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Effects of conventional boiling on the polyphenols and cell walls of pears

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Abstract: Pears of the cultivar Gieser Wildeman were cooked for up to 24 h and changes in polyphenol and cell wall components were monitored. The main polyphenols were flavan-3-ols (epicatechin and its procyanidin oligomers), with an average degree of polymerisation of 6, and caffeoylquinic acid. Upon cooking, flavan-3-ols were retained in the pear tissue while the hydroxycinnamic acids were partially leached into the cooking water. After 1 h of cooking, 65% of the original flavan-3-ols and 40% of the original caffeoylquinic acid were still detectable in the pear tissue; the cooking water contained only 2% of the flavan-3-ols but 24% of the caffeoylquinic acid. Cell walls represented 23 g kg^{-1} of the fresh pear and were composed of cellulose, pectins and xylans. The pectic fractions was degraded during cooking while xylans and cellulose were not affected.

Keywords: pear; *Pyrus communis* L; flavanol; hydroxycinnamic acid; pectin; cell wall; phenolics; flavonoids; caffeoylquinic acid; procyanidin; processing; domestic

INTRODUCTION

Fruits are often advertised as healthy foods, their health promoting effects being most often linked to three types of constituents:[1]

- ions: the presence of K^+ facilitates urinary excretion of sodium and pH homeostasis
- vitamins and micronutrients, with anti-oxidative properties, in particular ascorbic acid, polyphenols
- dietary fibres, which may have intestinal or systemic effects.

Of these three types of constituents, ions are mostly impervious to further processing of the fruit or vegetable, except for losses by leaching, while vitamins and micronutrients as well as dietary fibres are susceptible to heat degradation. Pears do not contain high amounts of vitamins[2] and their nutritional interest is centred on K^+, polyphenols and dietary fibre.

There has been a number of investigations on the changes of dietary fibres and cell walls in plant tissue during cooking, mainly concerned with potatoes,[3–6] carrots[7–10] and green beans,[11,12] and the influence of preheating on texture change and pectinmethylesterase activation.[6,13,14] Thermal treatment affects pectins in the first instance, owing to their susceptibility to β-elimination[15] and the almost ubiquitous presence of pectinmethylesterases in plants.[16]

Even less information is available on the changes of the phenolics of plant tissues, in spite of the interest in their anti-oxidant properties. Estimates of polyphenol intake generally consider only the compositions of fresh fruits and vegetables,[17–19] and thus do not take into account losses during cooking, though they appear significant and most plant foods are processed. Most interest has been focused on flavonols, for which a major cause of losses is peeling,[20] as these compounds are usually concentrated in peels. Boiling has been shown to induce flavonol losses of 20% and 40% in onions and asparagus, respectively.[21] Price *et al*[22] reported that canning did not result in chemical breakdown of flavonol glycosides but that leaching into cooking water was significant and depended on the conjugate. Cooking by boiling or frying of onions[23] led to a 25% loss of flavonol glycosides. Ewald *et al*[20] reported no significant variations between the different modes of cooking for onions, beans or peas, in contrast to Vallejo *et al*[24] for broccoli. Microwaving led to disappearance of 97% of flavonoids, 74% of sinapic acid derivatives and 87% of caffeic acid derivatives from broccoli inflorescence, while steaming had minimal effects.[24] During conventional boiling and high pressure boiling,[24] decrease of the content of phenolic compounds in the florets was due to both leaching into the cooking water and thermal degradation. Andlauer *et al*[25] showed that the proportion of cooking water influenced the final

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content of phenolics in zucchini, beans and carrot pieces, using as marker compounds the flavonol glycosides rutin and quercitrin, the hydroxycinnamic acid caffeic acid and its quinic ester, chlorogenic acid, again due to leaching.

Although proanthocyanidins form a significant proportion of the polyphenol intake, no study of their fate during cooking has been carried out. They are known to be susceptible to degradation, particularly in acidic conditions,[26] forming coloured compounds. As we have shown that they are able to bind non-covalently with cell walls,[27] we were interested in following their fate during the tissue disruption caused by cooking. We chose pears because their phenolic composition is simple and they are particularly rich in procyanidins.[28,29] Pears of a traditional 'cooking' variety were used as they can be supposed to be relatively resistant to tissue disintegration and therefore allow longer durations of treatment.

EXPERIMENTAL

Plant material

Commercially ripe pears of the cultivar Gieser Wildeman, traditionally used in The Netherlands as 'cooking' pears (stoofperen), were purchased in a local market. The pears were peeled, cored and cut longitudinally into pieces of less than 1 cm width. For cooking they were immersed in an equal weight of hot distilled water in hermetically closed Erlenmeyer flasks in a boiling water bath. After a given cooking time, the pieces were collected on a sieve and weighed. An aliquot was freeze-dried and another aliquot was ground in 960 g kg^{-1} ethanol (4 ml g^{-1}). The cooking water was collected and weighed; an aliquot was freeze-dried and another aliquot poured into cold 960 g kg^{-1} ethanol (4 ml g^{-1}).

Cell wall preparations

Fresh pears

Cell walls were isolated as alcohol-insoluble solids (AIS) as described in Renard *et al*.[30] Pear sections were boiled for 30 min in ethanol (3 ml g^{-1}) (temperature: 82–85 °C). They were then cooled to room temperature and filtered off on a sieve. The resulting thick paste was ground by six successive bursts of 15 s in a Braun kitchen processor and suspended in cold 700 ml l^{-1} ethanol. After standing for 20 min with intermittent gentle stirring, the solids were recovered on a G3 glass filter. This process was repeated until the filtrates were sugar-free (presence of sugars tested by the phenol–sulfuric method[31]). They were then dried by solvent exchange with 960 g kg^{-1} ethanol (three times) and acetone (three times), then overnight in an oven at 40 °C.

Cooked pears

The hot ethanol treatment was deemed not necessary as endogenous enzymes would have been inactivated during cooking in water. The recovered sections were directly ground in cold ethanol and further treated as above.

Sequential pectin extraction

Each cell wall preparation (1 g) was extracted sequentially by 0.05 M ammonium oxalate, pH 5 (100 ml, 2 h at 40 °C, repeated three times) followed by NaOH (0.05 M) with $NaBH_4$ 1 g l^{-1} (100 ml 1 h at 40 °C, three times). After each period of treatment, the extract and the cell wall were separated by filtration under vacuum on a G3 sintered glass filter. The three extracts from each treatment were pooled and dialysed against purified water (three times) prior to freeze-drying. The final residue was washed with distilled water and dried by solvent exchange as described for the AIS.

Analytical

The individual neutral sugars were analysed by gas chromatography (capillary column of 30 m × 0.25 mm id coated with DB225, 0.15 μm film thickness, J & W Scientific, Folsom, CA, USA) at 215 °C, using hydrogen as carrier gas, after sulfuric acid hydrolysis (1 M, 3 h, 100 °C) and derivatisation to alditol acetates.[32] The freeze-dried extracts (2–5 mg) were hydrolysed in 1 ml of sulfuric acid (1 M). Cell wall preparations (*ca* 10 mg) were submitted to prehydrolysis in 0.25 ml of 13 M sulfuric acid (1 h, room temperature) and diluted to 2.95 ml for hydrolysis.[33] Myo-inositol was used as internal standard. To determine the degree of methylation, samples were saponified with 6 ml of 0.2 M KOH, 2 h, at room temperature. Methanol was determined in the supernatants according to Klavons and Bennet.[34] Galacturonic acid was determined by the automated *m*-hydroxydiphenyl[35] assay on an Alliance instruments (Méry/Oise, France) autoanalyzer after acid hydrolysis (cell walls) or saponification (extracts). The degree of methylation (DM) was calculated as molar ratio of methanol to galacturonic acid. Nitrogen was analysed by the Kjeldahl method.[30] Proteins were calculated as N × 6.25. Polyphenols were measured by HPLC-DAD on a Purospher RP-18 endcapped (60 Å, 5 μm, 250 × 4 mm) column (Merck, Darmstadt, Germany) after dissolution in methanol (using *ca* 100 mg of the freeze-dried solids in the cooking water or 25 mg of pear/pear section and AIS dissolved/suspended in 1.2 ml of either methanol or the thioacidolysis reaction medium).[36] Standard errors of the means were calculated for series of replicated measurements using the sum of individual variances weighted by the individual degrees of freedom.

The molecular weight distribution of polysaccharides was determined using a HPLC system involving a laboratory data control (LDC Riviera Beach, FL, USA) programmable pump equipped with four Bio-Gel TSK columns (300 × 7.8 mm each) in series (50, 40, 30 and 25 PWXL; Tosohaas, Stuttgart, Germany), in combination with a TSK XL guard column (40 × 6 mm) at 35 °C. Solutions

(200 µl) of the extracts (2 g l^{-1}) were injected. They were eluted with 0.4 M sodium acetate buffer pH 3.6 at 0.8 ml min^{-1}. Anion-exchange column Pro-Gel TSK DEAE 5PW (75 × 7.5 mm, Sigma, St Louis, MI, USA) connected to an HPLC system (Waters 625 LC, Milford, MA, USA) was used for high-performance anion-exchange chromatography (HPAEC). The column was eluted with 0.05 M ammonium acetate buffer pH 6, followed by a linear gradient of 0.05–0.5 M ammonium acetate buffer pH 6 at 0.6 ml min^{-1}. Solutions (300 µl) of the extracts (2 g l^{-1}) were injected. Eluates from high-performance solvent exchange chromatography (HPSEC) and HPAEC were continuously monitored using the automated *m*-hydroxydiphenyl[35] and orcinol[37] assays on an Alliance instruments (Méry/Oise, France) autoanalyser.

RESULTS

Characterisation of the Gieser Wildeman pears

The Gieser Wildeman pears contained about 0.65 g kg^{-1} (fresh weight) of polyphenols (Table 1), mainly flavan-3-ols, with an average degree of polymerisation (DP) of 6 and containing mainly (−)-epicatechin (97 mol%). Hydroxycinnamic acids accounted for about 1/6th of the polyphenols and were represented mainly by caffeoylquinic acid (CQA; aka chlorogenic acid), the remainder being *p*-coumaroylquinic acid (PCQ). Caffeoylquinic acid has been previously reported as the main hydroxycinnamic acid in pears, and its content in Gieser Wildeman pears was comparable with literature values for table pears[2,38,39] and San Bartolomeu pears.[28] The presence of *p*-coumaroylquinic acid as a minor hydroxycinnamic acid in some pear cultivars has been reported by Amiot *et al*[39] and by Spanos and Wrolstad.[40] Arbutin, a phenolic compound characteristic of pears,[41] was present at about 0.13 g kg^{-1} dry weight, ie 16 mg kg^{-1} of fresh pear, lower than that reported for San Bartolomeu pear.[28]

Few data are available in the literature about the polymerised procyanidins of pear. Epicatechin is by far the main catechin in pear flavan-3-ols, whether as free monomer,[39] terminal unit or extension unit.[28,29] In comparison with San Bartolomeu pear (3.6 g kg^{-1} (fresh weight)[28]), the Gieser Wildeman pear was much poorer in flavan-3-ols and their average degree of polymerisation was lower (6 versus 14 for the flavan-3-ols of San Bartolomeu pear, 24 for procyanidins). Higher contents of flavan-3-ols and degrees of polymerisation have been found for perry pears.[29]

Isolation of cell wall material as alcohol-insoluble solids (AIS) was chosen as pears are almost devoid of starch. The yield of AIS from Gieser Wildeman pears (Table 2) was lower than the values reported by Jermyn and Isherwood[42] or Martin-Cabrejas *et al*,[43] both of which obtained AIS yields above 30 g kg^{-1} fresh weight. Sirisomboon *et al*[44] showed AIS values around 20–30 g kg^{-1} fresh weight for japanese pear (*Pyrus serotina*) close to harvest. Ferreira[45] obtained extremely low yields of cell wall material from fresh San Bartolomeu pears, at 10.7 g kg^{-1} fresh weight. The main sugars in our AIS were glucose, uronic acids and xylose. Arabinose and galactose were present in lower amounts while rhamnose, fucose and mannose were minor sugars. This composition is close to that reported by Martin-Cabrejas *et al*[43] for Blanquilla pear, the only difference being a higher galactose content here, while Ahmed and Labavitch[46] detected more arabinose than xylose in cell walls from Barlett pears, with major decreases in arabinose and uronic acid contents during maturation. High xylose and glucose contents in the AIS reflected the presence of stone cells which present thickened, lignified cell walls rich in xylans and cellulose.[43,47]

A simplified sequential extraction of pectic substances was carried out, as described above, on the AIS from fresh pears, including a chelating agent step (oxalate) and a dilute alkali step (0.05 M NaOH). Low yields were obtained with oxalate, comparable with the data reported by Ferreira[45] for CDTA extracts of

Table 1. Changes of the polyphenols of Gieser Wildeman pear, in pear sections and cooking water at various cooking times[a]

		Flavan-3-ols			Hydroxycinnamic acids			
Cooking time	Sample taken	(mg g^{-1})	(% ini)	DP	CQA (mg g^{-1})	CQA (% ini)	PCQ (mg g^{-1})	Derivatives (mg g^{-1})
0 h	Fresh pear	4.32		6	0.60		0.03	nd
20 min	Pear sections	5.18	80	13	0.34	38	0.03	nd
	Cooking water	0.26	2	2	0.35	18	0.05	nd
1 h	Pear sections	4.20	65	10	0.39	40	0.05	nd
	Cooking water	0.28	2	2	0.50	24	0.07	0.02
7 h	Pear sections	0.27	2	3	0.22	18	0.02	0.10
	Cooking water	0.11	1	1	0.21	17	0.03	0.08
24 h	Pear sections	0.11	1	1	0.25	16	nd	0.20
	Cooking water	0.09	1	1	0.12	10	nd	0.15
SEM		0.05		0.4	0.01		0.00	0.01

[a] Concentrations are given as mg g^{-1} of dry matter.

DP: degree of polymerisation; CQA: caffeoylquinic acid (chlorogenic acid); PCQ: *p*-coumaroylquinic acid; % ini: percentage of amount initially present; SEM: standard error of the mean (on the whole series); nd: not detected.

Table 2. Yields and composition (mg g^{-1} fresh weight) of cell walls and their fractions (including the ethanol precipitate from cooking waters) of the fresh and cooked Gieser Wildeman pears

		Yields relative to		Composition									
Cooking time	Fraction	AIS	Initial pear	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	GalA	Proteins (N × 6.25)	Procyanidins (DP)
Fresh pears	AIS		23.0	8	4	57	111	11	24	208	198	35	4.6 (29)
	Ox	59	1.4	2	0	11	2	1	7	3	685		
	NaOH	102	2.4	3	0	24	4	1	6	0	623		
	Residue	648	14.9	9	10	79	189	20	32	327	85		
20 min	Cooking water	12	0.3	10	4	49	18	15	64	44	271		
	AIS		25.3	8	4	56	111	12	21	215	200	42	7.2 (30)
	Ox	43	1.1	2	0	11	2	2	6	3	892		
	NaOH	135	3.4	3	0	27	4	0	6	0	607		
	Residue	610	15.4	9	8	73	178	22	32	355	83		
1 h	Cooking water	53	1.5	6	1	48	8	6	27	33	466		
	AIS		27.3	7	5	56	118	13	23	221	169	47	6.9 (30)
	Ox	66	1.8	3	0	13	1	1	5	3	620		
	NaOH	138	3.8	4	0	30	5	0	9	0	524		
	Residue	639	17.4	6	3	34	240	13	19	306	60		
7 h	Cooking water	186	3.7	11	1	125	22	0	31	7	518		
	AIS		19.9	6	6	35	145	14	23	262	98	63	1.2 (14)
	Ox	92	1.8	5	1	25	5	1	9	2	479		
	NaOH	26	0.5	9	0	57	11	1	15	1	398		
	Residue	739	14.7	6	8	28	197	22	28	375	38		
24 h	Cooking water	361	4.0	14	2	71	37	0	36	7	523		
	AIS		11.0	4	6	17	164	15	22	329	56	57	tr
	Ox	53	0.6	5	0	21	10	1	11	2	548		
	NaOH	15	0.2	5	0	90	7	1	10	1	221		
	Residue	806	8.9	6	9	9	192	21	25	389	31		
SEM				2	2	2	8	1	1	8	42		

AIS: alcohol-insoluble solids; Ox: 0.05 M ammonium oxalate extraction; DP: degree of polymerisation; SEM: standard error of the mean (on the AIS series).

Table 3. Global evolution during cooking: ratios of recovered to initial weight of the pear sections, recovered to initial liquid volume and repartition of the dry matter in the two compartments

		Pear sections				Cooking water			
Cooking time	Initial pear/water ratio (weight)	R/I weight	R/I dry matter	Dry matter (g kg^{-1})	AIS (g kg^{-1})	R/I volume	R/I dry matter	Dry matter (g l^{-1})	AIS (g l^{-1})
No cooking				128	23.0				
20 min	0.96	1.00	0.66	85	25.3	0.88	0.31	43	0.3
1 h	1.00	0.98	0.62	81	27.9	0.90	0.29	41	1.6
7 h	1.01	0.78	0.49	81	25.5	1.12	0.49	56	3.6
24 h	0.93	0.64	0.39	78	17.6	1.27	0.52	49	2.9

R/I: recovered/initial; AIS: alcohol-insoluble solids.

San Bartolomeu pear. The value obtained is 0.96 g anhydrouronic acid (AUA) kg^{-1} fresh weight, in the range of values given by Bartley *et al*[48] for soluble pectins of Conference pears, higher than reported by Murayama *et al*[49] for EDTA extracts from Marguerite Marillat and La France pears (after a water extraction) but lower than that reported by Kondo and Takano[50] for La France pears after extraction by hexametaphosphate at 90 °C. Higher yields were obtained with NaOH extraction, again in agreement with the data of Ferreira.[45] The extracts were rich in uronic acids and had very low contents of neutral sugars, of which arabinose had the highest content. Higher neutral sugar contents have been reported by Ferreira,[45] Martin-Cabrejas *et al*[43] and by Dick and Labavitch,[51] but always with arabinose as the main neutral sugar.

Changes of the pear sections during cooking

When the pears were immersed in hot boiling water, there was no marked disintegration of the sections until very long cooking times: after 7 h of cooking, the sections had shrunk but were still intact. Use of a sieve gave satisfactory recoveries (>900 g kg^{-1}) throughout. There was a fast diffusion of the smaller solutes to the liquid phase (Table 3), resulting in a decrease of the dry matter content of the pears and the appearance of soluble solids in the water. However equal values of soluble solids (ie dry matter minus

AIS) in the two phases were not reached. The content of AIS (relative to the fresh weight), which can be considered as representative of the cell wall fraction of the fruit, increased for the shorter cooking times. A marked decrease was only noted after 24 h of cooking, at which point the recovery was about half of that of the fresh pear. However by that time the pear sections had begun disintegrating and total recovery was not possible. The AIS represented 180 mg g^{-1} of the dry matter of the fresh pear. The proportion of the dry matter of the pear sections accounted for by the AIS increased to about a third for a cooking time of 7 h, and decreased to about 230 mg g^{-1} of the dry matter after 24 h of cooking. The proportions of proteins and procyanidins increased in the AIS during cooking (Table 1). After 24 h, the AIS was very poor in galacturonic acid and arabinose (Table 2), with contents reduced by 65–75%, while the rhamnose content was only halved. This AIS was also enriched in glucose, xylose and mannose. The amount of ethanol-precipitable material in the cooking liquid increased slowly: it represented only 5% of the AIS after 1 h of cooking, and up to 36% after 24 h. In terms of the weight of pears used, this represented less than 0.3 g kg^{-1} of the fresh weight after 20 min of cooking increasing to 4 g kg^{-1} after 24 h. Between 7 h and 24 h there was very little increase relative to the pear fresh weight.

Changes of the polyphenols

During the initial stages of cooking, the hydroxycinnamic acid concentrations in the pear sections decreased sharply while these molecules appeared in the liquid phase (Table 1). The total amounts detectable in the fruit and cooking water decreased to 56% of the initial values in 20 min but rose to 64% after 1 h. This initial decrease might be linked to oxidation by the endogenous enzymes of the fruit.[39] The proportion of caffeoylquinic acid that was leached represented about one-third of the caffeoylquinic acid present at 20 min and after 1 h, ie there was no equalisation of the concentrations in the pear pieces and liquid. This has been ascribed by Andlauer *et al*[25] to binding to the plant matrix. A covalent linkage to the proteins such as they proposed seemed unlikely here as the pH was too low (*ca* 3.5) to induce autooxidation[52] and the reaction products should not be extractable in methanol. The partition of the flavan-3-ols was very inhomogeneous: most stayed in the pear section (>95% of the detectable amounts), with increased average molecular weights, while only a low concentration was obtained in the cooking water, with a low degree of polymerisation. A weight balance showed a decrease in detectable flavan-3-ols of about 20% after 1 h of cooking, and of 30% for the hydroxycinnamic acids. No time courses are reported in the literature, but this decrease is in the range reported for flavonols after 1 h cooking of onions and asparagus[21] and for caffeoylquinic acid in carrots.[25] After prolonged cooking procyanidins disappeared, while hydroxycinnamic acids persisted (about 40% of the initial amounts), partially as new forms. At the same time, the pear sections turned from whitish-beige to pink, indicating conversion of the procyanidins to anthocyanin-like compounds. However this colour was limited to the sections, and no identifiable anthocyanins appeared in the cooking liquid. It seemed likely that the chemical degradation at the pH (*ca* 3.5) was not low enough to lead to depolymerisation of the pear sections.[26] Covalent linkages might be formed with the plant matrix via cleavage of the B-type linkage between epicatechin units, as reported by Beart *et al*.[53]

Changes of the cell wall polysaccharides

These changes were followed by three indicators: appearance of pectins in the cooking liquid (ethanol-precipitable material), changes in the yields of the fractional extractions (Table 2) and evolution of the size-exclusion and ion-exchange chromatography patterns of the solubilised and extracted pectins (Fig 1). For the shorter cooking times, most of the ethanol-precipitable material in the cooking water was not of polysaccharidic origin, but pectins became its main component from 1 h of cooking time (Table 2), as indicated by its sugar composition, with more than half the sugar content being galacturonic acid. Use of an ethanol precipitation step at relatively low ethanol concentrations and at room temperature ensured that only pectic polymers were recovered. In those conditions, pectic oligomers[54] and arabinans remain ethanol-soluble. This might explain the losses in galacturonic acid throughout cooking as well as the decrease in arabinose content for the cooking time of 24 h.

The pectic fraction of the pear cell walls could be divided into three parts: the freely soluble subfraction, represented by the pectic material in the cooking water, a subfraction retained by ionic links and extracted by oxalate, and more strongly bound subfractions represented by the NaOH soluble material and the residue. The proportion of the recovered uronic acids represented by the oxalate fraction was little modified between the fresh pear (200 mg g^{-1}) and 7 h of cooking (230 mg g^{-1}). During the same time, the fraction in the cooking water increased to half the total uronic acids, while the proportion in the NaOH extract decreased from 320 mg g^{-1} to 50 mg g^{-1}, and that in the residue from 270 mg g^{-1} to 140 mg g^{-1}. Transfer to the cooking water thus seemed to be mainly at the expense of the more retained pectin fractions, as already observed.[10,12,55]

Observation of the HPSEC and HPAEC traces for cooking-water pectins and oxalate-extracted pectins (Fig 1) however indicated that this interpretation may be too simplistic. The traces for the oxalate-extracted pectins seemed to represent mainly the same material as the cooking-water pectins. Only a minor fraction, eluted at high ionic strength in HPAEC and at high K_{av} values for HPSEC,

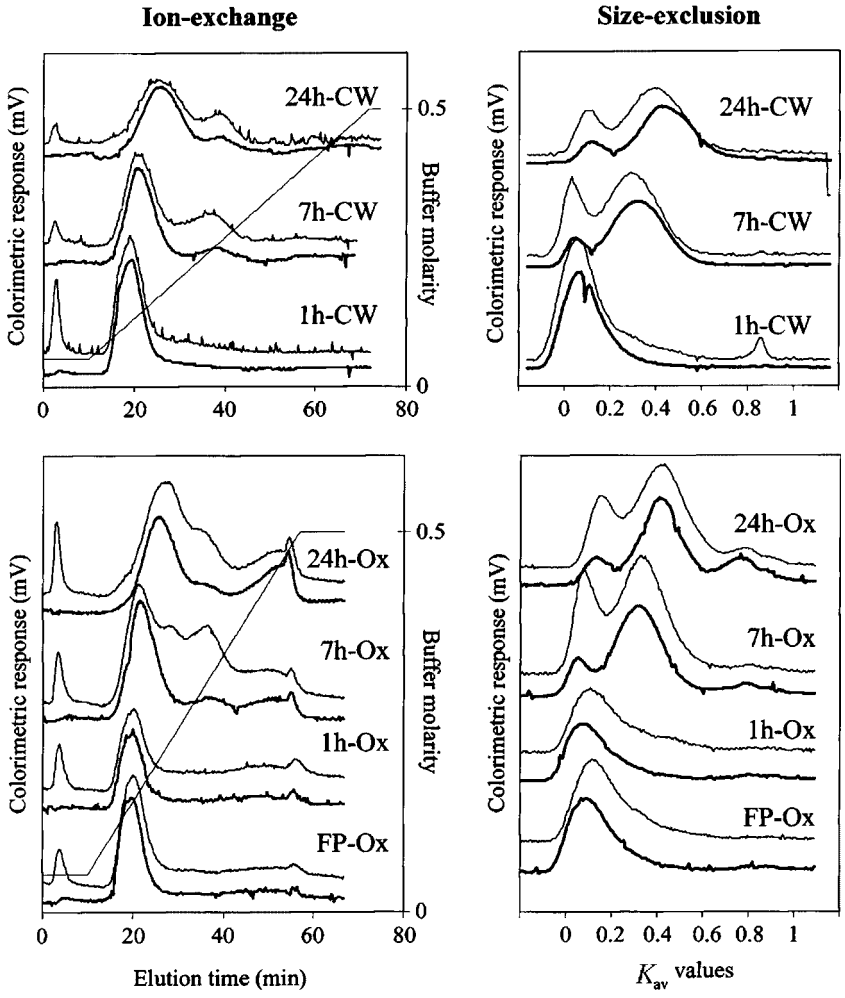

Figure 1. Change of the ion-exchange and gel-permeation patterns of the pectins solubilised in the cooking liquid (CW) and extracted by oxalate (Ox) from the alcohol-insoluble solids (AIS) of fresh pears (FP) or pears sections after different durations of boiling. Ion-exchange chromatography was performed on a ProGel TSK DEAE 5PW column eluted by ammonium acetate buffer pH 6. Gel-permeation chromatography was performed on 50, 40, 30 and 25 PWXL Bio-Gel TSK columns eluted by 0.4 M sodium acetate buffer pH 3.6. The thicker traces represent the uronic acids (*m*-hydroxydiphenyl signal) and the thinner traces the total sugars (orcinol signal).

appeared to be unique to the oxalate extracts. The fraction retained by ionic interactions in the cell walls thus appeared to contain more and more small homogalacturonans (high K_{av}), of low degree of methylation (high ionic strength of eluant). The degree of methylation of the oxalate-extracted pectins decreased during cooking, through acid-catalysed demethylation: initially very high (95), 77 after 7 h and 54 after 24 h. This was also compatible with the HPAEC traces: in the fresh pears, the bulk of the uronic acids eluted very early in the gradient; after 24 h, the peak eluting at the higher ionic strength, ie of high charge density and low degree of methylation, represented approximately one-third of the uronic acids.

The sugar compositions of the cooking-water pectins and the oxalate-extracted pectins differed, with higher proportions of neutral sugars in the cooking water. After 20 min of cooking, all neutral sugars were extracted to a similar extent. After 7 h, arabinose became prevalent. The neutral sugars did not appear as specific peaks in the traces of the cooking-water pectins, but as a higher proportion of neutral sugars in the HPSEC peak at the excluded volume ($K_{av} = 0$) and the HPAEC shoulder at intermediate ionic strength of eluant. This would correspond to the results obtained by β-elimination of pectins[3,15] or their treatment with enzymes degrading the homogalacturonan chains.[56]

DISCUSSION

Three different phenomena can be involved in the changes of the polyphenols and their partition between the two phases:

- oxidation: upon peeling and cutting, polyphenols, polyphenoloxidase and oxygen come in contact and chlorogenic acid can be transformed into its quinone; however, browning was limited here by the nature of the fruit and by short times between cutting and processing
- diffusion: the hydroxycinnamic acids and the smaller flavan-3-ols tend to equilibrate between the solid and liquid phases

- adsorption: procyanidins adsorb on plant cell walls by weak interactions:[27,57] this explains the preferential retention of the larger procyanidins in the pear sections
- degradation and other chemical reactions: for prolonged cooking durations, the bulk of the procyanidins are lost while a slightly pink colour develops, marking their conversion to anthocyanin-type structures.[26] Similarly the original hydroxycinnamic acids disappear while other molecules of near UV spectra appear.

Oxidation requires enzyme activity and is therefore only relevant in the first few minutes. Diffusion and adsorption seem to occur within the first 20 min of cooking, while chemical modifications, under the mild conditions used here, proceed slowly. Vallejo *et al*[24] have also reported the rôle of leaching in the loss of hydroxycinnamic acids from broccoli inflorescences after high pressure or conventional cooking, with steaming being the best domestic cooking methods for preservation of hydroxycinnamic acids and flavonoids if one considers only the edible parts, and high pressure when both edible parts and cooking water are consumed. This was confirmed by Andlauer *et al*[25] for zucchini and carrots. Boiling of grains has also been shown to be the most efficient technique to reduce their total extractable phenolics.[58] Procyanidins, in contrast with chlorogenic acid and flavonols, did not diffuse into the cooking water. This is in accordance with our observations[27,57] for interactions between apple procyanidins and cell walls: in model solutions, procyanidins, especially those of a high degree of polymerisation, are adsorbed onto cell walls by physico-chemical interactions.

Gieser Wildeman pears are valued as cooking pears, and, as such, are known to keep an acceptable appearance after prolonged cooking. This was confirmed here, with disintegration of the sections occurring only for a cooking time of 24 h. There was no equalisation of the soluble solids between the remaining solid phase and the exterior liquid, in spite of prolonged cooking times. Apparently some molecules could not diffuse throughout the cell walls in spite of loss of cell membrane integrity and cell wall alterations. The appearance of macromolecules (ethanol-precipitable material) in the cooking liquid was even slower, and concerned essentially degraded pectin molecules.

Changes in the cell wall compartment were fairly limited; this may be linked to the pH, which was close to that of maximum stability of pectins,[8,59] rather than to the intrinsic properties of the pear pectins, to the structure of the cell walls or to the presence of a high proportion of stone cells with their secondary cell walls (and high xylose content). The proportion of AIS, usually taken to be representative of the cell wall, actually increased early in the cooking. This might be due to the observed (co)-precipitation of intracellular material such as proteins and procyanidins, or increased substitution of the pectic charges by potassium and calcium instead of sodium. The pectins appear to be degraded globally by heat treatment, their solubilisation and leaching into the cooking water depending on whether each fragment retained bonds (ionic or otherwise) with the rest of the cell wall. The pH in the pear is such that both β-elimination and acid-catalysed cleavage are minimised,[59] but their effects are manifest after prolonged treatment. There are no structurally distinct pectin fractions which were not retained, retained by ionic bonds or retained by covalent bonds, but individual fragments of pectin molecules were present. The proportion of the uronic acid retained by ionic bonds changed little here, perhaps because the limiting factor might be high charge-density regions and ions. This would agree with observations on the protective effects on fruits and vegetable texture of treatments which decrease pectin susceptibility to β-elimination and/or increase its susceptibility to calcium reticulation: alkaline soak,[60] blanching at 60–80 °C, calculated to activate endogeneous pectinmethylesterases,[6,12,14] addition of divalent cations[3] to the cooking liquid.

CONCLUSION

The behaviour of procyanidins upon conventional boiling appeared different from that of chlorogenic acid or flavonols. There was no leaching into the cooking water, and it is thus likely that a high fraction of these polyphenols will be preserved in processed foods. Cell wall degradation concerned mainly pectins and for these molecules the main mechanisms appear to be cleavage of the intramolecular bonds.

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