Carbohydrate Polymers **20** (1993) 131–138

Effect of oxidative browning of apple pulp on the chemical and enzymatic extraction of cell wall polysaccharides

C. Ella Missang, P. Massiot,* A. Baron & J.F. Drilleau

Institut National de la Recherche Agronomique, Station de Recherches Cidricoles, Biotransformation des Fruits et Légumes, BP 29, 35650 Le Rheu, France

(Received 18 May 1992; revised version received 20 August 1992; accepted 24 August 1992)

Cell wall polysaccharides from cider apple pulp which is non-oxidized or oxidized for 2 min or 4 h were isolated as alcohol-insoluble solid (AIS) or water-insoluble solid (WIS). They were successively extracted with water, ammonium oxalate, hot dilute acid, cold dilute alkali and concentrated alkali or by enzymes such as pectin methylesterase and polygalacturonase together with endoglucanase alone or a combination of pectinases and cellulases. The soluble fraction of pectins decreased with the time of oxidation, especially with the presence of oxidized polyphenols (dark brown pigments) in cell wall materials. The results indicated a hindrance of cell wall polysaccharide hydrolysis by the oxidized polyphenols, but not enough to explain the limited yield using enzymatic treatment of oxidized apple pulp. The different behaviours of apple AIS and WIS towards extraction were discussed.

INTRODUCTION

In apple processing, cell wall polysaccharide-degrading enzymes are used as processing aids to alter the wall structure and improve the yield of juice and the quality characteristics of apple products (Pilnik & Voragen, 1991). Nevertheless, it is found in the industry that the enzymatic treatment of pulp from apples rich in phenolic compounds which have been submitted to oxidation with air, produces a very low increase in the yield of juice. In addition the enzymatic extraction of pectins from oxidized apple pomaces is very difficult (Joslyn & Deuel, 1963). Experiments in our laboratory show that yields of enzymatic liquefaction of cider apples were 95 and 63% for non-oxidized and oxidized pulp, respectively. This observation was similar with oxidized carrot pulp, with a fall of yield from 76 to 25%.

Polyphenols in apple juice as well as their oxidized forms are known to inhibit the pectinolytic enzymes, pectin methylesterase and polygalacturonase (Mitek & Drzazga, 1989). However, the limits of cell wall degradation in the enzymatic treatment of apple pulp or pomace seemed to be related to the oxidation state of the cell wall material. De Vos *et al.* (1972) observed changes in the structure of oxidized apple tissues, in particular the presence of many coagulate-like particles on the cell walls. This could be explained by the formation of insoluble condensates of polyphenols alone or as a complex with protein bodies. This hypothesis implies that solubilization and depolymerization of the cell wall polysaccharides could be modified with oxidation.

We now report a study on the effects of oxidation of apple pulp on the chemical extraction and enzymatic hydrolysis of cell wall polysaccharides. From cider apple pulp with different degrees of oxidation, cell wall polysaccharides were isolated as alcohol- or water-insoluble materials and characterized after their chemical or enzymatic solubilization.

MATERIALS AND METHODS

Preparation of the cell wall material

Ripe apples (var. Peau de chien) were harvested in October 1990 in the Mayenne department, France.

*To whom correspondence should be addressed.

Carbohydrate Polymers 0144-8617/93/$06.00

Starch-free apples (negative iodine test) were peeled and the cores removed.

Preparation of alcohol-insoluble solids (AIS)
The cortical tissue (350 g) was cut into pieces, homogenized in 200 ml of cold water in a Waring blender for 15 s and poured into boiling aqueous 96% ethanol (2 litres) immediately (sample A), after 2 min (sample B) or 4 h of oxidation (sample C). After 10 min, the mixture was filtered through a G3 sintered-glass filter. The insoluble materials were washed with ethanol until the filtrate was colourless and gave a negative reaction in the phenol–sulphuric acid test (Dubois *et al.*, 1956). The residues were washed with acetone and air-dried at 35°C, then stored at 4°C.

Preparation of water-insoluble solids (WIS)
Cortical tissue (350 g) was sliced and immersed in 200 ml of a mixture of 1% ascorbic acid, 1% citric acid, 0·1% sodium chloride (sample A) or distilled water (samples B and C) then homogenized in a Waring blender for 15 s. Immediately (sample A), after 2 min (sample B) or 4 h of oxidation (sample C), the mixture was filtered through a G3 sintered-glass filter. The water-insoluble materials were washed with the 1% ascorbic acid, 1% citric acid, 0·1% sodium chloride solution until the filtrate was colourless and gave a negative reaction in the phenol–sulphuric acid test (Dubois *et al.*, 1956). The residues were freeze-dried and stored at 4°C. The water-soluble fractions (WSF) were concentrated under reduced pressure at 40°C, treated with 4 volumes of aqueous 96% ethanol, and kept overnight at 4°C. After centrifugation (4500 ***g***, 20 min), the pellets were dispersed in aqueous 80% ethanol and centrifuged again (3 times). Precipitates were dissolved in distilled water and freeze-dried.

Enzymes

CPE, pectin methylesterase-rich preparation (from *Aspergillus niger*) was provided by Gist Brocades (the Netherlands). UM10, polygalacturonase-rich preparation (from *Aspergillus niger*) and Celluclast, cellulase-rich preparation (from *Trichoderma reesei*) were provided by Novo Industri (Denmark). The endoglucanase (EG) was purified from *Trichoderma reesei* (Massiot, 1992).

Chemical extraction

AIS and WIS were fractionated by sequential extraction with water at 25°C, ammonium oxalate 1% at 25°C, 0·05 M HCl at 85°C, 0·05 M NaOH at 2°C, then 4 M NaOH at 25°C, as described previously (Baron *et al.*, 1991). This extraction provided fractions containing water-soluble pectin (WSP), oxalate-soluble pectin (OXP), acid-soluble pectin (HP), alkali-soluble pectin (OHP) and hemicelluloses (HC), respectively.

Enzymatic extraction

Apple AIS or WIS (200 mg) were suspended in 20 ml of 0·02 M sodium acetate buffer pH 4. For the kinetic studies, the following enzymes or enzyme combinations were added: (I) CPE + UM10 containing pectin methylesterase (10 nkat) and polygalacturonase (10 nkat); (II) endoglucanase EG (10 nkat); (III) CPE + UM10 + Celluclast containing pectin methylesterase (10 nkat), polygalacturonase (10 nkat) and endoglucanase (10 nkat). To reach the degradation limit, the amounts of enzymes used were 250 nkat. Enzymes were omitted for the control. The reaction mixture was incubated at 30°C for 20 h in reaction vials equipped with magnetic stirrers. At different times of reaction, the mixture was heated at 90°C for 5 min by microwaves for enzyme denaturation then filtered through a G3 sintered-glass filter. The residue was dried at 70°C under reduced pressure. The filtrate was concentrated, treated with 4 volumes of aqueous 95% ethanol and kept overnight at 4°C. After centrifugation (4500 ***g***, 20 min), the pellet was resuspended in aqueous 80% ethanol and centrifuged as before. The process was repeated three times. The last pellet, dissolved in a minimum volume of water and freeze-dried, formed the polysaccharides fraction (P) solubilized with enzymes. The ethanol-soluble fraction, dried at 40°C under reduced pressure consisted of mono- and oligosaccharides (MO) (Mort *et al.*, 1991).

Analytical methods

AIS and WIS were ground (3 min) in a Retsch MM2 mixer mill. The neutral sugar composition of the samples was determined by gas chromatography (capillary column of 30 m × 0·25 mm i.d. with DB 225, 0·15 μm film thickness, J&W Scientific, at 215°C) using hydrogen as carrier gas, after sulphuric acid hydrolysis (Saeman *et al.*, 1954) and derivatization to alditol acetates (Hoebler *et al.*, 1989). *Myo*-inositol was used as internal standard. Galacturonide content was estimated colorimetrically with *m*-hydroxydiphenyl (Blumenkrantz & Asboe-Hansen, 1973) after sulphuric acid hydrolysis. In the water-soluble fractions, galacturonic acid and neutral sugar (expressed as arabinose) were determined by the automated *m*-hydroxydiphenyl (Thibault, 1979) and orcinol (Tollier & Robin, 1979) methods, respectively, the latter being corrected for interfering galacturonic acid.

Nitrogen was determined according to Moll *et al.* (1975) and protein content was estimated as N × 6·25 (AOAC 7·016).

Phenolic compounds were extracted from ethanol used for AIS preparation (after 2, 20 min and 4 h of oxidation) and analysed according to the method of Delage *et al.* (1991).

Enzyme activities were determined according to

Versteeg *et al.* (1978) for pectin methylesterase, and according to Baron *et al.* (1988) for polygalacturonase and endoglucanase.

High-performance ion-exchange chromatography

A base anion-exchange column TSK DEAE 5PW (75 × 7·5 mm, Toyosoda, Japan) connected to an HPLC system (Kontron) was used for high-performance ion-exchange chromatography (HPIEC) separation. Eluent was 0·05 M sodium acetate pH 6 then a linear gradient 0·05–0·5 M (μ = 0·047–0·47) of sodium acetate pH 6 with a flow rate of 0·6 ml/min. A sample of 200 μl containing 0·2–0·4 μmol of galacturonic acid was injected. The eluate was continuously monitored using the automated *m*-hydroxydiphenyl (Thibault, 1979) and orcinol (Tollier & Robin, 1979) methods.

High-performance size-exclusion chromatography (HPSEC)

The molecular weight distribution of polysaccharides was determined using an HPLC system involving a Laboratory data control (LDC) programmable pump equipped with three Bio-Gel TSK columns (each 300 × 7·8 mm) in series (40 PWXL, 30 PWXL and 25 PWXL; Bio-Rad Labs) in combination with a TSK XL guard column (40 × 6 mm) at 35°C. Eluent was 0·4 M acetic acid/sodium acetate pH 3·6 with a flow rate of 0·6 ml/min. The eluate was monitored using a thermostatted (40°C) Erma ERC 7512 refractive index detector.

RESULTS AND DISCUSSION

Phenolic compounds from oxidized apple pulp

The phenolic compounds extracted from pulp after 2, 20 min and 4 h of oxidation were analysed as catechins, chalcones, cinnamic acid derivatives, *p*-coumaric acid derivatives (Table 1) with relative standard deviation lower than 5% (Delage *et al.*, 1991). Amounts of phenolic compounds decreased as the enzymatic oxidation of phenols leads first to quinones and then further to brown pigments (Janovitz-Klapp *et al.*, 1990). There were 22 times more phenolic compounds in sample B (2 min) than in sample C (4 h). The choice of time of oxidation was 2 min and 4 h for the low and high contents of brown pigments, respectively. So, the colour of samples (AIS and WIS) were white (A), beige (B) and dark brown (C).

Isolation and characterization of cell wall polysaccharides

The yields of AIS and WIS from sample A (control) were 10·0 and 11·4 g per 100 g of dry pulp, respectively (Table 2). The relative standard deviation of the preparation of cell wall materials was 3·4%. The yields of AIS are in agreement with previous reported results on apple cell walls (Voragen *et al.*, 1983; Stevens & Selvendran, 1984; Renard *et al.*, 1990). With oxidation, AIS values increased corresponding in part to the evolution of protein contents. WIS values decreased but the amount of polysaccharides (WSF) solubilized during preparation of WIS increased with time of oxidation. This solubilization was probably due to the action of endogenous apple enzymes as pectin methylesterase, exopolygalacturonase, β-galactosidase (Bartley & Knee, 1982) released with the grinding of tissues. In this way, the sum of WIS and WSF increased by 10% after 4 h of oxidation.

The polysaccharide content (79·6%) in AIS (A) (Table 2) was lower than that (84·6%) in WIS (A). This proportion in AIS decreased after 4 h of oxidation (sample C). These results are inconsistent with those of Voragen *et al.* (1980), who recorded 90·2% and 45·0% for AIS and WIS, respectively. They used 0·5 M potassium phosphate buffer in the preparation of WIS which could extract more pectic substances than the 1% ascorbic acid, 1% citric acid, 0·1% sodium chloride solution. The yields of galacturonic acid in AIS and WIS decreased with the time of oxidation.

Table 3 gives the sugar composition of pectic and hemicellulosic fractions extracted from AIS, WIS and WSF. The total amounts of extracted galacturonic acid were 9·1–12·2 mmol per 100 g of dry pulp with no significant difference between AIS and WIS. This content decreased with the time of oxidation. Nevertheless, the total contents of WIS + WSF galacturonic acid were similar (13·5 mmol per 100 g of dry pulp) in the three oxidation conditions. Soluble pectins (WSP + OXP) decreased with time of oxidation, especially in

Table 1. Content of phenolic compounds of apple pulp after oxidation

Oxidation time	Fractions (mmol/100 g dry pulp)			
	Catechins	Chalcones	Cinnamic acid derivatives	*p*-Coumaric acid derivatives
2 min	122·7	16·3	99·3	15·0
20 min	8·0	5·7	36·6	9·9
4 h	2·9	1·6	4·7	2·4

Table 2. Yield and composition of alcohol-insoluble solid (AIS) and water-insoluble solid (WIS) from apple pulp non-oxidized (A) or oxidized 2 min (B) and 4 h (C)

Fractions	Yield (g/100 g dry pulp)	Protein (g/100 g dry fraction)	Sugar composition (g/100 g dry fraction)								
			Gal A[a]	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Total sugars
AIS (A)	10·0	3·9	26·4	1·1	0·7	9·5	5·6	2·1	6·0	28·2	79·6
AIS (B)	10·2	7·6	23·2	1·3	0·7	13·6	5·3	2·3	6·0	30·5	82·9
AIS (C)	10·7	6·7	19·0	1·1	0·6	10·9	4·4	1·9	5·3	27·7	70·9
WIS (A)	11·4	3·2	21·2	1·4	0·8	14·6	5·7	2·3	6·7	31·9	84·6
WIS (B)	11·0	2·9	21·3	1·4	0·8	14·8	6·1	2·4	5·9	33·0	85·7
WIS (C)	10·8	2·3	21·1	1·3	0·9	14·8	6·0	2·4	6·7	34·2	87·4
WSF (A)[b]	1·0		22·3								32·5
WSF (B)	1·5		23·1								33·2
WSF (C)	2·8		21·9								28·9

[a]Gal A, Rha, Fuc, Ara, Xyl, Man, Gal, Glc denotes galacturonic acid, rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose, respectively.
[b]Water-soluble fraction isolated with WIS preparation.

Table 3. Sugar composition of pectins and hemicelluloses obtained by sequential extraction of AIS and WIS from apple pulp non-oxidized (A), oxidized 2 min (B) or 4 h (C)

Fractions	AIS (A)		AIS (B)		AIS (C)		WIS (A)		WIS (B)		WIS (C)	
	Gal A[a]	NS[b]	Gal A	NS	Gal A	NS	Gal A	NS	Gal A	NS	Gal A	NS
Water-soluble pectin (WSP)	2·7 (0·4)	2·3 (0·1)	1·0 (0·3)	0·7 (0·2)	0·9 (0·1)	0·8 (0·4)	1·3 (0·5)	0·6 (0·2)	1·2 (0·5)	0·4 (0·2)	0·8 (0·2)	0·5 (0·1)
Oxalate-soluble pectin (OXP)	1·9 (0·7)	1·2 (0·7)	0·4 (0·4)	0·3 (0·2)	0·4 (0·3)	0·2 (0·1)	1·4 (0·5)	0·5 (0·1)	1·6 (0·4)	0·4 (0·1)	1·2 (0·4)	0·4 (0·1)
Acid-soluble pectin (HP)	4·9 (0·9)	8·2 (0·5)	6·4 (1·7)	14·6 (0·8)	6·4 (0·7)	12·8 (0·6)	6·5 (0·9)	16·9 (0·7)	5·3 (2·3)	13·8 (6·7)	5·8 (0·4)	15·2 (1·1)
Alkali-soluble pectin (OHP)	0·7 (0·3)	0·8 (0·9)	0·9 (0·1)	0·5 (0·2)	1·1 (0·1)	0·4 (0·1)	2·6 (0·5)	0·4 (0·2)	2·5 (0·2)	0·3 (0·1)	1·9 (0·5)	0·5 (0·2)
Hemicelluloses	0·4 (0·3)	4·6 (0·2)	0·5 (0·5)	4·1 (0·7)	0·3 (0·1)	3·6 (0·1)	0·4 (0·2)	5·1 (0·3)	0·6 (0·4)	4·8 (0·1)	0·4 (0·2)	5·0 (0·5)
Total	10·6 (0·7)	17·1 (1·7)	9·2 (1·4)	20·2 (1·4)	9·1 (0·7)	17·8 (1·1)	12·2 (1·3)	23·5 (1·2)	11·2 (1·8)	19·7 (7·1)	10·1 (0·3)	21·6 (1·1)
Water-soluble fraction (WSF)							1·3	0·7	2·4	1·0	3·4	1·3

[a]Galacturonic acid in mmol/100 g of dry pulp with standard deviations in parentheses.
[b]Neutral sugars expressed as arabinose in mmol/100 g of dry pulp with standard deviations in parentheses.

AIS (reduction of 71% between A and C), suggesting a hindrance of pectin extraction by the brown pigments. The decrease was lower (22%) for hemicelluloses from AIS but was significant.

The WSP fractions were submitted to anion-exchange chromatography (Fig. 1). The elution patterns of galacturonic acid were similar, with a main population eluted with the sodium acetate gradient (0·14 M). However, WSP from WIS (A) exhibited a heterogeneous distribution. The molecular weight distribution of neutral and acid fractions of WSP from WIS are shown in Fig. 2. The molecular weights of the water-soluble acid polysaccharides were higher after 2 min (B) and 4 h (C) of oxidation. In the same way, the molecular weights of the water-soluble neutral polysaccharides increased after 4 h (C). These results suggest that either the pectic fractions extracted from samples B and C were different populations from those extracted from sample A, implicating the action of endogenous enzymes in solubilizing high molecular weight fractions from protopectin, or there was an interaction between the polysaccharides and other macromolecules after extraction. The aggregation of polysaccharides and soluble polymerized quinones has been suggested earlier (McManus *et al.*, 1985; Mitek & Drzazga, 1988). But, after only 2 min of oxidation (B) the acid polysaccharides could interact with *o*-quinones, the first compounds formed as a result of the oxidation reaction.

Enzyme hydrolysis of cell wall polysaccharides

Figure 3 shows the limits of hydrolysis with 250 nkat of pectin methylesterase, polygalacturonase and endoglucanase after 20 h of reaction. The soluble material was divided into buffer-soluble (control without enzymes) and enzyme-soluble (total soluble material minus control) fractions. According to the WSP fractions, the amounts of buffer-soluble polysaccharides decreased with the degree of oxidation. Only 4–5% of the samples A and B were not solubilized by the enzyme treatment. The samples C, AIS and WIS, were more resistant to hydrolysis since 29 and 13%, respect-

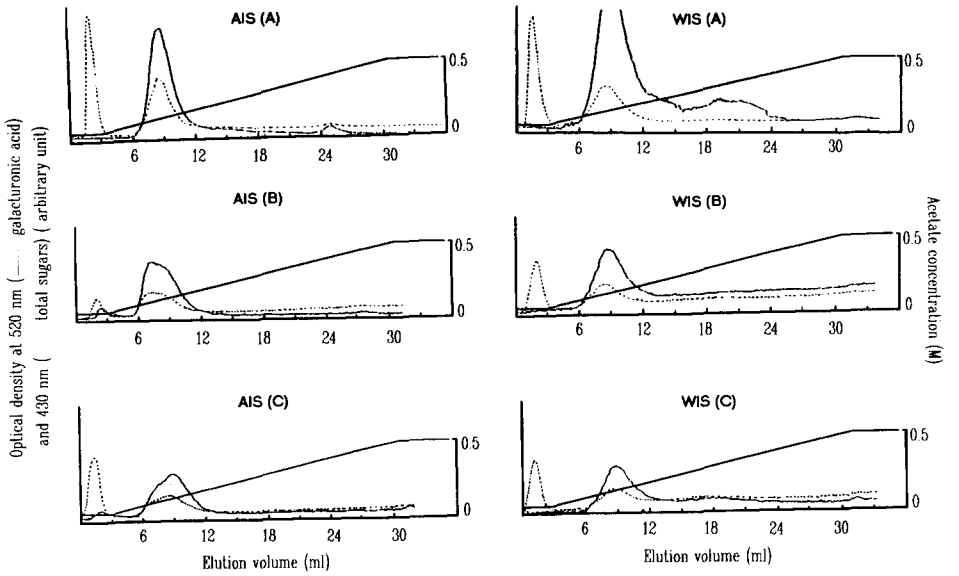

Fig. 1. HPIEC of water-soluble pectin (WSP) of AIS and WIS from apple pulp non-oxidized (A), oxidized 2 min (B) or 4 h (C).

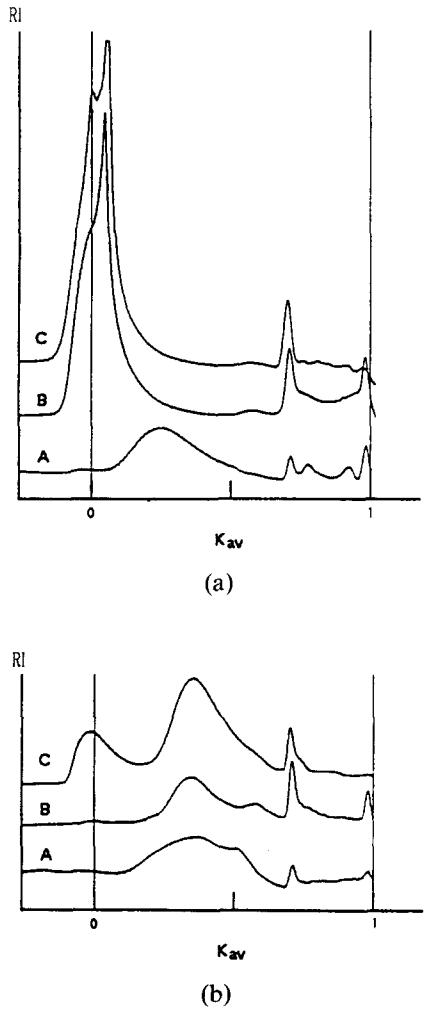

Fig. 2. HPSEC of acid (a) and neutral (b) fractions of water-soluble pectin (see Fig. 1) of WIS from apple pulp non-oxidized (A), oxidized 2 min (B) or 4 h (C). RI: Refractive index.

ively, were not solubilized. Therefore, the cell wall polysaccharides were still accessible by enzymes after 2 min of oxidation but some of them were not solubilized if brown pigments were present (sample C).

Figure 4 shows the kinetics of solubilization of galacturonic acids from WIS. The release of galacturonic acid from sample C was lower than that from samples A and B. After 10 and 20 h of reaction with 250 nkat (end points), the enzyme mixture I (pectinases) released 11·5–13·0 mmol of galacturonic acid per 100 g dry pulp and the enzyme II (endoglucanase) only 4·9–6·9 mmol. A synergistic effect was observed with the enzyme mixture III (pectinases and cellulases) with a release of 16·8–21·2 mmol of galacturonic acid per 100 g dry pulp.

In the same way, the enzymatic solubilization of neutral polysaccharides was slower and less extensive for sample C (Fig. 5). The pectinases solubilized 20·8, 20·6 and 13·8 mmol of neutral sugars per 100 g of dry pulp while the endoglucanase released 12·3, 11·7 and 8·0 mmol from WIS A, B and C, respectively. Pectinases and cellulases acted also with synergy because the end points were 30·2–31·2 mmol per 100 g of dry pulp. In the three cases, no difference was observed between samples A and B.

After hydrolysis of WIS with enzyme mixtures I and II, soluble polysaccharides (P) and mono- and oligosaccharides (MO) were analysed (Table 4). After 2 h of reaction, the pectinases released mainly pectic polysaccharides corresponding to 51·8, 44·1 and 30·8% of galacturonic acid content in WIS A, B and C, respectively. After 20 h, the presence of galacturonic acid, arabinose and galactose in the MO fractions and the decrease of galacturonic acid/rhamnose molar ratio of P fractions indicated that the galacturonan

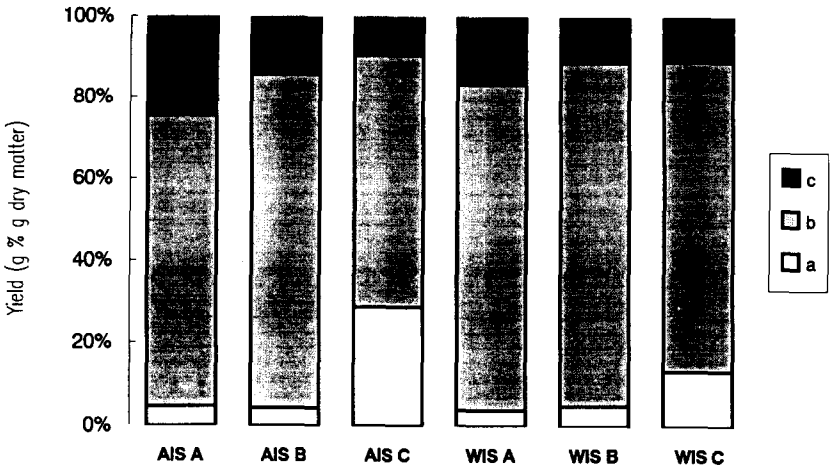

Fig. 3. Yield of solubilization of AIS and WIS from apple pulp non-oxidized (A), oxidized 2 min (B) or 4 h (C) after 20 h of reaction with pectin methylesterase, polygalacturonase and endoglucanase (250 nkat each). Insoluble (a), enzyme soluble (b), buffer soluble (c) fractions.

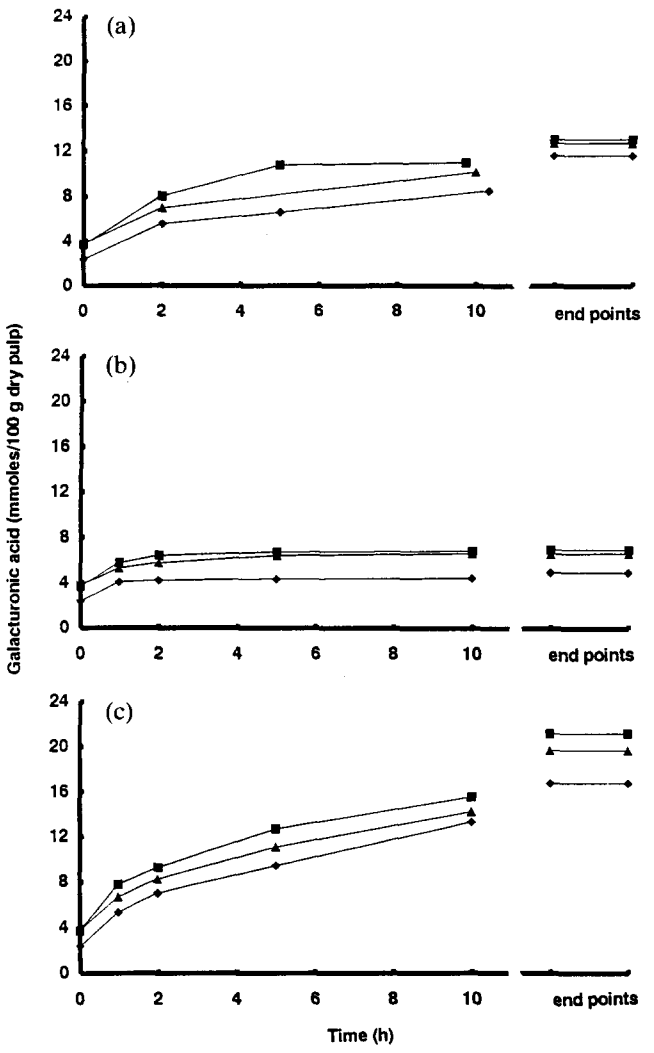

Fig. 4. Content of galacturonic acid in the soluble fraction after hydrolysis of WIS with pectinases (a), endoglucanase (b) and pectinases and cellulases (c) with 10 nkat of each activity. End points correspond to the degradation limit with 250 nkat of each activity after 10 and 20 h, respectively. ■, WIS (A); ▲, WIS (B); ♦, WIS (C).

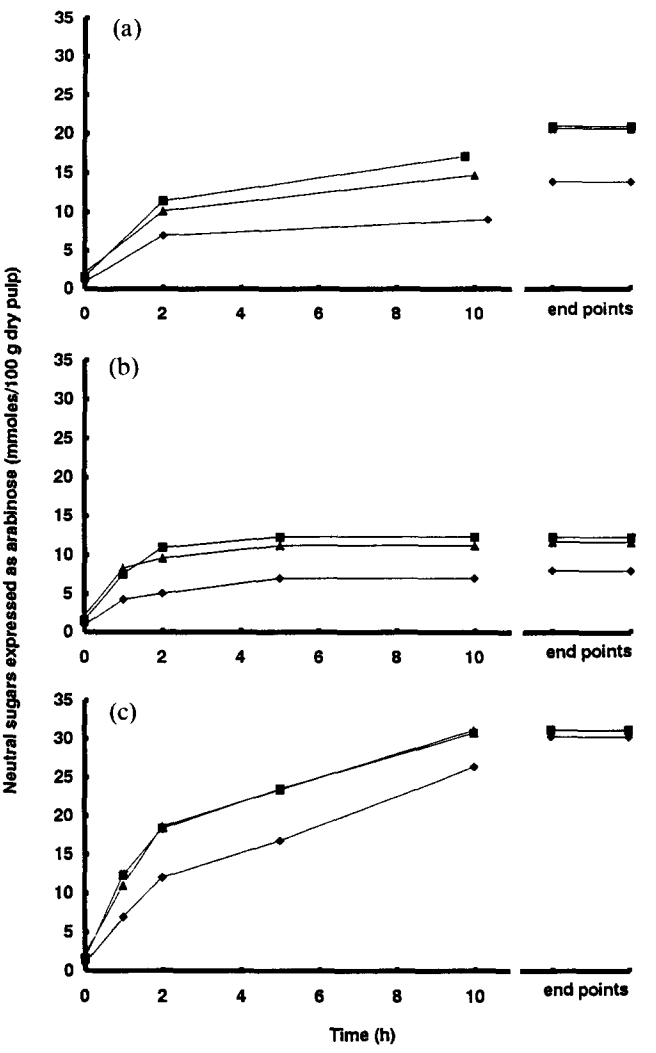

Fig. 5. Content of neutral sugars in the soluble fraction after hydrolysis of WIS with pectinases (a), endoglucanase (b) and pectinases and cellulases (c) with 10 nkat of each activity. End points correspond to the degradation limit with 250 nkat of each activity after 10 and 20 h, respectively. ■, WIS (A); ▲, WIS (B); ♦, WIS (C).

Table 4. Sugar composition of soluble polysaccharides (P) and mono- and oligosaccharides (MO) after enzymatic hydrolysis of water-insoluble solid (WIS) from apple pulp non-oxidized (A) or oxidized 2 min (B) or 4 h (C)

Enzyme	Reaction time (h)	Fractions	Molar composition (%)							Total sugars (g/100 g dry WIS)
			Gal A	Rha	Ara	Xyl	Man	Gal	Glc	
I (pectinases)	2	P (A)	51·0	2·4	32·1	2·5	—	7·7	4·3	20·1
		MO (A)	19·5	6·3	60·9	—	4·0	3·9	5·5	7·3
		P (B)	48·9	2·4	34·8	2·7	—	7·1	4·0	17·9
		MO (B)	5·6	5·1	78·9	—	4·9	—	5·6	7·1
		P (C)	47·2	3·1	34·4	2·5	—	7·3	5·3	12·8
		MO (C)	19·4	6·3	65·4	—	0·2	4	5·1	5·8
	20	P (A)	14·6	4·7	59·8	4·9	—	13·6	2·5	24·6
		MO (A)	57·6	0·7	11·8	9·1	0·8	4·0	16·0	24·2
		P (B)	19·4	4·7	56·9	4·8	—	11·7	2·4	25·6
		MO (B)	58·4	1·0	12·9	8·5	0·7	3·8	14·7	24·0
		P (C)	18·3	4·5	52·8	6·4	2·3	11·0	4·8	21·9
		MO (C)	64·5	1·0	14·2	5·4	1·0	3·0	10·7	17·5
II (endoglucanase)	1	P (A)	39·3	2·5	33·5	6·4	—	9·8	8·5	16·6
		MO (A)								trace[a]
		P (B)	35·9	3·0	35·9	6·9	0·5	9·5	8·4	17·4
		MO (B)								trace[a]
		P (C)	37·9	2·7	33·7	6·5	—	9·2	10·0	11·2
		MO (C)								trace[a]
	20	P (A)	38·1	3·4	42·3	3·9	—	9·7	2·6	27·6
		MO (A)	1·6	1·8	21·2	28·1	—	10·8	36·6	6·6
		P (B)	36·8	3·6	44·3	4·0	0·4	8·5	2·4	24·9
		MO (B)	ND[b]	1·8	25·8	28·7	—	10·2	33·7	5·9
		P (C)	37·0	3·8	43·1	4·7	—	8·3	3·1	34·2
		MO (C)	4·9	1·5	18·5	27·4	1·6	10·5	35·7	3·9

[a]Composition not determined.
[b]ND, not determined.

backbone of pectins was depolymerized; the soluble polysaccharides (P) were mainly pectic side-chains rich in arabinose. The yields of MO and P fractions after hydrolysis of WIS C were lower, indicating a limitation of the solubilization and the depolymerization of pectic polysaccharides. Nevertheless, no specific difference was observed between the composition of the reaction products.

After 1 h of hydrolysis, the endoglucanase released small amounts of pectic polysaccharides rich in neutral sugars but no oligosaccharides. After 20 h, there was mainly a solubilization of pectic side-chains (fractions P) and a depolymerization of β-glucans (fractions MO). The solubilization of pectic polysaccharides by cellulases was previously described in apple (Renard *et al.*, 1991) or carrot (Massiot & Thibault, 1989) cell walls. After 20 h of reaction, the solubilization of pectins from sample C by the endoglucanase was not hindered by the insoluble pigments but the depolymerization of cellulose decreased since the content of glucose from the MO fraction reduced to 25% in comparison with the control (sample A).

The residual activity of polygalacturonase in the soluble phase during the enzymatic treatment of WIS was determined with a relative standard deviation lower than 3%. In the control (without WIS), the activity decreased by 35% after 5 h, probably due to a lack of stability in the absence of substrate. With the cell wall materials, the activity immediately decreased by 35, 40 and 52% for samples A, B and C, respectively. This suggested the adsorption of enzyme onto the cell walls, increased in the presence of brown pigments. Moreover, in this assay the activity decreased with the time of reaction. The inhibition of enzymes by soluble phenolic compounds (Mitek & Drzazga, 1988) is unlikely since only the insoluble derivatives were isolated with cell walls.

CONCLUSIONS

The results described in this paper show that oxidation of apple pulp was concomitant with a solubilization of pectins. This was thought to be due to the action of endogenous enzymes. Voragen *et al.* (1980) pointed out such an enzymatic degradation of pectins during the preparation of water-insoluble solids of apple. On the other hand, an association probably occurred between the cell wall polysaccharides and the phenolic derivatives, and limited the chemical extraction of pectins.

The formation of quinones in the early stages of oxidation (sample B) had no significant effect on the solubilization rate and the nature of the reaction

products after hydrolysis of WIS by polysaccharidases. In contrast, the occurrence of brown pigments (sample C) limited the action of enzymes on the acid and, especially, on the neutral polysaccharides. In this case, the depolymerization of cellulose by the endoglucanase was hindered and the action of pectinases was limited. Moreover, a reduction of polygalacturonase activity was observed in the presence of WIS (C). These results suggest that the oxidation of phenolic compounds and their condensation on the cell wall surface (DeVos *et al.*, 1972) hinder the accessibility of cell wall polysaccharides.

Nevertheless, the decrease of the enzymatic action on the cell wall polysaccharides was too small to explain the high diminution of the yield when oxidized pulp was treated with polysaccharidases. The effect of interactions between the polysaccharides and brown pigments on the surface of the cell wall would be less in comparison to the inhibition of enzymes by the phenol derivatives (Mitek & Drzazga, 1989) in the soluble phase of the pulp.

The behaviour of AIS and WIS preparations towards chemical and enzymatic extraction were different. In particular, the extraction of soluble pectins and the limits of enzymatic hydrolysis of AIS were weaker than those of WIS. The ethanol treatment alters the cell wall structure with the formation of collapsus (Selvendran & O'Neill, 1987) between cell wall macromolecules such as polysaccharides, proteins and quinone derivatives. Moreover, the content of polysaccharides determined in AIS after sulphuric acid hydrolysis was low compared to that of WIS. This fact is probably due to a lesser accessibility of cell wall polysaccharides. For these reasons, WIS were used to study the kinetics and analyse the reaction products of enzymatic hydrolysis.

The amounts of pectin extracted from WIS A, B and C with pectinases and cellulases were 21·2, 19·7, and 16·8 mmol per 100 g of dry pulp, respectively, which were higher than those released by chemical extraction (12·2, 11·2 and 10·1 mmol per 100 g of dry pulp, respectively). This confirms that enzyme treatment of cell wall is a good method for solubilizing total pectins (Voragen *et al.*, 1980) and determining their sugar composition.

ACKNOWLEDGEMENTS

The authors thank Claudette Prioult and Nathalie Marnet for their skilful technical assistance.

REFERENCES

Baron, A., Saulnier, B., Prioult, C. & Drilleau, J.F. (1988). *Lebensm. Wiss. u.-Technol.*, **21**, 92–5.

Baron, A., Massiot, P., Prioult, C. & Marnet, N. (1991). *Sci. Aliments*, **11**, 627–39.

Bartley, I.M. & Knee, M. (1982). *Food Chem.*, **9**, 47–58.

Blumenkrantz, N. & Asboe-Hansen, G. (1973). *Anal. Biochem.*, **54**, 484–9.

Delage, E., Bohuon, G., Baron, A. & Drilleau, J.F. (1991). *J. Chromatogr.*, **555**, 125–36.

De Vos, L., Van Buren, J., Van Deelen, W. & Pilnik, W. (1972). In *International Federation of Fruit Juice Producers*, **12**, 199–210.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. (1956). *Anal. Chem.*, **28**, 350–6.

Hoebler, C., Barry, J.L., David, A. & Delort-Laval, J. (1989). *J. Agric. Food Chem.*, **37**, 360–5.

Janovitz-Klapp, A.H., Richard, C.D., Goupy, P.M. & Nicolas, J.J. (1990). *J. Agric. Food Chem.*, **38**, 1437–41.

Joslyn, M.A. & Deuel, H. (1963). *J. Food Sci.*, **28**, 65–83.

McManus, J.P., Davis, K.J., Beart, J.E., Gaffney, S.H., Lilley, T.H. & Haslam, E. (1985). *J. Chem. Soc. Perkin Trans.*, **2**, 1429–38.

Massiot, P. (1992). *Lebensm. Wiss. u.-Technol.*, **25**, 120–5.

Massiot, P. & Thibault, J.F. (1989). *Carbohydr. Res.*, **190**, 121–36.

Mitek, M. & Drzazga, B. (1988). *Acta Alimantaria Polonica*, **14 38**(2), 175–85.

Mitek, M. & Drzazga, B. (1989). *Acta Alimantaria Polonica*, **15 39**(1), 3–13.

Moll, M., Flayeux, R. & Lehuede, J.M. (1975). *Ind. Agric. Alim.*, **6**, 631–3.

Mort, A.J., Moerschbacher, B.M., Pierce, M.L. & Maness, N.O. (1991). *Carbohydr. Res.*, **215**, 219–27.

Pilnik, W. & Voragen, A.G.J. (1991). *Flüssiges Obst*, **58**, 125–9.

Renard, C.M.G.C., Voragen, A.G.J., Thibault, J.F. & Pilnik, W. (1990). *Carbohydr. Polym.*, **12**, 9–25.

Renard, C.M.G.C., Schols, H.A., Voragen, A.G.J., Thibault, J.F. & Pilnik, W. (1991). *Carbohydr. Polym.*, **15**, 13–32.

Saeman, J.F., Moore, W.E., Mitchell, R.L. & Millett, M.A. (1954). *Tappi*, **37**, 336–43.

Selvendran, R.R. & O'Neill, M.A. (1987). In *Methods in Biochemical Analysis*, Vol. 32, ed. D. Glick. Academic Press, London, pp. 25–153.

Stevens, B.J.H. & Selvendran, R.R. (1984). *Carbohydr. Res.*, **135**, 155–66.

Thibault, J.F. (1979). *Lebensm. Wiss. u.-Technol.*, **12**, 247–51.

Tollier, M.T. & Robin, J.P. (1979). *Ann. Technol. Agric.*, **28**, 1–15.

Versteeg, C., Rombouts, F.M. & Pilnik, W. (1978). *Lebensm. Wiss. u.-Technol.*, **11**, 267–74.

Voragen, A.G.J., Heutink, R. & Pilnik, W. (1980). *J. Appl. Biochem.*, **2**, 452–68.

Voragen, A.G.J., Timmers, J.P.J., Linssen, J.P.H., Schols, H.A. & Pilnik, W. (1983). *Z. Lebensm. Unters. Forsch.*, **177**, 251–6.