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Studies on Enzymic Hydrolysis of Polysaccharides in Sugar Beet Pulp

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ABSTRACT

Beet fibres have been prepared from sugar beet pulp and degraded with three enzymic preparations (SP249 from Aspergillus aculeatus, *Driselase from* Irpex lacteus *and Onozuka from* Trichoderma viride*) which have been characterised for their activities towards polysaccharides. The overall extent of hydrolysis was ~ 48% with Onozuka and up to ~ 80% with the other preparations. These values may be increased to ~ 90% by some chemical pretreatments including persulphate, chlorite or hydrogen peroxide.*

INTRODUCTION

Sugar beet pulp is an important by-product from the sugar refining industry which is to date practically only used in cattle feeding (Kelly, 1983). It is especially rich in polysaccharides, mainly pectic substances and suitable as a dietary fibre source (Michel *et al.*, 1985, 1988). These fibres have been extensively studied (Bertin *et al.*, 1988) and exhibit interesting properties such as water retention and ion exchange capacity, which have some important nutritional effects (Eastwood & Mitchell, 1976). Their enzymic degradation, which can occur in the large bowel, will also have nutritional consequences.

These large amounts of plant cell-wall materials can be processed to yield fermentable sugars (Beldman *et al.*, 1984; Sidi Ali *et al.*, 1984; Considine *et al.*, 1988). The conversion of polysaccharides into monomeric compounds is usually obtained by enzymic hydrolysis and

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the yield depends on the presence of suitable activities and the accessibility of the substrate (Ladisch *et al.*, 1983). For example, it has been reported that the matrix of pectic substances may hinder the degradation of cellulose fibrils by cellulases in the cell wall (Voragen *et al.*, 1980; Ben-Shalom, 1986; Massiot *et al.*, 1989). It was also found that mechanical, thermal or chemical pretreatments were sometimes necessary to achieve complete enzymic hydrolysis, especially when non-parenchymatous tissues are treated (Ladisch *et al.*, 1983; Cunningham & Carr, 1984).

In this paper, we report on the hydrolysis of the polysaccharides in fibres prepared from sugar beet pulp by different enzyme preparations. Various pretreatments of the substrate were used with the aim of obtaining maximum conversion and of determining the factors limiting degradation.

MATERIALS AND METHODS

Sugar beet pulp

Sugar beet pulp was obtained in a dried form from the Sugar Factory in Artenay (France). Pulp was ground in an IK grinder and sieved. The starting material for this study consisted of particles of diameter between 500 μm and 250 μm and was called RF (Bertin *et al.*, 1988).

Enzyme preparations

Experimental enzyme preparation SP249 (from *Aspergillus aculeatus*) was kindly provided by Novo Industri (Denmark). Driselase (from *Irpex lacteus*) was purchased from Fluka (Switzerland) and cellulase Onozuka (from *Trichoderma viride*) from Yakult Biochemicals (Japan).

Analytical methods

Total soluble sugars were measured by the automated orcinol method (Tollier & Robin, 1979) using arabinose as a standard and reducing sugars were determined according to the method of Nelson (1944). Neutral sugars were individually determined by GLC of their alditol acetates (Sawardeker *et al.*, 1965) after Saeman (Saeman *et al.*, 1954) hydrolysis or 1 M H_2SO_4 hydrolysis (Blakeney *et al.*, 1983) (100°C, 2 h) for insoluble or soluble materials, respectively. Uronic acids were measured by the *m*-phenylphenol method (Ahmed & Labavitch, 1977;

Thibault, 1979) using galacturonic acid as a standard. Methylation of the polysaccharides was carried out according to Lomax *et al.* (1983) on cryo-milled samples (3 min, Spex 6700). The methylated polysaccharides were hydrolysed by formic acid (100°C, 1 h) then trifluoroacetic acid (100°C, 3 h) and the resultant partially methylated sugars were reduced and acetylated by the procedure of Harris *et al.* (1984) and analysed using OV1 and CPSil 88 capillary columns. Proteins in enzymic preparations were determined by the Lowry *et al.* (1951) method using bovine serum albumin as a standard.

Enzymic activity assays

Activities of enzymic preparations were determined from the initial reaction velocity (except for filter paper activity and protease) and except for protease activity expressed in nanokatals (nkat). The substrates were polygalacturonic acid (ICN), commercial HM pectin (Unipectine, Redon, France), carboxymethylcellulose (CMC medium viscosity, Sigma), type I arabinogalactan prepared from lupin seeds (Jones & Tanaka, 1984), type II arabinogalactan (Serva), arabinan prepared from pea (Brillouet & Carré, 1983), xylan, laminarin and lichenan (Sigma). All the experimental conditions were as previously described (Massiot *et al.*, 1989).

Enzymic hydrolysis

Fibres (250 mg) were weighed in 100 ml erlenmeyer flasks and suspended in 50 ml of 10 mM sodium acetate buffer, pH 5·0, containing 0·01% (w/v) sodium merthiolate. Enzymes were added so that the reaction mixture contained 1 nkat of polygalacturonase activity per mg of fibre and the reaction was carried out at 25°C with magnetic stirring. After 48 h hydrolysis, a double amount of enzyme was added to the flasks in order to ensure that no inhibition of the enzymes by the products occurred and the reaction continued for a further 72 h. At appropriate periods of time, the contents of a flask were centrifuged (9000 *g*, 20 min) and the pellet was extensively washed over a G4 sintered glass with distilled water and then dried by solvent exchange (ethanol, acetone and ether). It was then dried under reduced pressure at 40°C and weighed. This residual material was ground in liquid nitrogen and analysed for neutral sugars, uronic acids or polysaccharides structure. Blanks were processed in exactly the same way except that enzyme additions were omitted. Hydrolysis was calculated by the dif-

ference between the polysaccharides content before and after enzymic reaction.

Preparation and pretreatments of the fibres

The fibre RF was extensively washed with distilled water over a G4 sintered glass filter in order to remove soluble materials. When the conductivity of the effluent was less than 20 μS, the residual fibre (WRF) was dried by solvent exchange. Ammonium oxalate-extracted RF (OEF), hydrochloric acid extracted-fibre (AEF) and sodium hydroxide extracted-fibre (BEF) were prepared as previously reported (Bertin *et al.*, 1988). Half of each fraction was dried by solvent exchange and the other half was kept undried in water suspension, containing 0·01% sodium thimerosal.

RF was also pretreated by ammonium persulphate, sodium chlorite and hydrogen peroxide:

2·7 g of RF were stirred for 24 h at ambient temperature with 500 ml of 0·04 M or 0·02 M ammonium persulphate solutions. The residual fibres were extensively washed with distilled water, dried by solvent exchange and weighed.

1 g of RF was suspended in 50 ml of 0·05 M hydrochloric acid solution and stirred for 30 min at 75°C. Solid sodium chlorite was then added to reach 0·2% (w/v) concentration in the medium. After addition of a drop of *n*-octanol as an anti-foaming agent, the reaction continued for 30 min at 75°C (Whistler & BeMiller, 1963). Then the delignified fibres were extensively washed with distilled water, dried by solvent exchange and weighed.

1 g of RF was stirred for 3 h at ambient temperature with 50 ml of 1% (v/v) aqueous hydrogen peroxide containing 1·2 ml of 12 M sodium hydroxide (final pH: 11·5) (Cunningham & Carr, 1984). The treated fibres were washed, dried and weighed as above.

The swelling of the modified fibres was measured in pure water as described previously (Bertin *et al.*, 1988).

RESULTS AND DISCUSSION

Preparation and analysis of the fibres

RF accounted for 60% of the crude beet pulp. WRF was obtained by extraction of RF with distilled water in order to avoid the spontaneous

release in the medium of the soluble part of the material during the enzymic reaction. The yield of WRF was 83·7% of RF whereas those of OEF, AEF and BEF were 84·9%, 44·2% and 35·5% of RF, respectively; these values are close to those previously reported for a similar preparation (Bertin *et al.*, 1988), indicating the good reproducibility of the procedure.

The compositions of polysaccharides of the different fibres (Table 1) are close to those of Bertin *et al.* (1988). The total amounts of sugars

TABLE 1
Sugar Composition of the Different Fibres (Per cent of Dry Matter)

Anhydrosugars	*Fractions*				
	RF	*WRF*	*OEF*	*AEF*	*BEF*
Rhamnose	0·8	1·0	1·0	1·0	0·8
Fucose	0·0	0·1	0·2	0·2	0·2
Arabinose	16·8	18·8	19·2	2·9	2·5
Xylose	1·6	1·5	1·4	2·6	3·5
Mannose	1·5	1·0	1·0	1·6	2·3
Galactose	3·9	4·4	4·4	4·2	3·9
Glucose	21·4	19·5	19·8	36·3	52·9
Galacturonic acid	19·6	21·2	20·6	18·3	9·9
Total	65·6	67·5	67·6	67·1	66·0

were about 65–68%, the remaining being ashes, proteins, lignin, methanol, acetic or phenolic acids (Bertin *et al.*, 1988). The polysaccharidic composition of WRF is very close to that of OEF or RF. Results from methylation (Table 2) showed that the structures of the neutral polysaccharides in WRF or in RF are similar and correspond to mainly cellulose, branched arabinan, galactans, xylans, mannans and xyloglucans, in association with rhamnogalacturonans. AEF and BEF have been mainly devoid of arabinan and enriched in cellulose whereas the content in galacturonic acid has been only decreased in BEF. All these results are in agreement with previously published results (Bertin *et al.*, 1988).

Activities of enzyme preparations

The main enzymic activities of the commercial preparations were measured in order to have a better understanding of the degradation of

TABLE 2
Partially Methylated Alditol Acetates Obtained from WRF after Different Enzymic Treatments (Per cent of Neutral Polysaccharides)

Derivatives	*Controls after*		*After treatment with*		
	0 h	*120 h*	*SP249*	*Driselase*	*Onozuka*
3-Me 1 Rha[a]	2·6 (2·1)[b]	2·0 (2·5)	0·8 (1·1)	0·9 (0·8)	3·8 (3·9)
2.3.5.-Me 3 Ara	11·8	14·2	2·0	1·1	2·3
2.3.-Me 2 Ara	14·0	14·1	1·2	1·1	31·7
2.5.-Me 2 Ara	0·6	0·6	0·6	0·4	0·8
2.-Me 1 Ara	11·7	9·8	0·4	0·3	0·8
Ara	tr	tr	—	—	—
Total Arabinose	37·7 (40·6)	38·0 (43·8)	4·2 (6·9)	3·0 (5·2)	35·7 (28·2)
2.3.4.-Me 3 Xyl	0·8	0·6	0·5	0·2	0·8
2.3.-Me 2 Xyl	3·3	2·3	4·2	4·2	1·6
3.-Me 1 Xyl	—	—	0·7	0·7	—
Total Xylose	4·1 (3·2)	2·9 (3·2)	5·4 (8·9)	5·1 (6·9)	2·5 (3·5)
2.3.4.6.-Me 4 Man	tr	tr	tr	tr	tr
2.3.6.-Me 3 Man	2·4	2·2	3·9	0·8	1·4
Total Mannose	2·4 (2·1)	2·2 (1·9)	3·9 (3·7)	0·8 (0·6)	1·4 (2·1)
2.3.4.6.-Me 4 Gal	0·7	0·5	0·4	0·3	1·5
2.4.6.-Me 3 Gal	1·2	1·2	tr	tr	1·5
2.3.6.-Me 3 Gal	4·6	5·1	1·9	1·0	6·4
2.3.4.-Me 3 Gal	0·9	1·0	0·6	0·6	1·1
2.6.-Me 2 Gal	0·9	1·0	0·2	0·2	0·3
Total Galactose	7·6 (9·6)	8·6 (8·6)	3·0 (4·4)	2·0 (3·3)	10·8 (13·7)
2.3.4.6.-Me 4 Glc	0·3	0·3	1·1	2·7	0·4
2.3.6.-Me 3 Glc	40·9	41·8	75·2	80·0	40·3
2.6.-Me 2 Glc	0·5	0·5	1·3	0·9	0·8
3.6.-Me 2 Glc	1·5	1·5	2·5	0·5	1·9
2.3.-Me 2 Glc	2·1	2·1	2·5	3·8	2·3
Total Glucose	45·3 (42·2)	46·3 (39·5)	82·6 (75·9)	88·1 (81·9)	46·0 (48·1)

[a]3-Me 1 Rha denotes 3-mono-O-methyl-1,2,4-tri-O-acetyl rhamnitol.
[b]Values in parenthesis were obtained by direct alditol acetates analysis.

the beet fibres. SP249 was supplied as a liquid preparation whereas Driselase and Onozuka were powders. The protein contents are ~27% (w/w) and ~12·5% (w/w) for Onozuka and Driselase, respectively and SP249 contains 65 mg of proteins per ml of enzymic solution. The values of the different activities measured in the preparations are reported in Table 3. SP249 was highly active towards polygalacturonic acid, arabinan and arabinogalactan, and attacked also xylan and a

TABLE 3
Enzymic Activities[a] and Proteins in Commercial Preparations

	SP249[b]	*Driselase*	*Onozuka*
Polygalacturonase	270	2	5
Pectin-lyase	10	0	0
Pectate-lyase	< 1	0	0
Pectinesterase	87	0	0
CMCase	19	62	52
FPase	0	2	5
β-D-glucosidase	2	3	4
α-D-galactosidase	10	2	< 1
β-D-mannosidase	< 1	< 1	< 1
β-D-xylosidase	2	4	2
α-L-arabinofuranosidase	14	5	2
β-D-cellobiohydrolase	< 1	1	1
Activities towards:			
arabinan	173	36	14
arabinogalactan I	42	5	4
arabinogalactan II	9	2	3
xylan	4	66	192
laminarin	4	23	7
lichenan	7	57	74
Protease	97	84	72

[a]nkat/mg proteins with the exception of protease activity expressed in arbitrary units/g of proteins.
[b]Values of Massiot *et al.* (1989).

soluble derivative (CMC) of cellulose. However, it was not able to degrade crystalline cellulose since less than 10% of the substrate was solubilised during the FPase assay. Driselase contained all the cell-wall degrading enzymes tested including FPase but was very poor in pectolytic activities. Onozuka was especially active towards hemicelluloses (xylan) and cellulose; its pectinase activity was very low.

Degradation of WRF by SP249

The enzymic hydrolysis was carried out at 25°C in order to limit non-enzymatic degradation of the polysaccharides and especially β-elimination reactions of pectins, and the blanks showed that the non-enzymatic solubilisation was always less than 5% (w/w) of the fibre. For example, when WRF was stirred for 120 h in the actetate buffer, the

amount of solubilised material was ~3·5% of the initial sample. A methylation analysis of the insoluble fraction showed only minor differences in the structure of the polysaccharides as compared to WRF (cf. Table 2).

The evolution of the weight loss, total polysaccharides and individual monomers during the hydrolysis of WRF by SP249 is shown on Fig. 1. The total weight loss after 120 h was 72·5% (w/w) and the total polysaccharide solubilisation ~80%. Taking into account the aqueous extraction and the enzyme action, the cumulative weight loss and polysaccharide solubilisation were 79·4% and 82·2%, respectively. Rhamnose, arabinose, galactose and galacturonic acids, originating from pectic polymers, were quickly and almost totally removed by SP249 (average extent of hydrolysis = 95%); this suggests the presence of a pectin acetyl-esterase in the preparation. In contrast, cellulose (glucose) and hemicelluloses (xylose, mannose, glucose) were degraded more slowly and to a lower extent (40–60%) (Table 4). The average degree of polymerisation of the soluble products calculated from the determina-

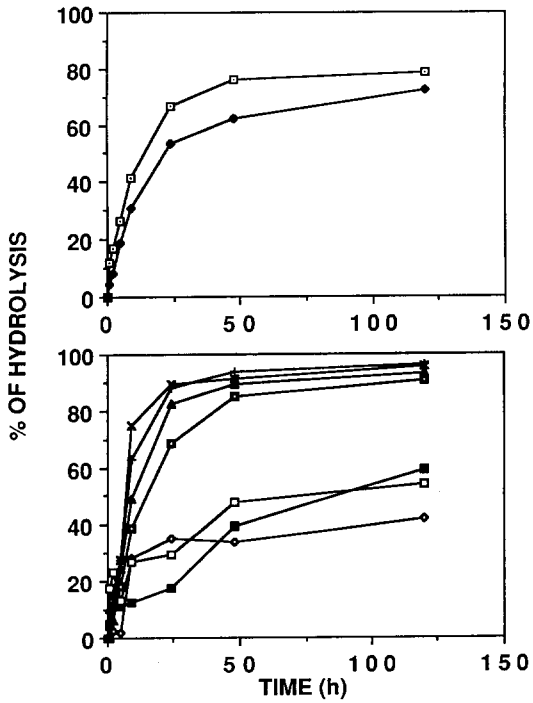

Fig. 1. Kinetics of hydrolysis of WRF by SP249 (⊡, total sugar hydrolysis; ◆, weight loss; ×, rhamnose; +, arabinose; □, galactose; ◇, xylose; ■, mannose; □, glucose; ▲, galacturonic acid).

TABLE 4
Extent of Polysaccharide Hydrolysis of WRF by Different Enzyme Preparations (Per cent of Initial Sugar Content)

	SP249	*Driselase*	*Onozuka*
Rhamnose	95·5	94·8	18·7
Arabinose	96·4	97·2	55·0
Xylose	42·0	55·1	43·4
Mannose	59·0	67·7	38·6
Galactose	90·6	92·6	9·9
Glucose	53·9	64·5	38·4
Galacturonic acid	93·3	95·1	37·0

TABLE 5
Sugar Composition (w/w) of the Residues Obtained by Different Enzymic Preparations

Anhydrosugars	*SP249*	*Driselase*	*Onozuka*
Rhamnose	0·3	0·3	1·8
Fucose	0·1	0·0	0·2
Arabinose	2·6	2·3	13·3
Xylose	3·5	3·7	1·7
Mannose	1·6	0·2	1·0
Galactose	1·4	1·5	6·5
Glucose	25·0	36·9	22·7
Uronic acids	5·1	4·8	21·6
Total	39·6	49·7	68·8

tion of both total and reducing sugars was 1·1. The predominant monomeric form of the products is probably due to the presence of glycosidase activities in SP249. (cf. Table 3).

Only ~ 40% of the residue left after 120 h hydrolysis was composed (Table 5) of polysaccharides; methylation analysis (Table 2) showed that cellulose was the major one and that the residue was enriched in xylose- and mannose-containing polymers. The proportion of arabinose was markedly decreased, especially the C_5-substituted residues, whereas a relative accumulation of C_3-substituted and terminal residues was observed. The appearance of doubly branched (C_2 and C_4) xylosyl residues and the decrease in terminal units may probably arise from the relative enrichment in xylans to the detriment of xyloglucans. An increase in terminal and 1,6-linked galactosyl residues with a decrease in 1,3-linked units was also noticed. In contrast, the distribution of the

different glucose residues was not significantly changed and more than 90% of the residues remained 1,4-linked. All these changes in the structure features are consistent with the presence in SP249 of arabinanase, arabinofuranosidase, galactanase and the absence of crystalline cellulose-degrading enzymes.

Hydrolysis of WRF by other enzymic preparations

The same experiments were carried out using Driselase and Onozuka. After 120 h, the weight losses were 77·2% and 37·5% (80·9% and 47·7% from RF), and the average polysaccharide hydrolyses were 84·1% and 38·3% (84% and 48·3% from RF) with Driselase and Onozuka, respectively. The extent of hydrolysis of each polysaccharide type is reported in Table 4. The solubilisation of pectic substances (galacturonic acid, rhamnose, arabinose, galactose) by Driselase is almost complete while hemicelluloses and cellulose are more resistant but more degraded than by SP249. The degradation of all the polysaccharides of WRF by Onozuka is very limited; pectic substances, cellulose and galactan are poorly degraded by this preparation. Explanations could be found in the low level of arabinan-degrading activity and/or in the absence of acetylesterase in this preparation. The average degree of polymerisation of the soluble products was found to be 1·0 with Driselase and 1·4 with Onozuka.

The composition and the methylation analysis of the residues (Table 5) showed that the action of Driselase left material similar in nature to that obtained by SP249. With Onozuka, the structure of the residual glucose- and galactose-containing polymers was unchanged in contrast to the arabinose- and xylose-containing molecules: ~90% of the arabinosyl units were found to be 1,5-linked, while terminal and doubly branched units content decreased markedly and terminal xylosyl residues markedly increased (Tables 2 and 5). This was ascribed to low arabinanase and high xylanase activities in this preparation.

When SP249 and Onozuka were used in admixture, 83·7% weight loss of WRF (86·3% from RF) was observed and nearly all the polysaccharides were solubilised. In particular, cellulose was hydrolysed to more than 90%.

Hydrolysis of OEF, AEF and BEF by SP249 and influence of the drying

OEF, AEF and BEF fibres have been kept either as dry powders or as (never-dried) aqueous slurries. Both forms were used in the following experiments (Table 6). OEF was solubilised roughly to the same extent as WRF due to their similar structures while AEF and BEF were less

TABLE 6
Extent of Weight Loss, Total Polysaccharides Hydrolysis and Monosaccharide Solubilisation after Action of SP249 on Different Fibres

	OEF		*AEF*		*BEF*	
	Dry	*Wet*	*Dry*	*Wet*	*Dry*	*Wet*
Weight loss (%)	75·3	74·7	56·6	57·5	40·9	44·3
Total polysaccharides (%)	80·5	77·6	59·3	59·3	40·5	43·9
Monosaccharide solubilisation (%)						
Rhamnose	85·3	84·7	71·9	77·7	58·7	62·0
Arabinose	98·1	97·8	83·6	82·6	75·1	74·5
Xylose	33·1	24·1	24·3	21·8	34·1	28·4
Mannose	46·1	44·6	16·5	22·6	24·6	21·9
Galactose	92·0	91·1	86·7	87·1	78·9	82·5
Glucose	49·9	41·4	40·6	40·9	28·5	33·2
Galacturonic acid	95·9	95·4	93·7	93·4	84·6	87·4

degraded. The sugar analysis of the residues showed that the decrease in degradation from OEF to BEF concerned all the monosaccharides, except xylose. However, galacturonic acid, arabinose and galactose remained very degradable even in the last fractions where their content was reduced. On the other hand, cellulose hydrolysis decreased regularly although its concentration was increased from WRF to BEF. The cumulative extents of solubilisation, taking into account both chemical (water, ammonium oxalate, hydrochloric acid, sodium hydroxide) and enzyme treatment are in the range 75–80%; the solubilisation of polysaccharide varied between 80 and 85%. The results show also that the drying had only a slight effect on the degradability of the fibres (Table 6).

Finally, 20–25% of the dry matter and 15–20% of the polysaccharides of the sugar beet fibres were not solubilised in these experiments. This may be ascribed to the presence of a highly crystalline cellulose fraction and/or polyphenolic material in the residues (Massiot *et al.*, 1989). Therefore, some oxidative pretreatments preceding the enzymic hydrolysis were carried out with the aim of increasing the extent of solubilisation.

Oxidative pretreatments and hydrolysis of beet fibres

Ammonium persulphate

The residue of SP249-hydrolysed WRF was first treated with 0·02 M ammonium persulphate then hydrolysed again by SP249. Ammonium persulphate caused by itself only a small weight loss (< 1%) and a more

appreciable (~ 6%) degradation of the polysaccharides. All the sugars were involved except glucose and xylose which accumulated (data not shown). Ammonium persulphate promoted an additional enzymic degradability of the residue which affected particularly mannose, xylose and glucose. The latter sugars were hydrolysed to 73·2%, 55·9% and 77·4%, respectively. The total weight loss and polysaccharide degradation increased therefore to 77·4% and 88·6%, respectively.

In other experiments, 0·02 M and 0·04 M ammonium persulphate were used directly on RF with yields of pretreated fibres of 79·6% and 80·0%,

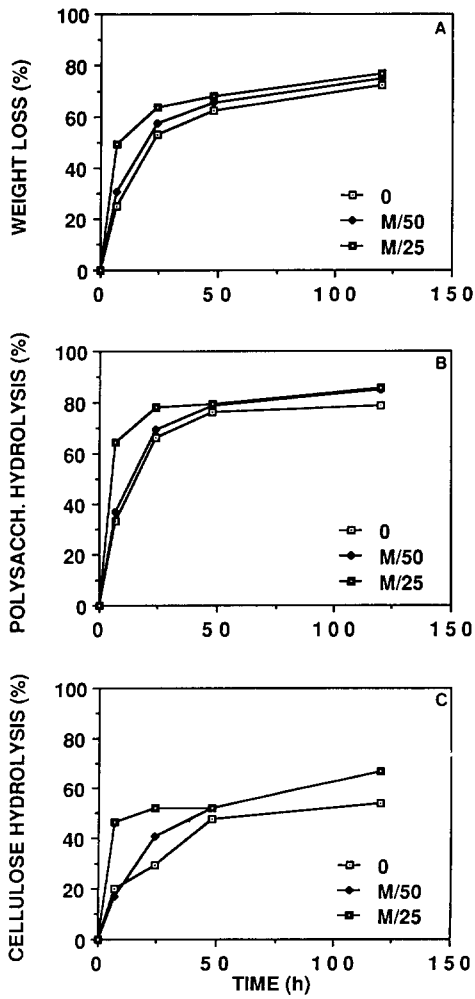

Fig. 2. Effects of ammonium persulphate concentration on weight loss (A), polysaccharide hydrolysis (B) and cellulose hydrolysis (C).

respectively. The material removed by persulphate was roughly similar in nature to the water-soluble one since the persulphate-treated fibres had a sugar composition very close to that of WRF (Table 7). After extensive action of SP249 (Fig. 2), weight losses from initial and 0·02 M or 0·04 M persulphate-treated fibres were ~75% and the total polysaccharide hydrolysis was similar (~85%) for the two application rates but higher than for the control (~80%). A more marked effect was observed for the cellulose hydrolysis and values of ~54%, ~67% and ~67% were obtained with untreated, 0·02 M persulphate-treated and 0·04 M persulphate-treated fibres, respectively. Moreover, the rate of degradation, particularly the polysaccharides hydrolysis, was increased after the persulphate action. Although a concentration of 0·02 M persulphate caused only a slight increase in hydrolysis rates, the effect was more pronounced with 0·04 M concentration since 7 h after enzyme addition, the weight loss, the total polysaccharides hydrolysis and cellulose hydrolysis were roughly twice that of the control. The cumulated cellulose hydroysis values were about 75% of the total cellulose.

TABLE 7
Effect of Various Pretreatments on Yield and Composition of the Fibres

Treatments	*Persulphate*		*Delignification process*		H_2O_2, *3 h*
	0·02 M	*0·04 M*	*Hot acid*	*Hot acid + $NaClO_2$ (10%)*	
Yield	79·6	80·0	58·7	50·6	65·0
Rhamnose + fucose	1·1	1:0	1·2	1·6	1·1
Arabinose	16·7	17·2	9·7	10·0	18·8
Xylose	1·6	1·5	2·4	2·8	1·4
Mannose	1·0	1·0	2·0	2·2	1·2
Galactose	4·3	4·2	6·5	5·7	4·6
Glucose	18·6	19·2	25·6	31·4	22·1
Galacturonic acid	20·5	20·0	24·4	20·1	12·5

Sodium chlorite

Treatments with sodium chlorite were carried out on RF in hot dilute acid as usually done in the delignification process. Preliminary experiments with sodium chlorite from 0·5 to 100% (w/w of sample) have shown that a rate of 10% gave white fibres exhibiting the highest water swelling (~75 ml/g). The specific effects of the treatment can be seen in Table 7. The hot acid treatment removed more than 40% of RF by

solubilising mainly arabinose (66% loss) and to a lesser extent galacturonic acid (~28%) and glucose (~30%). Sodium chlorite led to an additional weight loss so that the residue accounted for only half of RF and finally 43% of the initial polysaccharides had been lost. However, this residue was richer in polysaccharides (73·7%) than the other fractions. During the treatment, galacturonic acid was especially removed (total loss ~48%) but also galactose (total loss ~26%) whereas the contents of the other sugars were only very slightly modified.

When SP249 is applied for 120 h to the chlorite-pretreated fibre, extents of weight loss, total polysaccharide hydrolysis and cellulose hydrolysis were lower than or equal to the corresponding values obtained with control RF (68·7%, 77·4% and 53·8% versus 73·0%, 78·8% and 53·9%, respectively). However, cumulated values taking into account pretreatment and enzymic solubilisation were higher for the pretreated fibres: 84·2%, 88·6% and 76·6% versus 79·4%, 82·2% and 61·4%, respectively.

Hydrogen peroxide

In preliminary experiments, different reaction times (0–6 h) of 1% hydrogen peroxide at pH 11·5 were tested on RF at room temperature. A reaction time of 3 h was chosen because it furnished white fluffy fibres, exhibiting high swelling properties (~50 ml/g), with a yield of 65·0%. The effect of alkaline hydrogen peroxide on polysaccharide composition is shown in Table 7. The composition in neutral sugars of the residue was similar to that of RF and WRF but 60% of the galacturonic acid content was removed by the treatment, certainly because of β-elimination reactions.

SP249 was added to the H_2O_2-pretreated fibre and the hydrolysis course is characterised by a very rapid degradation during the first 10 h and then a slight increase up to 120 h. The weight loss, the polysaccharide hydrolysis, and the cellulose hydrolysis were 80, 83 and 60%, respectively; on a cumulated basis, these values were respectively ~87, 89 and 74%.

CONCLUSIONS

The results show clearly that sugar beet fibres can be easily degraded up to 80% by enzymic preparations rich in cell-wall degrading enzymes. The pectic polymers are solubilised to a very large extent by SP249 or Driselase whereas cellulose degradation was 90% when SP249 was used in admixture with Onozuka. The major residual material is apparently a

highly crystalline cellulose fraction which may be attacked by cellulases after chemical treatments such as persulphate, chlorite or hydrogen peroxide. Sugar beet fibre is a substrate with a low lignin content giving a relatively high yield in cellulose hydrolysis. It is well known that materials with a high lignin content are much less degraded by commercial enzymes and chemical or mechanical pretreatments have to be used to increase the extent of degradation.

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