Carbohydrate Research, 183 (1988) 311–319
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

CHANGES IN COMPOSITION AND STRUCTURE OF WHEAT BRAN RESULTING FROM THE ACTION OF HUMAN FAECAL BACTERIA *in vitro*

BARRY J. H. STEVENS AND ROBERT R. SELVENDRAN
AFRC Food Research Institute, Colney Lane, Norwich NR4 7UA (Great Britain)
(Received February 29th, 1988; accepted for publication, May 17th, 1988)

ABSTRACT

Cell-wall material of wheat bran was incubated with human faecal bacteria for 24–72 h and the resulting structural changes were studied by methylation analysis. Of the carbohydrate content, ~39% was degraded after 24 h, increasing to only 44% after 72 h. Arabinoxylans and mixed-linkage β-D-glucans from the aleurone layer were degraded preferentially. After treatment of the bran with alkali, the extent of degradation was increased three-fold as a result of saponification of ester cross-links which facilitated increased degradation of the polymers from both the aleurone and outer, lignified, layers. There was evidence that ester linkages between the glucuronosyl residues, attached to O-2 of the (1→4)-linked xylosyl residues, and phenolic groups of lignin were also saponified. The treatment with alkali also rendered the cellulose more susceptible to bacterial attack. The alkali-soluble acidic arabinoxylan fractions of the bran were degraded readily by bacterial action, but the xyloglucans cross-linked to arabinoxylans by phenolics were relatively resistant.

INTRODUCTION

The extent to which intestinal bacteria can degrade dietary fibre is important when studying its physiological effects and the metabolism of the intestinal microflora. An established property of dietary fibre is the capacity to increase faecal bulk[1,2], and this is dependent on the polymeric composition and solubility characteristics of the fibre[3,4]. Increase in faecal bulk is achieved by a combination of three factors: (*a*) increased mass from undegraded fibre, (*b*) increased bacterial mass, and (*c*) water retained by these components. The ability of bacteria to utilise complex substrates has been extensively studied, but few studies have been made of the detailed composition and structural features of the undegraded residues[5,6]. Such studies *in vivo* are difficult, largely because of interference from other components in the diet. *In vitro* systems also present problems of simulating conditions in the large intestine and in maintaining the natural composition of the microflora, but they do have the advantage that single, relatively pure substrates can be used, which facilitate chemical studies[7]. Wheat bran is a widely used source of dietary

0008-6215/88/$ 03.50

fibre which has a large faecal bulking capacity because it undergoes little degradation in the human alimentary tract[8] and, by virtue of its physical structure, has appreciable water-holding capacity[9].

The results of preliminary experiments on the degradation of apple and wheat bran cell-wall material (CWM) have been reported[10]. We now report on the structural features of wheat-bran polysaccharides that influence bacterial degradation.

EXPERIMENTAL

Wheat-bran CWM. — Wheat-bran CWM was prepared from commercial wheat bran by ball-milling with aqueous 1% sodium deoxycholate, followed by treatment in sequence[11] with phenol–acetic acid–water (2:1:1 w/v/v) and aqueous 90% methyl sulphoxide followed by dialysis.

Treatment with alkali. — A suspension of finely ground wheat-bran CWM (10 g) in oxygen-free distilled H_2O (375 mL) was stirred overnight at 1°, an equal volume of 2M KOH containing 20mM $NaBH_4$ was added, and stirring was continued for 2 h under Ar. Glacial acetic acid was then added to adjust the pH to ~5.5, and the suspension was dialysed extensively against H_2O at 1° and then freeze-dried.

Extraction with alkali. — Finely ground wheat-bran CWM was hydrated in the cold as described above and then extracted at 1°, under oxygen-free conditions, with M and 4M KOH, to leave a residue of α-cellulose[12].

Preparation of inocula and fermentation conditions. — The preparation of inocula from the faeces of volunteers, the media, and the fermentation conditions have been described[10]. In order to avoid autohydrolysis, the substrates were not sterilised prior to inoculation.

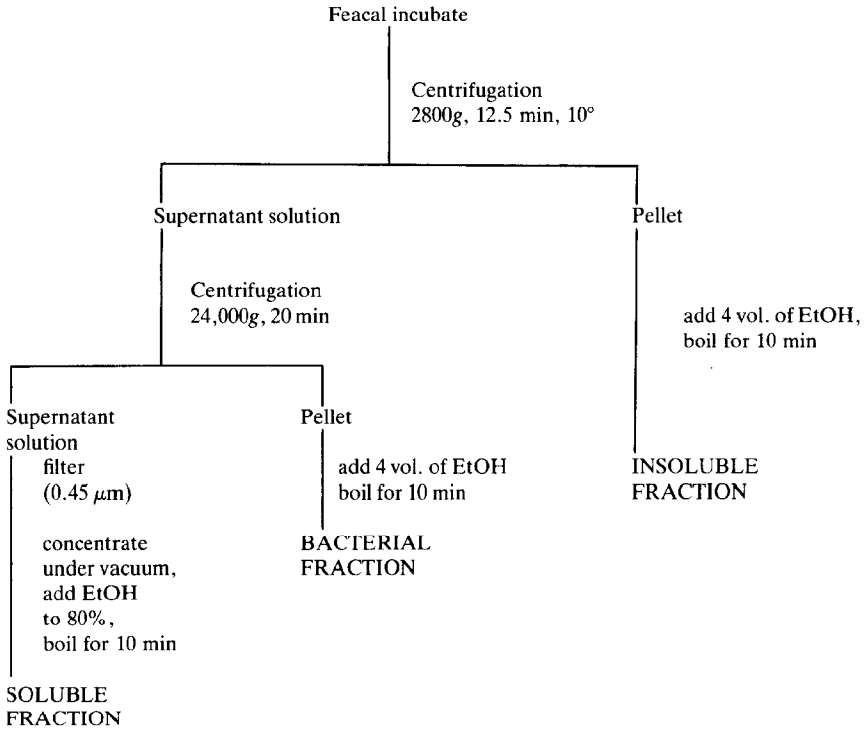
Isolation of the degraded substrates. — After incubation, the cultures were separated into substrate and bacterial fractions (Scheme 1).

Treatment with phenol. — In some experiments, the insoluble substrate (Scheme 1) was treated[10] with hot phenol–sodium dodecyl sulphate to partially remove bacterial protein.

Methods of analysis. — Neutral sugars were released by Saeman hydrolysis and determined[13] as their alditol acetates by g.l.c. Uronic acid was determined by a modification[13] of the method of Blumenkrantz and Asboe-Hansen[14]. Total nitrogen was determined as N_2 with a Carlo-Erba Model 1400 automatic nitrogen analyser.

Methylation analysis. — Polysaccharides were methylated by a modification of the Hakomori method and converted[11] into partially methylated alditol acetates (PMAA). G.l.c. was performed with a Carlo-Erba 4160 gas chromatograph, using on-column injection with a capillary column (10 m × 0.32 mm) of CP-Sil 43 (Chrompack). A packed column of ECNSS-M in a Pye 104 gas chromatograph was used to separate the (1→2)- and (1→4)-linked xylose derivatives from the terminal galactose derivative.

Scanning electron microscopy (SEM). — SEM of freeze-dried insoluble-residue fractions was carried out as described[10].



Scheme 1. Separation of substrate and bacteria after incubation with a faecal inoculum.

RESULTS AND DISCUSSION

Untreated wheat-bran CWM. — In the initial experiments, incubation for 24 h resulted in utilisation, by the bacteria, of 39% of the carbohydrate content of the insoluble fraction. After 72 h, the loss of carbohydrate increased to only 44%. SEM showed that, after incubation for 24 h, the aleurone layer was degraded preferentially[10]. This observation is supported by the ratios of the decrease in arabinose to that of xylose. From Table I, it can be seen that, after incubation for 24 h, the decreases in the contents of arabinose and xylose were 52.9 and 161.0 mg, respectively, which gives an arabinose:xylose ratio of 0.37:1. This is approximately the same as the weight-ratio (0.35:1) in the aleurone cell wall[15]. Likewise, after 72 h, the decreases in arabinose and xylose were 59.2 and 180.4 mg, respectively, which gives a ratio of 0.33:1. However, the decrease in glucose was relatively less than would have been expected if degradation of the aleurone cell walls had been complete. This finding suggests that mainly the arabinoxylans of the aleurone layer were degraded, although some degradation of the glucan also occurred.

TABLE I

COMPOSITION AND EXTENT OF DEGRADATION OF UNTREATED AND ALKALI-TREATED CELL-WALL MATERIAL OF WHEAT BRAN BY COLONIC BACTERIA

Treatment	*Incubation time (h)*	*Substrate fraction*	*Substrate recovered (g/g of initial substrate)*	*Protein (%)*[a]	*Total sugars recovered (mg/g dry wt. of initial substrate)*								*Carbohydrate degraded (%)*[b]
					Deoxy hexose	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total*	
Untreated	0[c]	residue	0.885	6.0	4.2	175.5	286.3	7.5	12.2	195.3	63	744.0	
Untreated	0	S/N[d] soluble	0.080	n.d.[e]	0.5	3.3	2.1	1.3	1.9	7.1	2	18.2	
Untreated	24[c,f]	residue	0.703	17.6	5.2	122.6	125.3	3.9	9.8	137.0	47	450.8	39.4
Untreated	40	residue	0.773	13.1	4.2	160.8	146.7	4.6	13.8	170.6	43	543.7	28.1
Untreated	40	S/N soluble	0.034	n.d.	1.1	0.2	0.2	0.1	0.6	1.1	1	4.2	
Untreated	72[c,f]	residue	0.653	15.0	7.4	116.3	105.9	3.7	9.7	126.7	48	417.7	43.9
Alkali-treated	0	residue	0.870	n.d.	1.9	182.2	319.5	3.6	10.2	210.7	39	767.1	
Alkali-treated	40	residue	0.298	52.6	4.9	17.1	11.3	1.2	3.4	26.4	6	65.4	91.5
Alkali-treated	92	residue	0.295	44	5.6	12.8	10.9	1.2	3.8	17.6	6	57.9	92.5
M KOH-soluble	0	residue	0.890	n.d.	1.6	266.1	561.6	2.1	9.9	59.2	20	920.5	
M KOH-soluble	40	residue	0.142	62.4	2.9	2.7	0.6	0.5	1.7	8.0	2	18.4	98.0
4M KOH-soluble	0	residue	0.878	n.d.	2.1	220.1	375.7	5.4	20.1	115.7	44	783.1	
4M KOH-soluble	40	residue	0.286	62.2	4.8	1.3	0.5	0.7	2.8	14.1	1	24.2	96.9
α-Cellulose residue	0	residue	0.980	n.d.	2.4	161.7	152.3	6.1	8.8	409.5	40	780.8	
α-Cellulose residue	40	residue	0.521	31.8	5.8	24.7	24.6	1.4	5.7	95.9	5	163.1	79.1

[a]N × 6.25. [b]Values are not corrected for possible contributions from sugars of bacterial origin. [c]Results for these have been reported in ref. 10. [d]Supernatant. [e]Not determined. [f]Inocula for 24 and 72 h were the same, but different from the remainder of the incubations.

The main linkages in the aleurone arabinoxylans and β-D-glucans are (in decreasing order of magnitude): (1→4)-Xyl*p*, (1→4)-Glc*p*, terminal Ara*f*, (1→2,4)- and (1→3,4)-Xyl*p*, and (1→3)-Glc*p*[13]. Methylation analysis of the bran CWM before, and after, bacterial attack (Table II) showed that the same types of arabinose and xylose residues as in the aleurone layer were the most depleted. The proportion of (1→3)-linked glucose in the undegraded CWM was relatively low, but none was detected after bacterial action. The increase in the proportion of (1→4)-linked glucose is largely due to the decrease in the proportions of arabinose and xylose, and the results in Table I show that some decrease had occurred. It should be borne in mind that most of the glucose in Tables I and II is from the cellulose present in the lignified layers. The proportions of terminal xylose and (1→3)-linked arabinose increased, which supports the finding with SEM that the lignified outer layers are resistant to attack, because these linkages are present in appreciable proportions in the lignified outer (beeswing-bran) layers[11] but at only low levels in the aleurone layer[15]. After 72 h and, with a different source of inoculum, after 40 h, the proportion of (1→4)-linked xylosyl residues was markedly lower, which indicated that the xylans in the beeswing-bran layer were also attacked, although the xyloglucan present[16] was relatively resistant as shown by the increased proportion of (1→4,6)-linked glucosyl residues.

After methylation, ~10% of the material remained insoluble in $CHCl_3/CH_3OH$ and the samples were re-methylated. With the possible exception of the terminal xylose residues, which decreased during incubation, the compositions remained similar; coupled with the resistance to methylation, this suggests that these are highly cross-linked polymers, rich in terminal arabinose and associated with lignin, which are resistant to bacterial attack.

The bulk of the deoxyhexose content of the degraded bran is rhamnose, which increased proportionally with incubation time and extent of degradation. Because the pectin content of bran CWM is very low[16], the rhamnose was probably derived from bacterial polysaccharides. The low levels of (1→3)- and (1→4)-linked glucosyl residues in the bacterially degraded fractions could also have been derived from bacterial exopolysaccharides.

The possibility that soluble oligomeric or polysaccharide fragments resistant to further degradation might be released was investigated by examining the dialysable and non-dialysable portions of the soluble substrate fraction. As reported previously[10], examination of the oligomeric fraction proved to be difficult owing to the presence of a large amount of amino acid-containing products. The compositions of some of the polymeric fractions in the supernatant solutions are given in Table I, and the results of methylation analysis of the supernatant fractions from the initial, undegraded CWM and after incubation for 40 h are given in Table II (columns 8 and 9). The carbohydrate contents of these fractions were very low (Table I) and were comparable to that of the material solubilised by 0.05M NaOH from CWM of beeswing wheat bran[16].

Alkali-treated bran. — Treatment of the wheat-bran CWM with M KOH, in

TABLE II

PARTIALLY METHYLATED ALDITOL ACETATES FROM POLYSACCHARIDES OF WHEAT-BRAN SUBSTRATES[a] BEFORE AND AFTER DEGRADATION BY COLONIC BACTERIA

Alditol acetates	*Relative mol %*[b]								
	Bran CWM (0 h)		*Bran CWM 24 h*	*Bran CWM 40 h*	*Bran CWM 72 h*	*Re-methylated*		*Supernatant fraction*[d]	
	H_2O washed	*Phenol extracted*				*Bran CWM 40 h*	*Bran CWM 72 h*	*0 h*	*40 h*
2,3,4-Me_3-Fuc[e]						5.0		2.9	2.3
2,3,4-Me_3-Rha									5.0
3,4-Me_2-Rha									5.6
2,4-Me_2-Rha									8.0
4-Me-Rha									4.8
2,3,5-Me_3-Ara	13.4	14.0	9.1	11.3	12.1	19.4	20.2	8.6	4.4
3,5-Me_2-Ara	0.7	0.8	1.4	2.1	2.2	3.2	4.0		1.2
2,5-Me_2-Ara	0.6	1.7	5.1	3.4	4.2	9.9	6.7		
2,3-Me_2-Ara	1.2	0.9	1.4	1.3	1.2	0.9	1.3	2.4	
5-Me-Ara	1.1	1.0	2.0	2.7	3.0	5.4	5.8		2.4
2-Me-Ara			1.3						
Arabinitol								0.6	1.7
2,3,4-Me_3-Xyl	0.7	2.0	4.3	4.4	4.3	6.2	6.9		0.9
3,4-Me_2-Xyl	1.9		Tr[f]	0.4		0.5	1.0		0.4
2,3-Me_2-Xyl	35.0	34.9	14.7	5.9	5.8	5.4	4.9	14.0	1.3
3-Me-Xyl		7.1[g]	5.1	5.0	5.6	Tr	9.9		
2-Me-Xyl	7.6					7.9			
Xylitol	2.0	2.6	3.2	3.7	3.0	7.7	8.8	3.2	2.8
2,3,4,6-Me_4-Gal						1.8	1.0		3.6
2,4,6-Me_3-Gal								2.3	5.6
2,3,6-Me_3-Gal								4.1	
2,3,4-Me_3-Gal									1.2
2,6-Me_2-Gal									1.6
2,3,4,6-Me_4-Glc								8.0	8.8
2,4,6-Me_3-Glc	2.5	2.0						5.7	8.5
2,3,6-Me_3-Glc	25.3	28.1	33.7	47.2	48.7	22.8	25.8	35.6	8.3
2,3,4-Me_3-Glc									5.8
2,6-Me_3-Glc	0.7	0.7	1.4						1.5
2,3-Me_2-Glc	1.3	2.1	5.1	5.2	4.1	0.9		3.4	1.7

[a]Phenol-extracted insoluble-residue fractions unless otherwise stated. [b]Minor proportions of other PMAA were also present. [c]Re-methylation of residue insoluble in $CHCl_3$/CH_3OH after first methylation. [d]Supernatant fraction (Scheme 1) soluble in aqueous 80% ethanol. [e]2,3,4-Me_3-Fuc = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylfucitol, *etc.* [f]Trace. [g]Mostly 3-Me-Xyl.

order to break phenolic ester linkages, resulted in a marked increase in degradability (Table I). With the same inoculum, extending the incubation time from 40 to 92 h produced no further overall degradation. It is clear from the compositions of the bacterially degraded substrates (Table I) that, in addition to arabinoxylans from the aleurone and lignified layers of the bran, an appreciable proportion of the cellulose was also degraded.

During the preparation of the alkali-treated bran, a small proportion of the low-molecular-weight arabinoxylans may have been lost during dialysis, together with the ferulic and *p*-coumaric acids. Studies with cell-wall polymers of beeswing wheat bran have shown that small but significant proportions of the arabinoxylans, presumably of low molecular weight, are soluble in aqueous 90% ethanol and some of this material may be lost on prolonged dialysis.

Comparison of the results of methylation analysis of the undegraded insoluble substrate fraction (Table III, column 1) with those of the bacterially degraded fractions (Table III, columns 2 and 3) shows appreciable losses of terminal arabinofuranosyl, (1→4)-linked xylopyranosyl, and (1→2,4)- and (1→3,4)-linked xylopyranosyl residues, with smaller losses of (1→3)- and (1→2,3)-linked arabinofuranosyl and (1→2,3,4)-linked xylopyranosyl residues. Although a large proportion of the cellulose was degraded, the relative increase in the proportion of (1→4)-linked glucosyl residues is due to the marked decrease in the variously linked xylosyl and arabinosyl residues. Interestingly, a small proportion of the (1→4,6)-linked glucosyl residues survived bacterial action. These residues were probably constitutents of complexes in which the xyloglucans are covalently linked to arabinoxylans, probably *via* phenolic cross-links. Most, if not all, of the xyloglucan in beeswing wheat bran exists in the form of these complexes (see ref. 16, Table VII, columns 4–6) which are not readily water-soluble.

The most notable feature of the above results is the marked decrease in the proportion of (1→2,4)-linked xylosyl residues. This result indicates that the glucuronosyl and 4-*O*-methylglucuronosyl residues, which are linked to O-2 of the (1→4)-linked xylosyl residues[11,16], are probably ester cross-linked to phenolic groups of lignin[17,18] and hydrolysis of the ester cross-links with alkali rendered the cross-linked arabinoxylans more degradable by the bacteria. This inference is corroborated by the results for the M and 4M KOH-soluble polymers which, after bacterial degradation, contained no detectable (1→2,4)-linked xylosyl residues (Table III columns 5 and 7).

M *KOH-soluble fraction.* — This fraction was rich in arabinoxylans, contained some mixed linkage β-D-glucans from the aleurone layer[15], and a small proportion of cross-linked xyloglucan from the beeswing-bran layer[16]. Most of the arabinoxylans were degraded by bacterial action but a small proportion of the xyloglucan was resistant. The considerable increase in the proportion of (1→4)-linked glucosyl residues can be accounted for by the decrease in the proportions of arabinosyl and xylosyl residues, offset by the increase in rhamnosyl residues. The appreciable proportion of variously linked rhamnosyl residues in the degraded material was

TABLE III

PARTIALLY METHYLATED ALDITOL ACETATES FROM POLYSACCHARIDES OF ALKALI-TREATED WHEAT-BRAN SUBSTRATES[a] BEFORE AND AFTER DEGRADATION BY COLONIC BACTERIA

Alditol acetates	*Relative mol %*[b]						
	Alkali-treated			M *KOH-soluble*		4M *KOH-soluble*	
	0 h	*40 h*	*92 h*	*0 h*	*40 h*	*0 h*	*40 h*
2,3,4-Me_3-Rha[c]					1.5		
3,4-Me_2-Rha		3.6			4.6		1.7
2,4-Me_2-Rha		3.3	3.0		8.1		4.4
4-Me-Rha		1.0			1.3		
2,3,5-Me_3Ara	15.6	11.1	6.7	19.7	4.8	17.7	1.3
3,5-Me_2-Ara	1.8	2.2	1.0	3.1		2.9	0.2
2,5-Me_2-Ara	3.7	1.7		4.8	Tr	6.3	
2,3-Me_2-Ara	1.0	1.8	0.6	2.3		1.0	
5-Me-Ara	4.0	Tr[d]		2.5		5.1	
Arabinitol	2.9		4.0			1.3	0.6
2,3,4-Me_3-Xyl	2.7	2.3	1.1	4.1		4.4	0.6
3,4-Me_2-Xyl		0.8					
2,3-Me_2-Xyl	23.0	9.9	13.0	39.3	1.9	23.8	1.8
3-Me-Xyl	10.5[e]	5.0[e]	Tr	11.3[e]		12.6[e]	
2-Me-Xyl							
Xylitol	6.1	1.5	3.0	6.1		1.3	
2,3,4,6-Me_4-Glc		3.3	1.3		9.9		7.0
2,4,6-Me_3-Glc		2.7	1.7	1.8	4.0		4.2
2,3,6-Me_3-Glc	26.9	40.8	39.3	4.4	38.8	13.2	47.2
2,6-Me_2-Glc			1.9				6.1
2,3-Me_2-Glc	1.8	3.1	2.9	0.4	3.0	3.8	5.9
Glucitol		1.3	15.8		1.3[f]		10.8

[a]Insoluble substrate residue fraction. [b]Minor proportions of other residues were also present. [c]2,3,4-Me_3-Rha = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol, *etc.* [d]Tr = Trace. [e]Mostly 3-Me-Xyl. [f]Galactitol, 14.0 mol%, was also present.

probably derived from bacterial exopolysaccharides. The terminal glucosyl residues in the degraded material may also have been of bacterial origin.

4M KOH-soluble fraction. — This fraction was also rich in arabinoxylans but contained a larger proportion of xyloglucan than the M KOH-soluble fraction. As with the M KOH-soluble fraction, most of the arabinoxylans were degraded but an appreciable proportion of the xyloglucan survived bacterial action. There is also evidence for the presence of bacterial exopolymers containing rhamnosyl and glucosyl residues.

α-Cellulose residue. — The residue remaining after extraction with alkali was incubated with a portion of the same inoculum used for fermenting the alkali-soluble fractions. After 40 h, ~80% of the cellulose and associated arabinoxylans were degraded (Table I). The action of the alkali, in addition to breaking ester

cross-linkages, probably aided bacterial attack by causing swelling of the denser regions of the cellulose complex, thereby facilitating penetration by the bacteria.

The following conclusions can be drawn. (*a*) When the CWM of wheat bran is exposed to faecal bacteria, the arabinoxylans and mixed-linkage β-D-glucans of the aleurone layer are preferentially degraded. In the untreated cell walls, cellulose is fairly resistant to bacterial action. (*b*) Treatment of the cell walls with alkali hydrolyses ester cross-links, which facilitates a significant increase in the degradation of the cell-wall polymers from both the aleurone and the lignified outer layers of the bran. There is evidence that the glucuronic acid and 4-*O*-methylglucuronic acid, which are linked to O-2 of the (1→4)-linked xylosyl residues, are ester cross-linked to phenolic groups of lignin and that hydrolysis of these cross-links considerably facilitates the bacterial degradation of the highly branched arabinoxylans. (*c*) Treatment of the bran with alkali renders the cellulose much more susceptible to bacterial attack, but the small proportions of cross-linked xyloglucans are less susceptible. (*d*) The alkali-soluble arabinoxylans are readily degraded by bacteria, but the cross-linked xyloglucans are resistant. (*e*) The faecal bacteria produce small amounts of exopolysaccharides containing variously linked rhamnosyl and glucosyl residues.

ACKNOWLEDGMENTS

This study was funded by the Ministry of Agriculture, Fisheries and Food. The authors thank Mrs. C. Bayliss, Miss N. Horn, and Miss K. A. Hamand for carrying out the fermentations and initial separations, Mr. J. Eagles and Mr. K. Parsley for performing the mass spectrometry, Mr. R. Turner for the electron microscopy, and Miss S. E. King for skilled technical assistance.

REFERENCES

1 M. A. Eastwood and W. G. Brydon, in M. Trowell, D. Burkitt, and K. Heaton (Eds.), *Dietary Fibre, Fibre-depleted Foods and Disease,* Academic Press, London, 1985, pp. 105–131.
2 J. L. Kelsay, *Am. J. Clin. Nutr.*, 31 (1978) 142–159.
3 R. R. Selvendran, *Am. J. Clin. Nutr.*, 39 (1984) 320–337.
4 R. R. Selvendran, B. J. H. Stevens, and M. S. Dupont, *Adv. Food Res.*, 31 (1987) 117–209.
5 A. A. Salyers, R. Arthur, and A. Kuritza, *J. Agric. Food Chem.*, 29 (1981) 475–480.
6 A. A. Salyers, F. Gherardini, and M. O'Brien, *Appl. Environ. Microbiol.*, 41 (1981) 1065–1068.
7 T. L. Miller and M. J. Wolin, *Appl. Environ. Microbiol.*, 42 (1981) 400–407.
8 A. M. Stephen and J. H. Cummings, *Nature (London)*, 284 (1980) 283–284.
9 J. A. Robertson and M. A. Eastwood, *Br. J. Nutr.*, 45 (1981) 83–88.
10 B. J. H. Stevens, R. R. Selvendran, C. E. Bayliss, and R. Turner, *J. Sci. Food Agric.*, 44 (1988) 151–166.
11 S. G. Ring and R. R. Selvendran, *Phytochemistry*, 19 (1980) 1723–1730.
12 S. G. Ring and R. R. Slevendran, *Phytochemistry*, 20 (1981) 2511–2519.
13 R. R. Selvendran and M. S. Dupont, in R. D. King (Ed.), *Developments in Food Analysis Techniques*, Vol. 3, Elsevier Applied Science, London, 1984, pp. 1–68.
14 N. Blumenkrantz and G. Asboe-Hansen, *Anal. Biochem.*, 54 (1973) 484–489.
15 A. Bacic and B. A. Stone, *Aust. J. Plant Physiol.*, 8 (1981) 475–495.
16 M. S. Dupont and R. R. Selvendran, *Carbohydr. Res.*, 163 (1987) 99–113.
17 N. N. Das, S. C. Das, A. S. Dutt, and A. Roy, *Carbohydr. Res.*, 94 (1981) 73–82.
18 O. Eriksson, D. A. I. Goring, and B. O. Lindgren, *Wood Sci. Technol.*, 14 (1980) 267–279.