ground and the fraction in the range between 250 and 500 μm , corresponding to $\sim 31\%$ of the native pulp weight, was kept for further experiments. This fraction was then washed several times with 70% ethanol in order to inactivate endogenous enzymes and to remove residual mono- and oligomers, until the filtrate was sugar-free. The resulting pulp was dried by solvent exchange (95% ethanol and acetone) and finally air-dried (28% of the native pulp weight was recovered). It is referred to as raw pulp in the text.

Modifications of the substrates

Saponification

The pH of a cold (4°C) raw beet pulp suspension (20 g l^{-1}) was adjusted to ~ 13 by adding 1 M NaOH. The suspension was stirred at 4°C for 2 h and neutralised by adding 0.1 M HCl. The pulp was then filtered off, washed with ultrapure water, dried by solvent exchange (95% ethanol, acetone) and finally air-dried. It is referred to as saponified pulp in the text.

Acidic form

The substrates were converted to the acidic form by stirring the suspension (10 g l^{-1}) at 4°C in aqueous solution of 0.01 M HCl overnight (Bertin *et al.*, 1988). Resulting acidic substrates were filtered off, washed with ultrapure water, dried by solvent exchange and then air-dried. More than 95% of the initial weight was recovered with each substrate.

Treatment by formaldehyde and epichlorohydrin

Five grams of dry pulp (raw or saponified) was suspended in 100 ml of 0.1 M H_2SO_4 at 50°C . Varying amounts of formaldehyde were added to obtain a range of molar ratios (formaldehyde/total sugars of starting pulp) from 0 to 100. The suspension was stirred under reflux at 50°C . After 4 h, the suspension was cooled to room temperature. The residue was filtered off and washed with ultrapure water until the filtrate reached $\text{pH} \sim 4$. It was then dried as described above.

Five grams of dry pulp (raw or saponified) was dispersed at 4°C in a mixture of 95% ethanol (60 ml) and 5 M NaOH (40 ml). After 15 min, varying amounts of epichlorohydrin at a range of molar ratios (epichlorohydrin/total sugars from 0 to 100) were added and the mixture was stirred under reflux at 45°C . After 1 h, the mixture was cooled to room temperature and the pH was adjusted to ~ 5 by adding acetic acid. The residue was filtered off and washed with ultrapure water. It was then dried as described above.

Blanks (ratio = 0) were used to determine the effect of

experimental conditions (temperature, pH, stirring, etc.) on the starting materials to be estimated.

Substrate characterisation

Analytical

After 1 h prehydrolysis in 72% H_2SO_4 at 20°C followed by 3 h hydrolysis in 1 M H_2SO_4 at 100°C, galacturonic acid content was determined by the automated methahydroxybiphenyl assay (Thibault, 1979) and individual neutral sugars were identified and quantified as their alditol acetate derivatives by g.l.c. (Englyst and Cummings, 1984). Methanol and acetic acid contents of the raw and the saponified pulps were determined by h.p.l.c. according to Voragen *et al.* (1986). The methanol content of the substrates treated with cross-linking reagents was determined by g.l.c. Substrate suspension (2 mg ml^{-1}) in 0.1 M NaOH was stirred at 25°C. After 2 days of saponification, methanol was quantified using a DB wax column (J.&W. Scientific) at 40°C. The degrees of methylation (DM) and acetylation (Dac) were calculated as the molar ratios of methanol and acetic acid to galacturonic acid content, respectively.

Hydration properties

Hydration properties were measured with substrates in the acidic form.

Swelling capacity (SC) was measured by the bed volume technique (Kuniak and Marchessault, 1972). Dry substrate (100 mg) was weighed in a glass cylinder and left overnight at 25°C in an excess of either water or 0.1 M NaNO_3 . The pH of the suspension was not changed (~ 3.5) or was adjusted to ~ 7 by adding 0.1 M NaOH. Results were expressed as millilitres of swollen substrate per gram of starting dry matter. Measurements were carried out in duplicate.

The water-retention capacity (WRC) was measured by centrifugation (MacConnell *et al.*, 1974). Dry substrates were soaked either in water or in 0.1 M NaNO_3 for 16 h at 4°C and centrifuged for 1 h at 18 000 g. The supernatants were carefully removed and used to measure the amount of solubilised galacturonic acid. The residues were left for 1 h on sintered glass (porosity from 40 to 90 μm) and were weighed, dried for 2 h at 120°C and weighed again. The WRC was expressed in g of water per g of the dry material, corrected for the amount of salts if necessary. Measurements were made in duplicate.

Specific surface area

Specific surface area was measured in duplicate on ~ 2 g of dry substrate by the BET method (Brunauer, 1945) using a Gemini III 2375 Surface Area Analyzer (Micromeritics Instruments Corporation, USA) with nitrogen.

Cation-exchange capacity (CEC)

The cation-exchange capacity corresponds to the total number of ionic sites per gram of product. It was determined by titration with 0.1 M NaOH on substrates in the acidic form

(100 mg in 50 ml of 0.1 M NaNO_3). It was expressed as meq g^{-1} of dry matter.

Pb^{2+} and Zn^{2+} binding experiments

Batch assays were performed as previously described (Dronnet *et al.*, 1996). Suspensions of the substrate in the acidic form at given concentrations were stirred to complete hydration in 0.1 M NaNO_3 at $25.0 \pm 0.1^\circ\text{C}$ for 1 h and then the pH was adjusted to ~ 7.2 by adding 0.1 M NaOH. Amounts of 0.1 M solution of Pb^{2+} (as $\text{Pb}(\text{NO}_3)_2$) or Zn^{2+} (as $\text{Zn}(\text{NO}_3)_2$) (referred to as Me^{2+} in the text) were added to the suspensions to reach a ratio $[\text{Me}^{2+}]_t/[\text{C}_p] = 1$ where $[\text{Me}^{2+}]_t$ and $[\text{C}_p]$ are the total metal cation concentration and the substrate concentration in eq l^{-1} , respectively.

The mixtures were stirred for 2 h to reach the binding equilibrium. The free cation concentration, $[\text{Me}^{2+}]_f$, was measured at equilibrium by potentiometry in the case of Pb^{2+} (Dronnet *et al.*, 1996) or by a dual-wavelength dye spectrophotometric method in the case of Zn^{2+} (Dronnet *et al.*, 1996, 1997). The difference between $[\text{Me}^{2+}]_t$ and $[\text{Me}^{2+}]_f$ allowed evaluation of the bound cation concentration, $[\text{Me}^{2+}]_b$. Metal cation concentrations were expressed in eq l^{-1} .

RESULTS AND DISCUSSION

Chemical characterisation and cation-exchange capacity

Raw and saponified pulp

The sugar characteristics of the raw and the saponified pulps are shown in Table 1. Beet pulp was rich in arabinose, galacturonic acid (pectic substances) and glucose (mostly from cellulose). The saponification (96% of the starting pulp weight was recovered) did not significantly alter the sugar composition, and led to a marked increase of the CEC (from 0.55 to 1.13 meq g^{-1}) due to the decrease of DM from 52 to < 2 ; the loss of acetyl substituents was efficient and the DAC decreased from 52 to < 1 . Similar compositions (Bertin

Table 1. Chemical characterisation and cation-exchange capacity of the raw and the saponified pulps (in the acidic form)

	Raw ^a	Saponified
Yield ^b	—	96
Rha (mg g^{-1})	22	24
Ala	229	226
Gal	56	63
Glc	206	244
GalA	200	205
DM (%)	52	< 2
Dac (%)	52	< 1
CEC _{exp} (meq g^{-1})	0.55	1.13
CEC _{theo} (meq g^{-1})	0.55	1.15

^aFrom Dronnet *et al.* (1997)

^b% of the starting pulp weight

Table 2. Sugar composition, DM and CEC of the pulp cross-linked with formaldehyde

Molar ratio	Raw pulp							Saponified pulp			
	0	0.1	1	5	10	50	100	0	1	10	100
Yield ^a	74	76	76	77	77	81	84	83	83	83	86
Rha (mg g ⁻¹)	25	25	25	26	25	27	23	19	21	20	20
Ara	131	127	129	136	150	172	160	114	117	101	138
Gal	63	62	63	63	60	58	51	56	55	59	50
Glc	279	270	285	270	260	242	225	234	238	242	237
GalA	206	228	228	203	223	216	212	185	206	207	180
DM (%)	50	49	45	50	50	51	51	< 1	< 1	< 1	< 1
CEC _{exp.} (meq g ⁻¹)	0.54	0.52	0.56	0.53	0.52	0.52	0.51	1.23	1.21	0.94	1.21
CEC _{theo.} (meq g ⁻¹)	0.58	0.66	0.71	0.58	0.63	0.60	0.59	1.04	1.16	1.16	1.01

^a% of the starting pulp weight

et al., 1988; Ralet *et al.*, 1991; Renard *et al.*, 1994) and CECs (Langenhorst *et al.*, 1961; Rouau *et al.*, 1987; Renard *et al.*, 1994) have already been reported. The experimental values found for the CEC were in very good agreement with the values calculated from the amount of GalA and the DM. It confirmed that only galacturonosyl units, carried by the pectic backbone, are responsible for the H⁺/Na⁺ exchange.

Pulp treated with formaldehyde and epichlorohydrin

Treatments were performed with the raw pulp and the saponified pulp, with molar ratios (reagent/total sugars of the starting substrate) from 0 to 100. The composition of the pulps treated with formaldehyde and with epichlorohydrin is shown in Table 2 and Table 3, respectively. The yields correspond to the ratio of the weight of the product recovered after the reaction to that of the starting material. It therefore takes into account the loss of some starting material as well as the addition of cross-linking reagents.

For the formaldehyde treatment, the yield of the blanks was 74 or 83% starting from raw or saponified pulp, respectively. The amount of arabinose was drastically decreased (by 25%–45%) compared to the starting material in agreement with the sensitivity of glycosidic linkages to acid

hydrolysis (Selvendran *et al.*, 1985). While the contents of rhamnose and galacturonic acid were similar, the addition of formaldehyde increased the yield only slightly (10% at a maximum) whatever the amount of formaldehyde used and the yields were higher with the saponified pulp as starting material. The sugar content was then mostly independent of the amount of formaldehyde introduced.

As the GalA content and the DM were constant whatever the molar ratio, and close to those of the starting materials, CECs were in the range from 0.6 to 0.7 meq g⁻¹ starting from raw pulp, and ~1.2 meq g⁻¹ starting from saponified pulp. The effects of the cross-linking by formaldehyde on sugar composition were minor and this treatment did not modify the CEC.

Cross-linking with epichlorohydrin takes place in severe alkaline conditions which dramatically affected the CEC and the sugar composition of the pulp. In the blanks, the yield was very low with the raw pulp (25%) but was higher when the pulp was previously saponified (43%). The loss of GalA reached 90% of the initial amount in the raw pulp. This extremely high loss of pectins may be ascribed to β -elimination of methyl-esterified galacturonosyl units in the pectic substances (Albersheim *et al.*, 1960). However, some other phenomena may also occur since a marked loss of GalA (31%) was also observed when the saponified pulp

Table 3. Sugar composition, DM and CEC of the pulp cross-linked with epichlorohydrin

Molar ratio	Raw pulp							Saponified pulp					
	0	0.1	1	5	10	50	100	0	1	5	10	50	100
Yield ^a	25	49	49	99	92	109	117	43	73	99	111	110	112
Rha (mg g ⁻¹)	7	14	22	18	17	11	9	15	21	17	16	12	13
Ara	40	159	246	98	67	14	10	114	204	130	50	17	20
Gal	13	39	52	31	27	6	4	37	54	36	25	12	15
Glc	633	296	301	159	142	66	48	444	254	196	131	86	114
GalA	75	148	136	202	193	156	120	150	202	177	176	171	193
DM (%)	11	6	7	5	4	< 1	< 2	< 1	< 1	< 1	< 1	< 1	< 1
CEC _{exp.} (meq g ⁻¹)	1.28	0.81	0.73	0.81	0.82	0.76	0.76	0.87	1.19	1.08	0.97	0.93	0.82
CEC _{theo.} (meq g ⁻¹)	0.38	0.79	0.72	1.09	1.05	0.88	0.67	0.84	1.14	1.00	0.99	0.96	1.09

^a% of the starting pulp weight

is treated. Glucose became the main sugar in the treated material due to the chemical stability of cellulose.

In contrast to formaldehyde, epichlorohydrin had a marked effect on the yields and sugar compositions. The yield was almost doubled when molar ratios exceeded 0.1 and 1 for the raw and the saponified pulps, respectively; they increased with increasing molar ratio, reached 100% at a molar ratio of 5 and were higher than 110% at the highest molar ratios. The treatment by epichlorohydrin reinforced the structure, even with a small quantity of reagent, and counteracted the alkaline degradation of the cell-wall polysaccharides.

When raw pulp was used as starting material, the DM decreased but the methyl-ester groups were not completely saponified, additionally, the loss of GalA was reduced when the saponified pulp was the starting material, so that the CECs of these latter substrates were higher. Experimental CECs were close to the theoretical values, especially when the treatment was performed on the saponified pulp.

Although excellent yields were reached when the pulp was treated with epichlorohydrin, the sugar contents were very low. At high molar ratios of epichlorohydrin/total sugars, the quantity of all neutral sugars decreased, until less than 10% of the weight of the cross-linked pulp could be accounted for as neutral sugars. This could be explained by the increased extent of the side reaction wherein sugars are substituted by epichlorohydrin as monoether derivatives and do not participate to the formation of inter-connections via diether cross-links (Kuniak and Marchessault, 1972; Rombouts *et al.*, 1979). Such etherified neutral sugars can not form alditol peracetates and therefore are not detected by our g.l.c. method, explaining the discrepancy between low sugar contents, notably in the case of glucose, and high yields. This decrease did not occur for GalA, detected by colorimetry.

Hydration properties

Raw and saponified pulp

The swelling capacity (SC) of the pulp was measured by the bed volume technique at a pH of either ~ 3.5 or ~ 7 , in pure water or in the presence of 0.1 M NaNO₃. Whatever the solvent conditions, the SC of the raw and the saponified pulps were similar, showing that saponification had little effect on the network as already shown with the pulp in the acidic form by Renard *et al.* (1994). The SC values were higher than values observed in literature for other natural ion-exchangers such as biomass of marine algae (6.2 ml g⁻¹ in water: Holan *et al.*, 1993) or for synthetic ion-exchange resin (AG 50W-X8 Bio-Rad resin: 3 ml g⁻¹).

SC was drastically affected by the solvent conditions (Table 4): a minimal value (~ 13 ml g⁻¹) was found at pH ~ 3.5 in the presence of 0.1 M NaNO₃ while a value of 32 ml g⁻¹ was reached in water at pH 7. The SC values were in agreement with previous reports on beet pulp (Rouau *et al.*, 1987; Ralet *et al.*, 1991; Renard *et al.*, 1994). For a

Table 4. Specific surface area and hydration properties of the raw and the saponified pulps

	Raw ^a	Saponified
Surface area (m ² g ⁻¹)	3.05	2.70
SC at pH ~ 3.5 (ml g ⁻¹)		
0.1 M NaNO ₃	13.4 \pm 0.2	12.6 \pm 0.3
0.1 M NaNO ₃ ^b	11.1 \pm 0.1	8.4 \pm 0.1
Water	17.8 \pm 0.4	21.9 \pm 1.0
SC at pH ~ 7 (ml g ⁻¹)		
0.1 M NaNO ₃	15.3 \pm 0.2	19.3 \pm 0.4
Water	32.0 \pm 0.1	32.4 \pm 0.1
WRC at pH ~ 3.5 (g g ⁻¹)		
Water	16.0 \pm 3.0	18.3 \pm 0.1
0.1 M NaNO ₃	23.9 \pm 0.5	8.3 \pm 2.0

^aFrom Dronnet *et al.* (1997)

^bIn the presence of varying [Me²⁺],

given pH, SC was higher in water than with salts as already reported for beet pulp (Renard *et al.*, 1994) or other substrates such as algae (Fleury and Lahaye, 1991). The higher the pH the higher the SC. These variations were attributed to the electrostatic interactions between ionic sites and their screening by adding external salts (Renard and Thibault, 1991; Renard *et al.*, 1994), extreme values being obtained for a completely dissociated network (pH ~ 7 in pure water) and a network with partially undissociated and screened charges (pH ~ 3.5 in 0.1 M NaNO₃) (Renard *et al.*, 1994). These authors also showed a greater effect of the pH conditions on the SC of the raw pulp than on that of the saponified form.

SC at pH ~ 3.5 in the presence of 0.1 M NaNO₃ was also evaluated for both pulps in the presence of varying amounts of metal cations (Ca²⁺, Cd²⁺, Cu²⁺, Ni²⁺, Pb²⁺ and Zn²⁺). The value of SC was lowered in the presence of metal cations, but the nature and the concentration of the metal cation had no significant effect on the SC values. The effect of the addition of metal cations on SC was more marked for the saponified pulp, confirming that ionic cross-linking takes place and restricts the swelling.

The water-retention capacity (WRC) was measured only at pH ~ 3.5 in water or in the presence of 0.1 M NaNO₃. WRC values were in agreement with literature (Bertin *et al.*, 1988; Ralet *et al.*, 1991; Renard *et al.*, 1994). WRC was lower in water than in the presence of salts for both pulps for unexplained reasons, and was lower for the saponified than for the raw form, as already mentioned by Renard *et al.* (1994). On the contrary, WRC values of beet pulp decreased with increasing concentrations of NaCl or CaCl₂ (Renard *et al.*, 1994) and were lower in the presence of 1 M NaCl than in water for fiber extracted from beet pulp (Bertin *et al.*, 1988). The alkaline conditions necessary for saponification would cause a slight loss of the coherence of the cell walls which can be related to the decrease of the specific surface area (11% after saponification). Alkali-treated cell walls of *Chlorella vulgaris* also exhibited a smaller specific surface area than the starting form (Ting *et al.*, 1995).

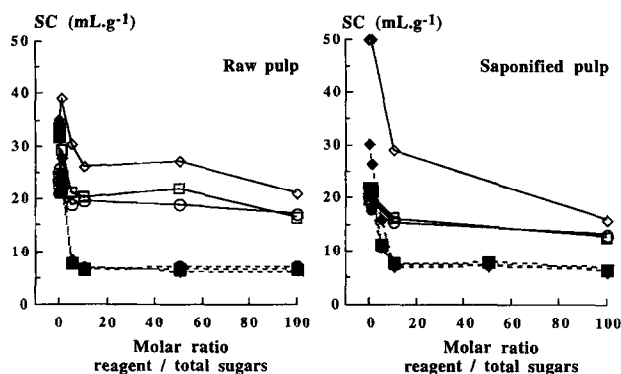


Fig. 1. Swelling capacity (SC) at different pH and ionic strength conditions of raw and saponified beet pulp treated with formaldehyde and epichlorohydrin. Open symbols represent pulp treated by formaldehyde: (○) pH~3.5, 0.1 M NaNO₃; (□) pH~3.5, water; (◇) pH~7, water. Filled symbols represent pulp treated by epichlorohydrin: (●) pH~3.5, 0.1 M NaNO₃; (■) pH~3.5, water; (◆) pH~7, water. Errors bars are not shown for better clarity

The saponification improved the CEC and the WRC but led to a detrimental increase of the SC under some conditions.

Pulp treated with formaldehyde and epichlorohydrin

Swelling (Fig. 1) and water-retention (Fig. 2) capacities of the raw and the saponified pulps treated either by formaldehyde or by epichlorohydrin were measured.

The formaldehyde treatment led to a limited decrease of the hydration properties (SC and WRC). With raw pulp as the starting material, the SC value decreased by only one-third to one-half (pH~3.5) for a molar ratio of 100. This decrease was even less marked for the WRC. The same SC values were obtained at pH~3.5 in water or in 0.1 M NaNO₃ and were always lower than at pH 7. WRC were lower in water than in 0.1 M NaNO₃, and the variation with increasing amount of formaldehyde was limited. With the formaldehyde-treated saponified pulp, hydration values higher than those of the starting material were obtained at

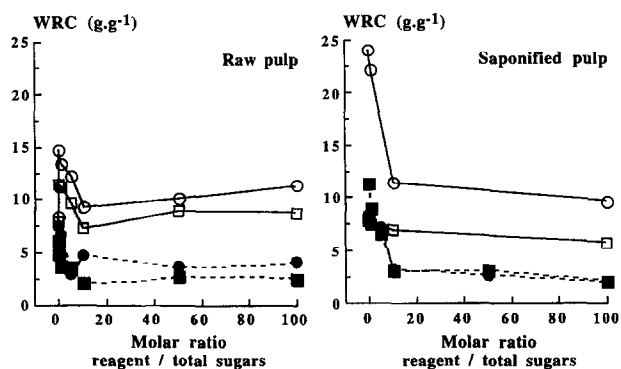


Fig. 2. Water-retention capacity (WRC) at pH~3.5 and different ionic strength conditions of raw and saponified beet pulp treated with formaldehyde and epichlorohydrin. Open symbols represent pulp treated by formaldehyde: (○) 0.1 M NaNO₃; (□) water. Filled symbols represent pulp treated by epichlorohydrin: (●) 0.1 M NaNO₃; (■) water. Errors bars are not shown for better clarity

Table 5. Solubilisation of galacturonic acid during WRC experiments (in % of the initial galacturonic acid content) of the raw and the saponified pulp and of some cross-linked pulps

	Raw		Saponified	
	0.1 M NaNO ₃	Water	0.1 M NaNO ₃	Water
Not modified	4.1 ^a	1.4 ^a	4.0	1.2
Formaldehyde				
0	4.1	2.2	4.2	1.7
0.1	3.5	2.1	—	—
1	3.0	1.1	3.8	1.6
10	3.1	1.7	4.1	1.5
100	3.4	1.1	3.8	1.0
Epichlorohydrin				
0	19.1	39.0	8.4	10.0
1	6.8	nd	8.3	8.7
10	3.9	tr	4.2	tr
100	5.2	tr	3.6	tr

^aFrom Dronnet et al. (1997); nd, not determined; tr, traces

the lower molar ratios, remarkably so in water. This suggests a destructuring effect of the acidic conditions. Increased formaldehyde levels again resulted in a limited decrease of the hydration capacities which reached 65%–50% of the values of the starting saponified pulp. Low performances of formaldehyde cross-linking were in contradiction with literature reports: a raw beet pulp treated with formaldehyde with a SC of 2.8 ml g⁻¹ (a decrease by a factor of 6.5 compared with the starting pulp) was obtained by Langenhorst *et al.* (1961). SC of a biomass of *Ascomyces nodosum* and *Fucus vesiculosus* was decreased 2.2 and 1.4 times by treatment with formaldehyde, respectively (Holan and Volesky, 1994). These new substrates exhibited SC lower than 2 ml g⁻¹ which was lower than the value obtained after treatment with divinylsulfone or glutaric dialdehyde (Holan *et al.*, 1993).

The effects of the epichlorohydrin treatment on the hydration properties were much more drastic. The SC and WRC values became low and independent of the pH and the ionic strength conditions for molar ratios ≥10, in agreement with results obtained for citrus peels by Hatanaka *et al.* (1990). The SC and WRC values decreased dramatically for molar ratios between 0.1 and 5, and became constant at a molar ratio of 10. This plateau corresponded to SC values of 6–7 and 6 ml g⁻¹ starting from the raw and saponified pulps, respectively. Such values agreed with those obtained for epichlorohydrin-treated citrus peels at a ratio epichlorohydrin/total sugars of 10 (Hatanaka *et al.*, 1990). Whatever the solvent conditions, the WRC values reached ~3–4 g g⁻¹ for the treated-raw pulp and 2 g g⁻¹ for the treated-saponified pulp. This corresponded to a decrease of ~10 times of the value of the raw pulp. Lignocellulosic apple residues also exhibited SC values ~7 ml g⁻¹ when treated with phosphorus(V) oxychloride (Maranon and Sastre, 1992).

Aqueous extraction of GalA after cross-linking

The loss of galacturonic acid by aqueous extraction during WRC experiments was measured on the raw pulp

and the saponified pulp, and on the cross-linked pulps (Table 5).

In the case of the raw and the saponified pulps, the loss of GalA was less than 4% of the initial amount of GalA in the presence of 0.1 M NaNO₃, with no significant difference between the raw and the saponified pulps, and less than 2% in water. After cross-linking treatments with high molar ratios, the loss of GalA remained less in water than in the presence of 0.1 M NaNO₃. On the contrary, Renard *et al.* (1994) found reduced GalA extraction from saponified pulp than from raw pulp, with higher extraction in 1 M NaCl than in water but lower in 1 M CaCl₂.

The loss of GalA was not drastically affected by treatment with formaldehyde in contrast to epichlorohydrin. Indeed, a marked loss of GalA was observed in the blanks, especially for the raw pulp, showing that strongly alkaline conditions caused loss of the cell-wall structure and may increase the amount of soluble material. The addition of epichlorohydrin drastically reduced this loss to the same level as in the starting material when the molar ratio reached 10 in the presence of 0.1 M NaNO₃. No extraction was detected in water beyond a molar ratio of 10, showing that the cross-linking treatment reduced the amount of GalA solubilised during WRC experiments.

Binding properties

The binding of Zn²⁺ and Pb²⁺ by the saponified pulp and by some cross-linked substrates was compared to the binding by the raw pulp (Table 6). Measurements were carried out with two different substrate concentrations either at a fixed charge number per volume (8 meq l⁻¹) or at a fixed weight per volume (14.6 g l⁻¹). The binding level was studied only for a ratio [Me²⁺]_i/[C_p] = 1 corresponding to the theoretical saturation of the anionic sites assuming a stoichiometry of two carboxyl functions for one bound divalent cation. To take into account the different CEC values, the binding level of the raw pulp treated by formaldehyde has to be compared to that of the raw pulp, whereas all the other substrates have to be compared to the binding level of the saponified pulp.

General rules for ionic binding already observed with native pulp (Dronnet *et al.*, 1997) were still followed after saponification or cross-linking treatment: the binding increased with the substrate concentration and Pb²⁺ was more strongly bound than Zn²⁺.

At 8 meq l⁻¹, the binding of Zn²⁺ by the raw pulp was slightly lower after treatment with formaldehyde while CECs were of the same order. At 14.6 g l⁻¹, the binding of Zn²⁺ was lowered after treatment of the saponified pulp with formaldehyde although the CEC of the treated substrates was higher. Although CECs of the modified pulp were higher, the binding of Pb²⁺ was much lower after treatment whatever the starting pulp and the substrate concentration. Treatment by formaldehyde caused a decrease of affinity. However, the binding level increased with the molar ratio applied to the starting substrate.

Table 6. Binding level of Zn²⁺ and Pb²⁺ for a ratio [Me²⁺]_i/[C_p] = 1 by raw, saponified and some cross-linked pulps at two different concentrations^a

	[Zn ²⁺] _i /[C _p]		[Pb ²⁺] _i /[C _p]	
	8 meq l ⁻¹	14.6 g l ⁻¹	8 meq l ⁻¹	14.6 g l ⁻¹
Raw pulp				
Not modified ^b	0.59	0.59	0.68	0.68
Formaldehyde				
1	0.43	0.50	0.49	0.48
100	0.44	0.59	0.57	0.59
Epichlorohydrin				
5	0.65	0.67	0.76	0.83
50	0.74	0.75	0.85	0.91
Saponified pulp				
Not modified	0.62	0.73	0.82	0.87
Formaldehyde				
1	0.64	0.63	0.62	0.47
100	0.64	0.64	0.71	0.70
Epichlorohydrin				
5	0.63	0.75	0.72	0.82
50	0.70	0.76	0.82	0.88

^a[C_p] (eq l⁻¹) = [pulp] (g l⁻¹) · CEC (eq g⁻¹)

^bFrom Dronnet *et al.* (1997)

The binding of Zn²⁺ increased when the pulp was treated with epichlorohydrin but no such increase was observed for Pb²⁺. As in the case of the treatment by formaldehyde, an increase in the binding level was observed with increasing amount of epichlorohydrin applied on the starting pulps. After treatment with epichlorohydrin, substrates had similar or even higher binding capacities than the saponified pulp. Thus, the pulp affinity towards metal cations was not affected by epichlorohydrin cross-linking, as previously observed by Kohn *et al.* (1976).

CONCLUSION

Saponification of beet pulp led to a two-fold increase in the cation-exchange capacity but the binding capacity of metal cations was only slightly improved. No decrease of SC whatever the solvent conditions was observed, though WRC was decreased. No modification of the sugar composition was involved by such a mild alkaline treatment. Formaldehyde treatment allowed the hydration capacities to be slightly decreased but led to a reduction in the metal cation binding capacity. The acidic conditions of this treatment involved a slight loss of sugars which was not prevented by the addition of formaldehyde.

On the contrary, epichlorohydrin treatment had drastic effects on sugar composition and on hydration properties. Cross-linking by epichlorohydrin took place in strong alkaline conditions, which led to a major loss of pectins. However, this loss was counteracted by efficient cross-linking, indicating that the new cross-links probably involved the galacturonosyl units of the pectic backbone. At the same time, SC of cross-linked substrates was decreased to a minimum value of 7 ml g⁻¹ and became independent of

the solvent conditions. The alkaline conditions allowed simultaneous saponification, and thus a increase of the CEC, which was another advantage compared to formaldehyde. No significant decrease in metal cation binding properties was observed after treatment by epichlorohydrin. This treatment did improve the binding properties of beet pulp per unit of hydrated volume, i.e. decreased its hydration properties and increased metal-binding capacities.

Cross-linking of beet pulp by epichlorohydrin was much more efficient than by formaldehyde. It produced new materials with low hydration properties, independent of pH and ionic strength, high CECs and maintained cation binding properties.

The next step consists in evaluating more finely the impact of this treatment on the selectivity and the characteristics of the binding, by studying the binding isotherms of metal cations to beet pulp after saponification and after cross-linking by epichlorohydrin.

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