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Oxidative gelation of sugar-beet pectins: use of laccases and hydration properties of the cross-linked pectins

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Abstract

Beet pectins are known to have feruloyl groups on their neutral side-chains. It is possible to cross-link the beet pectin through the oxidative coupling of these feruloyl groups leading to a new gelation process. We have investigated a new way of cross-linking by the use of a single enzyme, namely a laccase. Pectins have been extracted from beet pulp by various methods, including acid treatment and extrusion-cooking. Results showed that beet pectins can gel on the addition of laccases. More or less gellable pectins can be obtained but the gelation may be favoured using another enzyme, an arabinofuranosidase which make the feruloyl groups accessible to the coupling reaction. Chemical gels can therefore be obtained and the cross-linked pectins may be obtained as powders after drying. The degree of cross-linking, which can be modulated by the amount of pectin, of enzyme added and the reaction time, was found to be very critical for the hydration properties of the powders. Swelling capacities were typically in the range 25–50 ml/g of product, leading possibly to new applications for the beet pectins. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Pectins; Beet pulp; Laccases; Ferulic acid

1. Introduction

There has been a continuous interest in finding new applications for beet pulp, the residue left by the sugar industry (Broughton, Dalton, Jones & Williams, 1995). Beet pulp has for a long time been investigated as an alternative source of pectins, to apple pomace or citrus peels. The beet pectins however have poor gelling properties when compared with the citrus and apple pectins (Pippen, MacCready & Owen, 1950). Both their acetylesterification and their relatively low molar mass may prevent or hinder the close association of the chains, necessary for the gelation process either with calcium ions or in the presence of sucrose at acidic pH (Pippen et al., 1950; Rombouts & Thibault, 1986a).

Previous studies (Rombouts & Thibault, 1986a) on sugar-beet pectins have revealed the presence of feruloyl groups. It has been found that the feruloyl groups esterified some neutral sugars in the side-chains of the so-called “hairy” regions (Rombouts & Thibault, 1986b). The exact location of the ferulic acid was determined through NMR studies of purified feruloylated oligomers obtained after enzymic and acid degradations of the pectins. Of the ferulic acids, 50–60% are linked at the O-2 of arabinose residues, and 40–50% to the O-6 of galactose residues (Ralet, Thibault, Faulds & Williamson, 1994; Colquhoun, Ralet, Thibault, Faulds & Williamson, 1994; Micard, Renard & Thibault, 1997). It has also been shown that the feruloyl groups are bound directly on the backbone of arabinan side-chains rather than on the single units of arabinose substituting this backbone (Ralet et al., 1994; Colquhoun et al., 1994).

It is possible to take advantage of the presence of feruloyl groups in beet pectins. For example, enzymes can be used to release this phenolic acid and to achieve its biotransformation in natural vanillin (Micard, Renard & Thibault, 1994 and 1996; Lesage-Meesen et al., 1996). It is also possible to cross-link the (extracted) feruloylated pectins by carrying out coupling reactions. It has been reported that the mixture hydrogen peroxide–peroxidase and chemical oxidants such as ammonium persulphate (Rombouts, Thibault & Mercier, 1983; Thibault & Rombouts, 1986; Thibault, Guillon & Rombouts, 1991) are effective gel-forming agents. It has also been shown that the extraction process is important for the oxidative gelation of the beet pectins; non-gellable pectins can be obtained despite high contents in feruloyl groups. The accessibility of the feruloyl groups is

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apparently one critical parameter for the gelation, even though the quantification of accessible feruloyl groups is not simple (Thibault, 1988; Guillon & Thibault, 1987 and 1990).

In the present article, we show that the gelation can be enzymatically achieved very satisfactorily via the simple addition of only one polyphenol oxidase, namely a laccase. We used pectins extracted under conditions which can be easily scaled up, such as acidic extraction and aqueous extraction after extrusion-cooking of the pulp. The extracted bean pectins could be used to gel different systems by adding oxidants. As the gel is based on chemical bonds, it is also possible to recover the cross-linked pectins, after drying. Indeed, these cross-linked pectins have remarkable water-absorption capacities which can lead to new applications of the beet pectins (Thibault, 1986; Thibault et al., 1991). The hydration properties are investigated, mainly as a function of the degree of cross-linking which can be modulated by the variation of the amount of pectins, the amount of laccase and the reaction time.

2. Experimental

2.1. Beet pulp

The sugar-beet pulp (dry matter, 231 mg/g) from Danisco (Copenhagen, DK) was used without pretreatments.

2.2. Beet pectins

Nine different pectin samples, including one commercial sample, were used in this study.

- One pectin sample (CP pectin) (GENU pectin Type X-0905, batch 433791) was from Copenhagen Pectin Factory A/S (Skensved, DK).
- Five pectin samples (H1–H5) were obtained by acidic extraction of the pulp (ratio solid to liquid: 200 g/l); H1–H3 were extracted by hydrolysis at pH 1.5 (adjusted with 0.1 mol/l HCl), at 85°C during 1, 3 and 5 h, respectively; the exact conditions were described in Michel, Thibault, Mercier, Heitz and Pouillaude (1985). H4 and H5 were extracted (Kertesz, 1951), at pH 1.0 (adjusted with concentrated HCl), at 50°C during 16 and 24 h, respectively.

  The extracts were brought to pH ~ 4 with 2 mol/l NaOH, and concentrated by vacuum evaporation. They were either directly freeze-dried or dialysed before freeze-drying.
- Three pectin samples (E1–E3) were obtained by water extraction of the pulp after extrusion-cooking with a twin-screw extruder Clextral BC 45 (Ralet, Thibault & Della Valle, 1991). The barrel was 1 m long, the screw length/screw diameter ratio was 18. It included four heating and cooling zones. The screw configuration featured a reverse pitch element located 100 mm before the die; the die was made of two cylindrical tubes (diameter 4 mm, length 30 mm). The rate of added water (30% of solid feed rate) and the feed rate (36.5 kg/h) were kept constant. The pulp was treated at different barrel temperatures in the last section and with different screw speeds, leading to different specific mechanical energies measured as described before (Ralet et al., 1991). Three extruded pulps were obtained with different specific mechanical energy: 307 kWh/t (100°C, 150 rev/min), 252 kWh/t (150°C and 150 rev/min), and 296 kWh/t (130°C and 240 rev/min) from which E1–E3 were extracted, respectively.

For extraction, the extruded pulp (100 g) was stirred with distilled water (2 l) for 30 min at 20°C. The suspension was filtered on a cheese cloth, the liquid phase was centrifuged at 15 000 g at 10°C for 30 min and the supernatant was collected and concentrated by vacuum evaporation. The extracts were either directly freeze-dried or dialysed before freeze-drying.

2.3. Analysis

The amount of galacturonic acid was determined by the automated metahydroxybiphenyl method (Thibault, 1979). The pectins were deesterified (0.05 mol/l NaOH, 4°C, 30 min) prior to analysis. The total neutral sugars was estimated by an automated orcinol procedure using arabinose as a standard, after correction for uronic acid interference (Tollier & Robin, 1979).

The amount of feruloyl groups was measured by spectrophotometry from the absorbance at 375 nm of solutions of pectins in 0.1 mol/l glycine–sodium hydroxide buffer (pH 10), assuming a molar extinction coefficient of 34 300 (Micard et al., 1994).

All compositions are given on a moisture-free basis, as determined by drying the sample at 130°C for 2 h.

Viscosity measurements were performed at 25°C with an automatic capillary (diameter 0.46 mm) Ubbelholde viscometer (Amtec, France). The flow times of solutions of pectins in 0.1 mol/l NaCl at pH 7 were measured and the intrinsic viscosities were obtained by extrapolation to zero concentration by the Huggins and Kraemer equations (Axelos, Thibault, & Lefebvre, 1989).

2.4. Enzymes

Two laccases (*p*-diphenol-oxygen oxidoreductase, E.C. 1.10.3.2) from Novo Nordisk A/S (Bagsvaerd, DK) were used in this study (Budolfsen & Heldt-Hansen, 1996). The laccase P was from *Polyporus pinsitius* and the laccase M from *Myceliophtora thermophili*. They were used in solution with 200 and 182 laccase units (Lacu/ml). One Lacu is the amount of enzyme which under standard conditions ($pH = 5.5$ and 30°C) catalyses the conversion of 1 μM syringaldazine per minute.

Table 1
Yields and characteristics of the sugar-beet pectins after dialysis

Samples	Yield mg/g of pulp	[η] (ml/g)	Composition (mg/g of pectin)		
			GalA	Neutral sugars	Feruloyl residues
CP Pectin	—	275	737	92	5
Acid-extracted pectins					
H1	150	190	635	308	9
H2	140	275	719	129	6
H3	210	195	700	68	5
H4	170	285	664	316	9
H5	160	250	503	298	9
Water-extracted pectins after extrusion of the pulp					
E1	85	nd[a]	510	226	6
E2	135	nd[a]	500	248	7
E3	95	nd[a]	461	217	6

[a] nd: not determined.

SP 580 from Novo Nordisk A/S (Bagsvaerd, DK) was used as an *Aspergillus* sp. α-arabinofuranosidase (E.C. 3.2.1.55).

2.5. Oxidative gelation

The assays were performed on dialysed and undialysed laboratory extracted and commercial pectins. The reaction was carried out at room temperature, in unbuffered conditions (pH range 4.3–5.3). The solutions (volume in the range 5–50 ml) contained 10–50 g of pectins/l and enzymes.

Laccase P was used at a concentration of 1.25–2.5 Lacu/g of pectin whereas laccase M was used at a concentration of 5–10 Lacu/g of pectin.

The α-arabinofuranosidase was tested at pH 4.0 at a concentration of 0.01 mg of protein/g of pectin.

The gel state was estimated by visual inspection of the reaction mixture; periodically, the vessel containing the reaction mixture was tilted and the system was considered as gelled when it did not deform under its own weight.

2.6. Drying of the gels

The gels were disrupted with a glass rod on a sintered glass (porosity G_3) and washed with 4 volumes of acetone, or isopropanol, or 95% ethanol. They were left over night at ambient conditions in order to complete the removal of the solvent. The dried gels, referred to as cross-linked pectins in the text, were ground by hand in order to pass a 250 μm grid prior to further characterization.

2.7. Determination of the swelling capacity

Swelling was measured on 50 mg of cross-linked pectins, by the bed volume technique (Kuniak & Marchessault, 1972; Thibault, 1986) in glass cylinders after 24 h at 20°C, in an excess of distilled water or 0.1 mol/l NaCl. The results were expressed as ml of bed volume per gram of dry cross-linked pectins. Results were given at ±50%.

3. Results

3.1. Extraction and characterization of the pectins

For each type of extraction, the yield in pectins (mg of pectins after dialysis/g of pulp) was calculated and the compositions of the pectins were determined. The results are presented in Table 1.

The results showed that acidic extractions gave high pectin yields. The highest yield (210 mg/g) was obtained at 85°C and pH 1.5 after 5 h of extraction and the lowest (140 mg/g) at 85°C and pH 1.5 after 3 h of extraction. Extraction at pH 1 and 50°C gave high yields which decreased (from 170 to 160 mg/g) slightly when the time of extraction increased from 16 to 24 h. Apparently, the extracted pectins were different in nature and two types of pectins were obtained by varying pH and temperature of extraction. At high temperature (85°C) and pH 1.5, pectins were obtained with a rather low molar mass as indicated by the values of intrinsic viscosity (except H2), and neutral sugar and ferulic acid contents which decreased with increasing reaction time; after 5 h of extraction time, the amount of neutral sugars was very low while the amount of ferulic acid was still 5 mg/g. This fact indicated that the acidic treatment led to the removal of external fragments of the side-chains in which the amount of ferulic acid was low. In contrast, the content of galacturonic acid increased. The other type of pectins obtained at 50°C and at pH 1.0 was characterized by a higher molar mass and high contents of neutral sugars and ferulic acid (9 mg/g). When the reaction

Table 2
The test of gelation of the beet pectins upon addition of laccases and arabinofuranosidase

Samples[a]	Laccase M[b]		Laccase P[c]		Laccase P + arabinofuranosidase[d]	
	Gelation	Time	Gelation	Time	Gelation	Time
CP	Yes	< 5 min	Yes	< 5 min		
H1 dialysed	Yes	0.5–1 h	Yes	10–15 min		
H1 not dialysed	No		No			
H2 dialysed	Yes	5–20 min	Yes	< 5 min		
H2 not dialysed	No		No			
H3 dialysed	Yes	10–15 min	Yes	< 5 min	Yes	< 5 min
H3 not dialysed	No		No			
H4 dialysed	?, see text		?, see text	48 h	Yes	2–2.5 h
H4 not dialysed	No		No			
H5 dialysed	No		Yes	2–18 h	Yes	1.5–2 h
H5 not dialysed	No		No			
E1 dialysed	Yes	2 h				
E1 not dialysed	Yes	24 h				
E2 dialysed	Yes	2 h				
E2 not dialysed	Yes	24 h				
E3 dialysed	Yes	2 h				
E3 not dialysed	Yes	24 h				

[a] The solutions contained 20 g/l of dialysed pectins and 40 g/l of not dialysed pectins.
[b] Concentration: 10 Lacu/g of dialysed pectin and 5 Lacu /g of not dialysed pectin.
[c] Concentration: 2.5 Lacu/g of dialysed pectin and 1.25 Lacu/g of not dialysed pectin.
[d] Concentration: 2.5 Lacu/g of pectin and 0.01 mg of α-rabinofuranosidase/g of pectin added.

time was increased, the content of galacturonic acid and neutral sugars slightly decreased whereas the ferulic acid content remained unchanged.

By comparison with these pectins, the CP pectin (which probably is also acid-extracted) was characterized by a higher amount of galacturonic acid, a lower neutral sugar (except H3) and ferulic acid contents, and similar molar mass. The compositions of H1–H3 are close to those already published (Michel et al., 1985). CP and H2/H3 appeared to be very similar pectins.

The yields of pectin obtained after water extraction of the extruded beet pulp and their chemical composition are also presented in Table 1. The sample E2 gave the highest yield (135 mg/g); this sample was extruded with the lowest specific mechanical energy (∼ 250 kWh/t). The yields in pectins apparently decreased with higher specific mechanical energy. E2 gave a yield in pectins similar to that obtained by acidic extraction at 85°C and pH 1.5 during 1 or 3 h. However, these values are lower than those previously observed by Ralet et al. (1991) for similar specific mechanical energy, probably because of the differences in the geometry of the screws. The compositions of pectins E1–E3 were similar to the previously published data (Ralet et al., 1991). The ferulic acid content (6–7 mg/g) is slightly higher than that of the CP pectin (5 mg/g) and slightly lower than the acid-extracted pectins. They contained much more neutral sugars than CP, but less galacturonic acid, in agreement with previous results which showed that extrusion-cooking split more linkages than the acid in the “smooth” region of the pectins (Ralet et al., 1991; Ralet & Thibault, 1994).

3.2. Oxidative gelation of the pectins by the addition of laccases

All these pectins and the commercial pectin CP were tested for their gelling capacity in the presence of the laccases with or without a prior dialysis step. The results are shown in Table 2.

With laccases P and M, none of the undialysed samples gelled, except for E1, E2 and E3. For these latter samples, only weak gels were observed after 24 h of reaction. Increased concentrations of laccase were tentatively used to gel the acid-extracted undialysed pectins. A very weak gel was only observed with H3 after 48 h of reaction when a 10-fold concentration of laccase P was used.

Gelation was observed for the CP pectin and for all the pectins extracted by HCl, at 85°C and pH 1.5 after dialysis, whatever the nature of the laccase. It can be noticed that the time of gelation decreased significantly with increasing extraction time of the pectins. Longer times of gelation were also observed with laccase M than with laccase P although the number of units of laccase M added was higher. The CP pectin gelled very rapidly, whatever the nature of the laccase used.

Gels were more difficult to obtain with H4 and H5. Only some gelled material was reproducibly obtained from H5 with laccase P after 18 h reaction time; the reproducibility of the other results was rather low.

E1, E2 and E3 after dialysis also gelled with laccase M. The gelling time was much shorter (2 h) than for the non-dialysed pectins (24 h) and no visual difference could be observed between these samples.

Table 3
The swelling capacity (in distilled water and in 0.1 mol/l NaCl) of the acetone-dried cross-linked pectins (gelation with Laccase M, 10 Lacu/g of pectin, 16 h, room temperature)

Pectin concentration	Yield in cross-linked pectin (mg/g of initial pectin)	Swelling capacity (ml/g of cross-linked pectin)	
(g/l)		In water	In NaCl
10	745	—	115
20	910	120	55
50	925	35	20

In order to decrease the gelling time and to obtain gels irrespective of the samples, an α-arabinofuranosidase was added to the reaction mixture containing the laccase. Indeed, our previous studies have shown that non-gellable pectins can gel after the action of α-arabinofuranosidase (Guillon & Thibault, 1987 and 1990). The results (Table 2) showed that a positive effect of α-arabinofuranosidase can be observed with H4 and H5: gels were reproducibly obtained and their gelling times were very significantly reduced. The addition of α-arabinofuranosidase did not change the gelling behaviour of H3.

3.3. Effects of the pectin concentration on the yield in cross-linked pectins and their swelling capacities

Gels were obtained from solutions of CP at three concentrations (10, 20 and 50 g/l of distilled water). The laccase M was added for ∼ 16 h at a concentration of 10 Lacu/g of pectins.

The yields in cross-linked pectin are presented in Table 3. High yields (> 745 mg/g of initial pectin) were generally obtained. The lower yield was observed with gels made from 10 g/l solutions: a very weak gel was obtained and a part probably was lost during filtration.

The cross-linked pectins obtained after drying were tested for their ability to swell in water and in 0.1 mol/l NaCl solution. In the latter conditions, the charges of the galacturonic acid residues were screened and the electrostatic repulsions were reduced, leading to minimal values of the swelling (Thibault, 1986 and 1988).

Results obtained are presented in Table 3. High swelling capacities in water (120 ml/g) was obtained with an initial pectin concentration of 20 g/l. For the gels made from 10 g/l pectin solution, a non-clear separation between the gel and liquid phases was observed; it has probably a very high swelling capacity due to a very low degree of cross-linking. The high value (up to 115 ml/g) obtained for this gel in 0.1 mol/l NaCl confirmed this hypothesis. Gels made with 50 g/l always gave cross-linked pectins with lower swelling capacities in water as well as in NaCl.

It was observed that the swelling capacities of the cross-linked pectins significantly decreased with increasing the initial concentration in pectins. This fact can be related to the overlapping of the molecules, leading to a compact network at high pectin concentration. The more compact these networks are, the less they are affected by the ionic strength of the surrounding solution.

3.4. Effect of the time of action and the concentration of the laccase on the swelling capacity

These results showed that the degree of cross-linking may have a marked influence on the swelling capacity. Both the concentration and the time of the action of the laccases could be of importance in this respect; they were therefore studied. Gels were made from solutions containing 20 g/l of CP pectin, as the previous results showed that this concentration gave a high yield in cross-linked pectins with a high swelling capacity. Different times of action (15, 30 and 45 min, and 1, 2, 4, 6 and 16 h) and different concentrations (0.1, 1, 2, 5 and 10 Lacu/g of pectins) of the laccase M were tested. The gels were dried in acetone. The results are in Table 4.

With a laccase concentrations of 0.1 Lacu/g of pectin, only solutions with increased viscosity can be obtained. The pectins could not set up a three dimensional network. However, some cross-linking reactions occurred as shown by the fact that after drying, their water solubility was significantly decreased. They swelled but were not soluble anymore.

With higher concentration of laccase, gels appeared after a lag time of 15–30 min. The values of the swelling capacities in 0.1 mol/l NaCl were in the range 30–100 ml/g; these values were a complex function of the reaction time and concentration in laccase. For a given concentration of laccase, the swelling capacity of the cross-linked pectins can double during the course of action of the laccase. The values decreased after a maximum was reached and apparently all the values tended to a similar swelling capacity around 50–60 ml/g. Similar variations of the swelling capacities in water of the cross-linked pectins was observed; the values plateaued to 120 ml/g.

Probably, at a low degree of cross-linking, i.e. (short reaction time or low concentration of enzyme), the swelling capacity can be increased up to a critical degree beyond which the network becomes compact leading to reduced swelling capacities. For long time of action of the enzyme, it is likely that the enzyme could not diffuse anymore into the network or that all the available feruloyl groups were cross-linked.

Table 4
The swelling capacity (in distilled water and in 0.1 mol/l NaCl) of the dried cross-linked pectins made from CP pectin (20 g/l) as a function of the time of action and the concentration of the laccase M

Laccase concentration (Lacu/g of pectin)	Time of action of laccase	Gelation	Yield in cross-linked pectin (mg/g of initial pectin)	Swelling capacity (ml/g of cross-linked pectin)	
				In water	In NaCl
0.1					
	15 min	No	665	nd[a]	25
	30 min	No	760	nd[a]	35
	45 min	No	845	nd[a]	35
	1 h	No	830	nd[a]	30
	2 h	No	890	nd[a]	55
	3 h	No	930	nd[a]	55
	4 h	No	940	nd[a]	75
1					
	15 min	No	605	nd[a]	45
	30 min	No	910	nd[a]	50
	45 min	Yes	930	nd[a]	80
	1 h	Yes	930	nd[a]	80
	2 h	Yes	920	nd[a]	60
	3 h	Yes	925	nd[a]	50
	4 h	Yes	930	nd[a]	65
2					
	15 min	No	1000	nd[a]	30
	30 min	Yes	940	nd[a]	35
	45 min	Yes	1000	nd[a]	35
	1 h	Yes	930	nd[a]	50
	2 h	Yes	960	nd[a]	55
	3 h	Yes	900	nd[a]	55
	4 h	Yes	915	nd[a]	50
5					
	15 min	No	960	nd[a]	90
	30 min	Yes	945	nd[a]	35
	45 min	Yes	975	nd[a]	50
	1 h	Yes	960	nd[a]	70
	2 h	Yes	1000	nd[a]	65
	3 h	Yes	1000	nd[a]	60
	4 h	Yes	955	nd[a]	50
10					
	15 min	No	965	180	100
	30 min	Yes	975	155	85
10	45 min	Yes	935	85	50
	1 h	Yes	985	120	65
	2 h	Yes	960	155	85
	4 h	Yes	880	120	60
	6 h	Yes	950	120	65
	16 h	Yes	930	120	55

[a] nd: not determined.

3.5. Swelling capacities of the different pectin samples

We have measured the swelling capacities of the cross-linked pectins obtained from all our pectin samples. Solutions were made with the different samples (concentration 20 g/l) and laccase M (10 Lacu/g of pectin) was added for different periods of time (15 min to 24 h); the gels were dried by acetone and the swelling capacities of the resulting cross-linked pectins were measured (Table 5).

Gels were observed for all samples; however, as previously observed, only weak gels were obtained with H4 and H5, and with the non-dialysed samples obtained after aqueous extraction of extruded pulps.

The lowest yields (425–630 mg/g) of cross-linked pectins were obtained with H1 (after 15 min of reaction time of the laccase) and with the non-dialysed E1–E3 samples; they were explained by the weak gels obtained from these samples. Values up to 910 mg/g can be obtained with the other samples.

For the gels made from acid-extracted pectins the swelling capacities of the cross-linked pectins was lowered by increasing the reaction time, as previously observed with CP

Table 5
The swelling capacity (in distilled water and in 0.1 mol/l NaCl) of the cross-linked pectins, obtained from beet pectins (concentration 20 g/l) and laccase M (10 Lacu/g of pectin)

	Reaction time of laccase	Gelation	Yield of cross-linked pectins (mg/g of initial pectins)	Swelling capacity (ml/g of cross/linked pectin)	
				In water	In NaCl
H1 dialysed	15 min	Yes	425	50	25
	4 h	Yes	855	20	20
H2 dialysed	15 min	Yes	910	60	35
	4 h	Yes	905	50	30
H3 dialysed	15 min	Yes	810	105	40
	4 h	Yes	835	40	25
H4 dialysed	15 min	Weak gel	810	*	*
	4 h	Weak gel	835	*s	*
H5 dialysed	15 min	Weak gel	705	60	25
	4 h	Weak gel	755	45	25
E1 dialysed	24 h	Yes	790	25	20
E1 not dialysed	24 h	Yes	625	55	35
E2 dialysed	24 h	Yes	815	20	20
E2 not dialysed	24 h	Yes	635	45	40
E3 dialysed	24 h	Yes	900	35	30
E3 not dialysed	24 h	Yes	635	55	45

[*] not reproducible values.

pectin, indicating an increased degree of cross-linking. Swelling capacity in 0.1 mol/l NaCl was 1 to 2 times lower than in water as previously observed with the commercial pectin. For some samples, the differences of the swelling capacities measured in water and in NaCl were more important. The highest swelling (105 ml/g in water, 40 ml/g in 0.1 mol/l NaCl) was obtained with the pectin (H3) extracted 5 h by HCl at pH 1.5 and cross-linked by the laccase for 15 min. These values were lower than those obtained with CP pectin (180 ml/g in water, 100 ml/g in 0.1 mol/l NaCl). All the other pectin samples extracted by acid gave cross-linked products with swelling capacities in water lower than that obtained from CP pectin in the presence of salt.

As mentioned before, H4 and H5 gave weak gels; the cross-linked pectins from H4 gave irreproducible and very low (range 2–25 ml/g) values of the swelling capacity. More reliable data were obtained with H5 but the values of the swelling capacities remained low (range 25–60 ml/g).

The values of the swelling capacities obtained in water and in 0.1 mol/l NaCl with cross-linked pectins from E1–E3 are also presented in Table 5. All the dialysed samples gave values slightly lower than the undialysed samples, whatever the ionic strength of the surrounding solution. The undialysed material however gave gels of lower strength. The sample E3, corresponding to a high specific mechanical energy of extrusion gave cross-linked pectins with the highest swelling capacities in water and in NaCl. However, the conditions of extrusion have apparently a low impact on the hydration properties of the cross-linked pectins.

The values of the swelling capacities obtained from undialysed pectins were in the same range as those obtained with acid extracted pectins, in water or in NaCl. In NaCl, they gave values close to those obtained with CP. In water however, the CP pectins gave cross-linked pectins with a higher degree of swelling.

4. Discussion

The results showed that feruloylated pectins from beet pulp can be gelled by adding a single enzyme, namely a laccase, instead of chemicals (ammonium persulphate for instance) or a combination of an oxidising agent (hydrogen peroxide, for example) and an enzyme for which the oxidizing agent is a substrate (peroxides, for example). Treatment with laccase therefore avoids these undesirable substances.

In fact, the presence of oxygen is needed for the laccase but molecular oxygen from the atmosphere is apparently sufficient. The two laccases used in this study were from different microorganisms; the results showed that their actions were not identical and the laccase from *Polyporus pinsitius* was, in our experimental conditions, more efficient. The reasons for this specificity, as well as the structure of the resulting dimeric phenolic cross-links, are not yet determined and methods already used for determining dimeric forms of ferulic acids in pectins within the beet pulp (Micard, Grabber, Ralph, Renard & Thibault, 1997) have to be applied.

The conditions used for the extraction of pectins generally gave gellable pectins; some samples however gave only weak and non-reproducible gels, even though it is extremely difficult to predict this behaviour from the chemical compositions, as previously stated (Thibault, 1988). It is also the first report of the oxidative gelation of pectins obtained by water extraction of extruded pulp. It must be noted that these pectins have a relatively low molecular weight (Ralet et al., 1991). In order to decrease the gelation time and/or to obtain more reproducible and reliable results whatever the pectin sample, we have shown that it is possible to add α-arabinofuranosidase to the laccase. Indeed, this enzyme removes some peripheral arabinose residues and make the feruloyl groups linked to the inner part of the side-chains more accessible (Guillon & Thibault, 1987 and 1990).

Some purification is needed for the pectins, except those extracted after extrusion-cooking and the pectins extracted by acid gelled only if they were previously purified by dialysis. These facts indicated that the presence of salts, rather than the presence of small sugars could hinder or inhibit the cross-linking reaction. The negative role of some salts in the cross-linking reactions has already been reported (Thibault, Garreau, & Durand, 1987).

The concentration of laccase, the time of the reaction as well as the concentration in pectin are the main parameters which controlled the degree of cross-linking. In order to obtain reproducible and constant results of swelling capacity, it is very important to let the laccase act for a long time and to use pectin solutions at concentrations which permit the overlapping of the chains. In these conditions, the network is rather compact and less sensitive to the variations of the ionic strength. The drying process was also shown to be a very important step for producing cross-linked pectins. Freeze-drying, drying under vacuum or solvent exchange could be used, and drying at high temperature must be avoided. Swelling capacities around 30 ml/g can be easily obtained at high salt concentration for the cross-linked pectins. These values can lead to new applications of the beet pectins.

Future work will involve other methods to measure hydration properties of the cross-linked pectins and the use of these new polymers in some applications, in foodstuffs (stabilising or thickening agents,…) or in non-food areas (super absorbants, drug encapsulation, biocontainer for slow release of active molecules…).

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