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Abstract

To determine the role of physico-chemical characteristics (gross porosity, microporosity, particle size, crystallinity) of dietary fibres rich in primary cell walls in their degradability by human flora, two sources of sugar beet fibres were submitted to various chemical and then dehydration treatments. Chemical extraction involving hot dilute acid followed by cold dilute alkali treatments resulted mainly in the removal of pectic polysaccharides (9–49% recovery) at the expense of cellulose (80–100% recovery). Harsh drying, when applied to simply hydrated fibre, did not result in dramatic changes of the pore volume (from 14.9 to 12.7 ml g^{-1} for the total pore volume, and from 10.4 to 7.8 ml g^{-1} for the pore volume accessible to bacteria). The presence of pectins in such materials may prevent pore changes at high drying temperatures. In contrast, when following chemical extraction, harsh drying induced a noticeable decrease in the total pore volume (from 14.9 to 6.1 ml g^{-1}) and especially in the pore volume accessible to bacteria (from 10.4 to 3.2 ml g^{-1}). This dramactic changes was ascribed to the distortion and shrinking of cells during drying. Harsh drying conditions following chemical extraction did not affect the crystallinity of cellulose in the fibre. Neither the particle size, nor the crystallinity of cellulose were major determinant factors in degradability of sugar-beet fibres. In contrast, pore volume accessible to bacteria in sugar beet fibres was highly correlated ($R = 0.88$) with its fermentability. This was consistent with polysaccharides degrading enzymes being closely associated with bacteria and illustrates the importance of matrix physical structure in the control of the physicochemical behaviour of fibre.

Keywords: Fermentability; Human faecal flora; Sugar beet pulp; Porosity

1. Introduction

There is an increasing awareness of the importance of colonic fermentation on the intestinal metabolic functions in man, but our understanding of the factors which control fermentation remain limited. Differences known to exist in fermentation pattern can result from both host and microbial physiology (e.g. host transit time, flora turnover) as well as from substrate availability. The major substrates for colonic fermentation are dietary fibres, resistant starch and endogeneous secretions produced by the gut mucosa. Hence, complex carbohydrates and in particular those from the plant cell-wall, are important contributors to fermentable substrates. Such fibres demonstrate a wide diversity both in their rate and extent of degradation and in the amount and profile of short chain fatty acids produced (McBurney and Thompson, 1990; Titgemeyer et al., 1991; Salvador et al., 1993). Although the influence of both the chemical and physico-chemical characteristics on fermentation is recognised, the features of fibre that are important for their fermentative profile have not been well established. Chemical and structural features of the cell-walls can influence the extent of breakdown: components such as pectins have been shown to be extensively degraded (Englyst et al., 1987; Robertson et al., 1987), whereas lignified tissues are poorly fermented. Lignification also limits the microbial hydrolysis of cell walls (Kerley et al., 1988). Physico-chemical factors, especially the hydration properties of fibre have been also related to the extent of bacterial degradation (McBurney et al., 1985; Auffret et al., 1993). In this context, the presence of water within the fibre matrix, representing the pore volume, is thought to facilitate the accessibility of enzymes and bacteria to the substrate, but assumes the water is contained in pores accessible to bacteria activity.

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Attention on fermentation in human nutrition has been focussed mainly on gross structural/chemical features and their relationship with hydration properties of fibre rather than microstructural features of the fibre matrix. However, complementary studies on industrial applications of lignocellulosic materials have highlighted the role of physical parameters such as particle size and specific surface area (Gharpuray et al., 1983), porosity (Stone and Scallan, 1968; Stone et al., 1969; Grethlein et al., 1984; Grethlein, 1985; Lin et al., 1985; Gama et al., 1994) and crystallinity (Fan et al., 1980; Lee et al., 1983) as important factors determining their rate and extent of degradation. Degradability has been studied after applying severe chemical and physical treatments namely ball-milling, steam explosion, pulping (Gharpuray et al., 1983; Grous et al., 1986; Wong et al., 1988). The major difficulty when studying lignocellulosic substrates has been to identify the limiting factor in controlling degradability. For example, it has been shown for lignocellulosic material, that the initial rate of enzymolysis depends on the pore volume and in particular, the diameter of pore large enough to accommodate the cellulase (Stone et al., 1969; Grethlein et al., 1984). The critical diameter for cellulase access has been estimated as approximatively 5 nm (Cowling and Kirk, 1976; Weimer and Weston, 1985). Similarly, the extent of enzymatic degradation of wheat straw has been related to particle size and available surface area (Gharpuray et al., 1983). Whilst fibre preparations in foods contain tissues more immature than in lignocellulosics, pore size and surface availability of fibre preparations can also be important, and cooking and processing activities may also influence the physical behaviour of fibre in foods and hence during fermentation.

In this paper, the objective was to determine how modification to fibre physical structure could influence fermentation. Selected physical parameters of well characterized sugar beet fibres (Bertin et al., 1988; Guillon et al., 1992) treated by chemical extractions and/or processed by severe and mild drying, were related to known differences in fermentability (Auffret et al., 1993). The physical parameters studied were porosity and cellulose crystallinity. The porosity was measured using two different methods in order to differentiate between macroporosity (>1 μm pore diameter) and microporosity (<1 μm pore diameter).

2. Materials and methods

2.1. Raw fibres

Sugar-beet pulp and commercial sugar-beet fibre were purchased from Agro-Recherche-Développement (Pomacle, France). Sugar-beet pulp (P) to indicate the P samples are pulp as opposed to the commercial fibre referred to as C.

2.2. Preparation of fibres (P) from the pulp

Samples of pulp were hammer milled (Culati) at a linear velocity of 60 m/s, passed through a 2 mm screen and extracted with water for 45 min at room temperature. This material is referred to as P. Different chemical extractions were applied to this fibre. The nomenclature of the resulting fibres is shown in Table 1.

2.3. Hydration and drying from the commercial fibre (C)

C samples were stirred with 30 vol of distilled water for 30 min at 15°C, then filterered through G3 sintered glass. The insoluble fraction recovered was spread in a 5 mm layer under drying lamps for 3 h (Mazda, 1 kW) until the dry matter was 80–85%. The fraction is referred to as C+.

2.4. Extractions and drying from C

Chemical treatments (Table 1) involved sequential extraction with 30 vol of 0.05 M HNO_3 for 30 min at 85°C, (×2), recovery and neutralisation of the insoluble residue, then extraction with 30 vol 0.05 M NaOH for 30 min at 0°C, (×2). The final water-insoluble residue was extensively washed to neutrality and recovered by filtration (100 μm sieve). A part of the residue was freeze-dried (GS 25 Sérail, 1.5 m^2, −70°C) for 48 h and another part was dried under lamps. These samples are referred to as CS− and CS+, respectively.

2.5. Extractions and drying from P

Chemical treatments (Table 1) involved a sequential extraction with removal of supernatant between extractions and washing the insoluble residue to neutrality before further extraction. The first procedure involved extraction

Table 1
Processing conditions and nomenclature of sugar beet fibre samples

Initial fibres	Chemical treatments	Drying conditions	Nomenclature
C	–	Drying under lamps, 100°C	C+
C	0.05 M HNO_3 (×2) + 0.05 M NaOH (×2)	Freeze-drying	CS−
C	0.05 M HNO_3 (×2) + 0.05 M NaOH (×2)	Drying under lamps, 100°C	CS+
P	0.05 M HCl (×3)	Solvent exchange and oven drying, 40°C	PH−
P	0.05 M HCl (×3) + 0.05 M NaOH (×3)	Solvent exchange and oven drying, 40°C	PS−
P	[0.075 M HNO_3 (×2) + 0.025 M NaOH (×2)	Pilot air-drying, 100°C	PS+

(×2) with 0.05 M HCl for 45 min at 85°C, then for 30 min at 85°C. The residue was divided into two samples. One sample was retained without further extraction (PH−). The second sample was subsequently extracted for 30 min at 4°C with 0.05 M NaOH (×3) to obtain the residue PS−. The second procedure involved extraction (×2) with 0.075 M HNO_3 for 45 min at 85°C and subsequently with 0.025 M NaOH for 45 min at 4°C with (×2) to obtain PS+. The water-insoluble residue remaining after extraction was recovered by filtration, extensively washed with water and dried, either under vacuum at room temperature (20°C) after solvent exchange (ethanol, acetone and diethyl ether) for residues resulting from acid extraction (PH−) and from the first sequential procedure (PS−) or by air drying (100°C) for residues from the second sequential extraction (PS+). To ensure homogeneity of residue, air-dried fibres (PS +) which formed crusts were ground for 4 min in a water cooled blade grinder (IKA).

2.6. Analysis

All values are given on a moisture-free basis, moisture being determined after drying in an air-oven at 100°C for 16 h. For sugar analysis, samples were dispersed in 72% sulphuric acid (1 h, 25°C), which was then diluted to 1 M (2 h, 100°C). Total neutral sugars were estimated by the automated orcinol method (Tollier and Robin, 1979) using glucose (for the starting material) or glucose and arabinose (for starting fibres) (1/1) as standards. The individual neutral sugars were analysed by gas liquid chromatography after derivatisation as alditol acetate derivates and, using inositol as internal standard (Hoebler et al., 1989). Uronic acids were quantified by the automated *m*-phenyl-phenol method (Thibault, 1979). All the residues of fermentation were analysed for total neutral sugars and uronic acids. The raw and treated fibre samples, and the residues of fermentation from the P serie were analysed for their monosaccharide composition.

2.7. Microscopy

The macrostructure of fibres was investigated by using a phase contrast light microscope (Vanox Olympus apparatus). Hydrated samples were frozen, thick-sectioned (5 μm, Microm, HM 500 OM apparatus), then placed on a thin plate, and directly observed.

2.8. Particle size

The particle size distribution of coarse fibres (> 250 μm, C and treated fibres from C) was performed with a set of eight RETSCH standard sieves with size ranging from 100 to 1000 μm according to a geometric progression, fitted with a pan and a cover. Samples (15 g) were placed on the top of stack (1000 μm) of tared screens and nested sieves shaken for 12 min. The screening rejects were then weighed separately. The cumulative percent undersize was plotted on a normal probality axis and the log of the corresponding sieve aperture plotted on the log axis. The geometric mean diameter and the geometric standard deviation were calculated according to AFNOR (1987), with the aperture through which 50% of the sample passed representing the mean particle size.

The particle size distribution of fine particles (P and treated fibres from P) was performed with a Coulter Counter model TA II. The counter was fitted with a 400 μm aperture tube and water-isopropyl alcohol solution (1:1, v:v) was used as carrier. The mean particle size was calculated according to AFNOR (1987)

2.9. Water-retention capacity

Water-retention capacity (WRC) was measured, in triplicate, by centrifugation. Samples (0.3 g dry weight) were soaked for 1 h in tubes containing 10 ml of NaCl (0.15 M), with gentle stirring in a water-bath at 37°C (mechanical stirring: 100 cycles per min). After centrifugation (20 min at 14,000 g), the residue was left to drain (G1 sintered glass; 30 min), fresh weight was determined and sample dried (16 h at 100°C). The WRC was calculated from the fresh and dry weight, corrected for the presence of the dissolved electrolytes. The WRC is an estimate of the total pore volume of the hydrated fibre matrix.

2.10. Porosity by suction pressures: macroporosity

The gross porosity of fibre samples was determined by measurements of water-absorption kinetics of fibres at different osmotic pressures (Robertson and Eastwood, 1981). The capacity of fibres to retain water was obtained by dialysing samples of hydrated fibre against solutions of polyethylene glycol (PEG) (MW 8000) of known suction pressure. A series of PEG solutions (0–140 g l^{-1}) of known suction potential (0–0.412 MPa) was used to provide a profile of the macroporosity. The water retained by the fibre matrix was related to pore size through suction pressure and surface tension according to the formula:

$$\text{Diameter} = \frac{4\bar{S}}{\Delta P},$$

where S is the surface tension of solute (water), ΔP the suction pressure (MPa) used to measure water held/pore volume and diameter is in mm. Surface tension of the solute was measured after hydration and taken to represent surface tension effective in the sample pores. The measured value was about 0.055 N m^{-1}, compared to 0.076 N m^{-1} for pure water (J. Mingins, pers. comm.). The pore volume measured has been distinguished as pore volume diameter >1 μm (accessible to bacteria) and <1 μm (inaccessible to bacteria/micropore volume).

Measurement of the water absorption at different suction pressures (Robertson and Eastwood, 1981) involved

presoaking fibre samples overnight at 4°C, transferring the equivalent of 0.2–0.3 g dry fibre, as hydrated fibres into dialysis bag (PolyLabo, ref 24006, 32 mm diameter), which was then sealed and placed in 100 ml of the PEG solution in a 250 ml conical flask. Sodium azide (0.002 g kg^{-1}) was added as a bacteriostat. Flasks were shaken on an orbital shaker for 72 h at 37°C (100 cycles min^{-1}) and the contents then quantitatively recovered, weighed and oven-dried for 16 h at 100°C to determine dry weight and, hence, water content. Each sample at each PEG concentration was tested in triplicate. Results (ml water g^{-1} dry sample) represent the total pore volume of sample at each suction pressure.

2.11. Porosity by solute exclusion technique: microporosity

The microporosity was measured using a solute exclusion technique (Stone and Scallan, 1968) as modified by Lin et al. (1987). The technique estimates pore volume from the relative change in the concentration of molecular probes incubated with hydrated fibre samples. Different PEG, MW 200–10 000 were chosen as molecular probes (Lin et al., 1987). Their solvated diameters have been calculated both from intrinsic viscosity measurements and solute exclusion chromatography (Neuman and Walker, 1992), as shown in Table 2.

The solute exclusion technique was performed in duplicate using 25 ml sealed bottles. 15 ml of 20 g l^{-1} PEG for each probe size, or distilled water as control blank, was added to 5 g hydrated fibres. Sodium azide (0.002 g kg^{-1}) was added as bacteriostat. Bottles were sealed and periodically hand shaken for 3 min over a one hour period then left overnight at 4°C. Supernatant was recovered from each bottle by filtration [G3 sintered glass then on 0.45 μm filters (Millex system, Millipore)], and analysed by differential refractometry (water apparatus). Each solution was analysed × 5 (Lin et al., 1987).

The inaccessible pore volume for each substrate was determined as ml g^{-1} dry weight from the following equation (Stone et al., 1969):

$$I = \left[\frac{W+q}{p} - \frac{W\,Ci}{p\,Cf}\right],$$

Table 2
PEG diameters measured by intrinsic viscosity

Molecular weight of PEG [g mol^{-1}]	Calculated molecular diameter [nm]	Molecular diameter according to Neuman and Walker (1992) [nm]
200	0.9	0.9
400	1.3	1.2
600	1.5	1.5
1450	2.3	2.3
3350	3.6	3.6
10 000	6.4	ND

ND: not determined.

where I is the inaccessible water (ml g^{-1}) for the PEG probe; W the mass of the PEG solution; q is the mass of the water in sample of solids; p is the sample dry weight; C_i the initial concentration of the PEG solution and C_f the final concentration of the PEG solution. The inaccessible pore volume for each probe was measured for micropore diameter sizes ranging from 0.9 to 7.1 nm. Two types of micropore volume have been distinguished in terms of accessibility. These are the enzyme inaccessible (<5 nm) and enzyme accessible micropore volume (>5 nm, but <1 μm).

2.12. Cellulose crystallinity

Crystallinity was assessed by X-ray diffraction analysis. X-ray diffraction patterns were obtained using an INEL spectrometer working at 40 kV and 30 mA operating in the Debye–Scherrer transmission method. The X-ray radiation was selected with a monochromator on Cu Kα (λ = 0.15405 nm) and detected by a curved positive detector (INEL CPS-120). The recording time was 2 h and the diffraction diagrams were standardized at the same total scattering between Bragg angles (2θ) of 3 and 30°. The relative crystallinity was determined following the method of Wakelin et al. (1959) using hydrolysed (HCl 2.5 mol.l^{-1}) cellulose of tunicin and regenerated (in water) cellulose from concentrated sulphuric acid solution, as crystalline and amorphous standards, respectively.

2.13. In vitro fermentation

The in vitro fermentation was carried out in triplicate for each sample according to the method of Barry et al. (1989). Fibres (400 mg) was anaerobically fermented in bottles at 37°C for 6, 12 or 24 h with 20 ml of inoculum. The inoculum was obtained after mixing three human faeces from strict H_2 producers with warmed (37°C) nutritive buffer (two parts of nutritive buffer to one part of faeces) and filtering the homogenate through gauze to remove fibre particules. A control blank (containing no fibres) was included. Fermentations were stopped at either 6, 12 or 24 h by addition of $HgCl_2$. The insoluble residue in each bottle was recovered, freeze-dried and weighed before analysis and quantification for residual sugars.

The fermentability coefficient, as the apparent percentage disappearance of sugars, was calculated at each incubation time as:

$$\%\ \text{of sugar disappearance} = \frac{[F+I]-R}{F+I} \times 100,$$

where F is the amount of sugars in the fibre sample, I, the amount of sugars in the faecal inoculum and R, the amount of sugars in the fermented residue. All fermentation trials were in triplicate.

2.14. Regression analysis

Fermentation data were first fitted by a simple linear regression model in which variables were time (T) of fermentation and porosity (P). Various variable changes were tested until the best fit was found. Then, fermentation data were fitted with a multilinear regression model (Mendenhall and Sincich, 1992) including both time (T) and porosity (P) as variable, changed according to the best fit found with the simple regression model. Stat-View SE Graphic Software (Abacus concept Inc, CA, USA) for Apple Macintosh microcomputer was used.

3. Results

3.1. Sugar composition

3.1.1. Sugar composition of the raw fibres

Total sugars accounted for 808–688 g kg^{-1} of the raw fibres C and P, respectively (Table 3). The raw fibres, C and P respectively, were mainly composed of glucose (243 and 206 g kg^{-1}; 99% of which as cellulose), uronic acid (232 and 202 g kg^{-1}, as galacturonic acid) and arabinose (236 and 191 g kg^{-1}). Galactose was present in significant but lower amount (54 and 46 g kg^{-1}) and other sugars were minor components.

3.1.2. Sugar composition of the treated fibres

The yields and composition of the treated fibres C+, CS−, CS+, PH−, PS−, PS+ are shown in Table 3. In contrast to the treatment with water (C+), acidic treatment alone or in sequence with alkaline extraction led to a large loss of pectic polysaccharides. The loss was due mainly to the extraction of arabinose and uronic acid, especially where alkali extraction followed acid extraction. This led to an enrichment in cellulose of the extracted residues (from 30% of the polysaccharides in the untreated samples up to 70% of the polysaccharides in the extracted residues). Recovery of glucose in residues (> 92%) indicated that the treatments did not affect cellulose except for PS+, where recovery was only 80%.

3.2. Microscopic observations

Figs. 1 and 2 show the histology of fibres C and P. The tissue in raw fibres was formed by a meshwork of void cells with thick walls and irregular shape. Chemical extraction followed by freeze-drying [Fig. 1(b)] or solvent drying [Fig. 2(b)] resulted in a packing of the meshwork. When chemical extraction was followed by a severe drying [Figs 1(c) and 2(c)], cells were more wrinkled and crumpled. These physical changes can have significant implications for WRC and porosity.

3.3. Particle size

Fibres C (430–580 μm) had an apparently much higher mean particle size than P (90–190 μm) (Table 3). The different treatments applied to C did not markedly affect the mean particle size. The lower particle size of fibre from P was ascribed to grinding performed after processing to get homogeneous fibres.

3.4. WRC

The WRC (Table 4) showed differences between the two raw fibres (C: 11.7 and P: 8.8 ml g^{1}). Chemical extractions and drying treatments modified the WRC. Chemically treated fibres dried under mild conditions (freeze-drying/solvent exchange) had a higher WRC than the raw fibres. Hydration of fibres then drying under lamps at 100°C (C+) did not modify the WRC, whereas chemical extraction of the cell-wall components led to a marked decrease in WRC when drying was at 100°C.

3.5. Porosity

3.5.1. Macroporosity (pore size > 1 μm): pore size estimation by suction pressure

Figs 3(a) and 4(a) show the pore volume of C, CS− and CS + and P, PH, and PS + . The total pore volume and the pore volume accessible to bacteria are summarized in

Table 3
Sugar composition and mean particle size of sugar-beet fibre samples

Fibres	C	C+	CS−	CS+	P	PH−	PS−	PS+
Yield (%)	100	98	46	46	100	61	35	35
Composition (g kg^{-1})								
Rhamnose	15	15	12	12	14	17	10	6
Arabinose	236	215	53	53	191	72	11	18
Xylose	14	11	25	25	16	29	36	43
Mannose	14	12	26	26	13	22	27	25
Galactose	54	52	45	45	46	51	23	28
Glucose	243	236	510	510	206	339	542	476
Uronic acids	232	231	120	120	202	162	49	54
Total sugars	808	772	791	791	688	692	698	650
Mean particle size (μm)	480	460	430	580	150	190	120	90

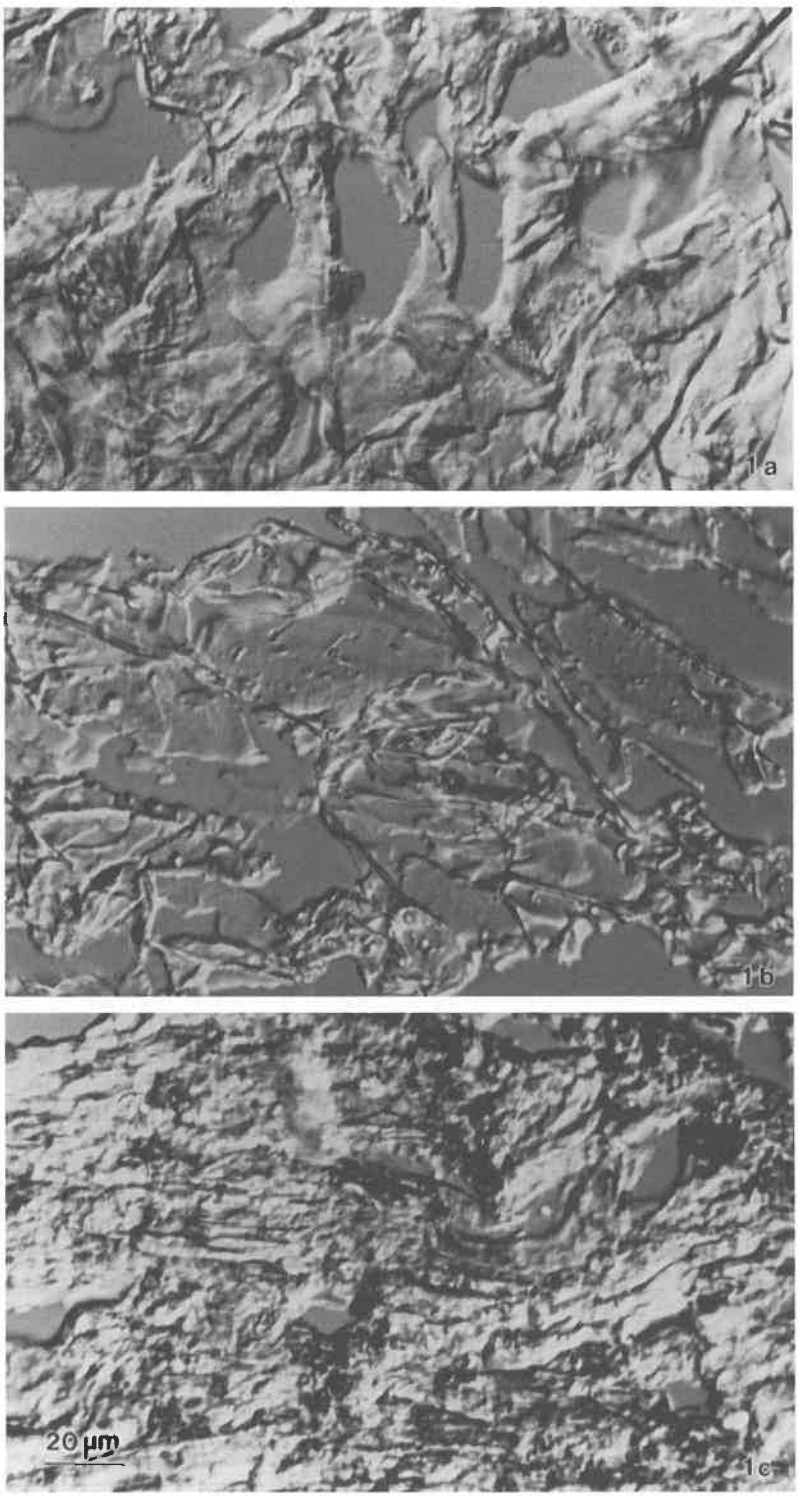

Fig. 1. Light micrographs produced by differential interference contrast microscopy of sugar beet fibre (a) C and corresponding selected residues: (b) CS−; (c) CS+. See Table 1 for description of treatments.

Table 4. The raw fibres, C and P, had similar profile of porosity. Treatments on the raw fibres which included a chemical extraction had a marked effect on their porosity. After extraction following by mild drying by solvent exchange (PH−), the total pore volume of the fibres and the volume accessible to bacteria were increased. Fibres freeze-dried (CS−) had a total pore volume, similar to the raw fibres. Extraction following by drying under high temperature (PS+ and CS+) induced a noticeable decrease of the total pore volume. These samples also had an altered profile of porosity, i.e. the drying process had led to collapse of larger pores.

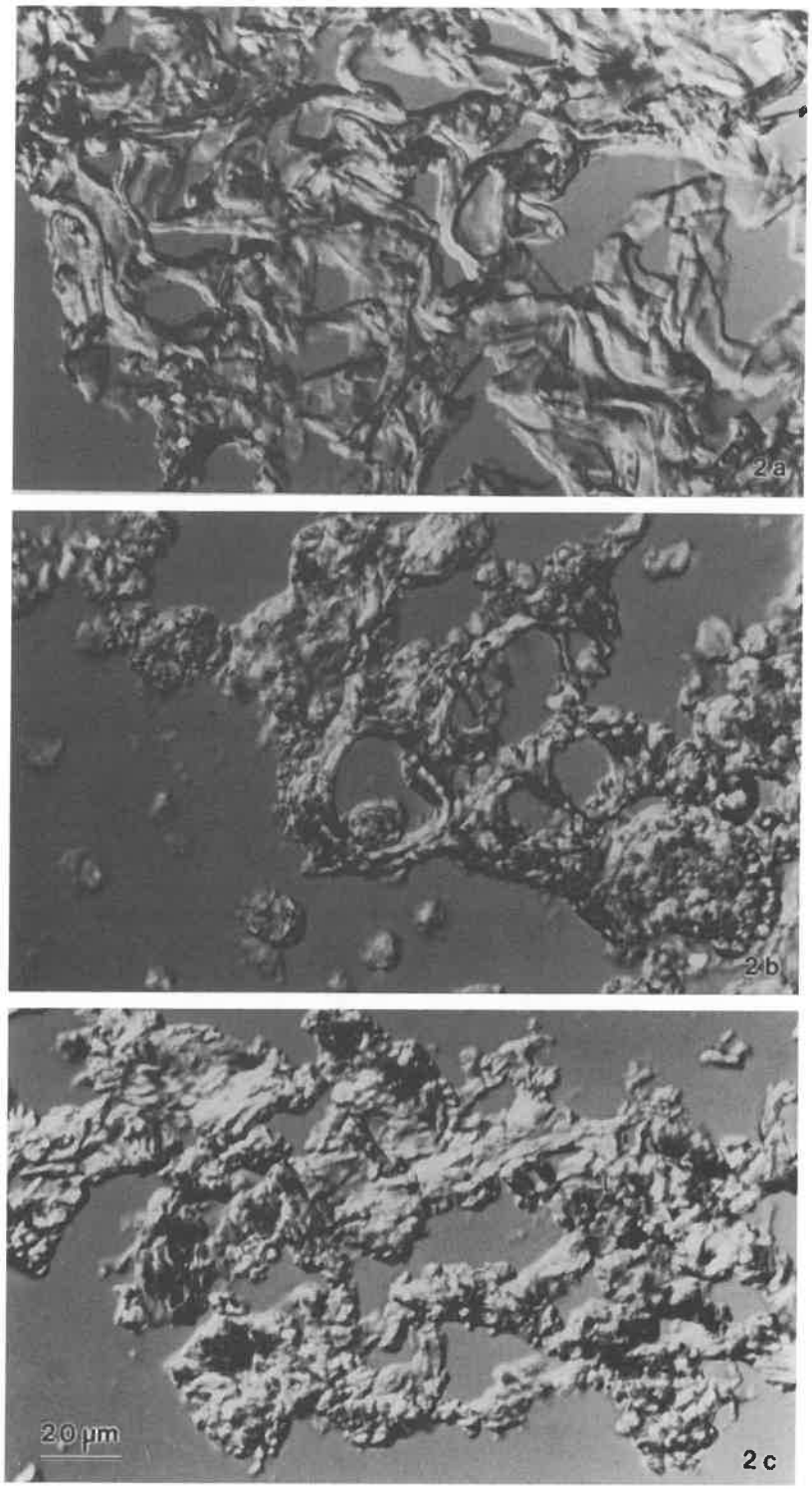

Fig. 2. Light micrographs produced by differential interference contrast microscopy of sugar beet fibre (a) P and corresponding selected residues: (b) PH−; (c) PS+. See Table 1 for description of treatments.

3.5.2. Microporosity (pore size < 1 µm): pore size estimation by solute exclusion

Figs 3(b) and 4(b) show the micropore volume of C, CS− and CS+ fibres and P, PH, PS+. The micropore volume and the pore volumes accessible to enzymes are given in Table 4. Approximatively, one third of the total pore volume of C and P fibres was accounted for by micropores. The micropore volume, taken as the fibre saturation point, (< 1 µm) was similar for C and P fibres and the bulk of micropore volume was accounted for by pores over 2.5 nm diameter. Above a value of 3 nm, the values of pore diameter for pore volume plateaued at a value corresponding to the pore

Table 4
Effect of experimental treatments on pore volume compartmentation in sugar beet fibre samples

Fibres	C	C +	CS −	CS +	P	PH −	PS −	PS +
Total pore volume [ml g^{-1}]								
WRC [± sem]	11.7 (0.2)	11.1 (0.3)	14.6 (0.4)	6.4 (0.0)	8.8 (0.3)	13.1 (0.3)	14.0 (0.3)	4.1 (0.1)
Water absorption capacity (T) [± sem]	14.9 (0.4)	12.7 (0.5)	15.2 (1.1)	6.1 (0.7)	13.8 (0.4)	18.3 (0.1)	nd	6.1 (0.1)
B = pore volume > 1 μm[a]	10.4	7.8	9.9	3.2	8.8	14.1	nd	3.6
I = pore volume < 5 nm[b]	2.9	3.9	3.6	2.3	2.9	3.3	nd	2.2
Pore volume 5 nm-pore vol 1 μm[c] [$E' = (T - B) - I$]	1.6	1.0	1.7	0.6	2.1	0.9	nd	0.3
Pore volume > 5 nm[d] ($E = T - I$)	12.0	8.8	11.6	3.8	10.9	15.0	nd	3.9

[a] Volume accessible to bacteria estimated from macroporosity [Figs 1(a) and 2(a)].
[b] Volume inaccessible to enzymes estimated from microporosity profile [Figs 1(b) and 2(b)].
[c] Volume accessible to enzyme, but not to bacteria.
[d] Volume accessible to enzymes.
nd: not determined.

volume for each sample at 1 μm measured as macroporosity. Differences between raw and treated fibres were observed most noticeably between 2 and 7 nm pore diameters for samples dried at 100°C (PS+ and CS+). These samples had a lower pore volume and also a smaller volume accessible to enzymes. The chemically treated fibres from P, dried under mild conditions (PH−), had apparent increase in total pore volume while micropore volume was not affected. The pore volume accessible to enzyme, but not bacteria (5 nm–1 μm), was between 1.6–2 for raw fibres. It was reduced to between 0.3 and 0.9 ml g^{-1} for P treated fibres and to between 1.0 and 0.6 for severe dried C fibres.

3.6. Cellulose crystallinity

X-ray diffraction measurements were performed on P and PH−, PS− and PS+ (Fig. 5). The spectrum of raw fibres

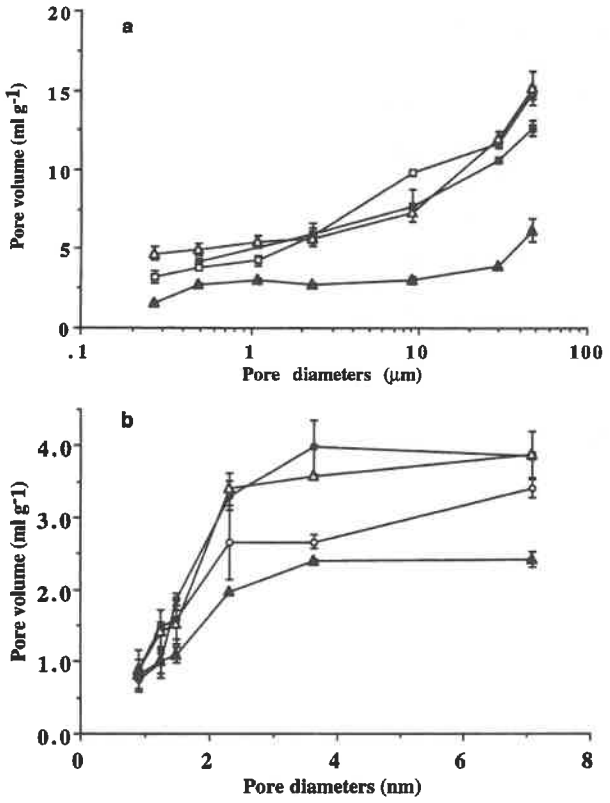

Fig. 3. Pore size distribution for C (– □ –), C+ (– ■ –), CS − (– Δ –) and CS+ (– ▲ –). (a) Macroporosity, pore size estimation by suction pressure; (b) Microporosity, pore size estimation by solute exclusion.

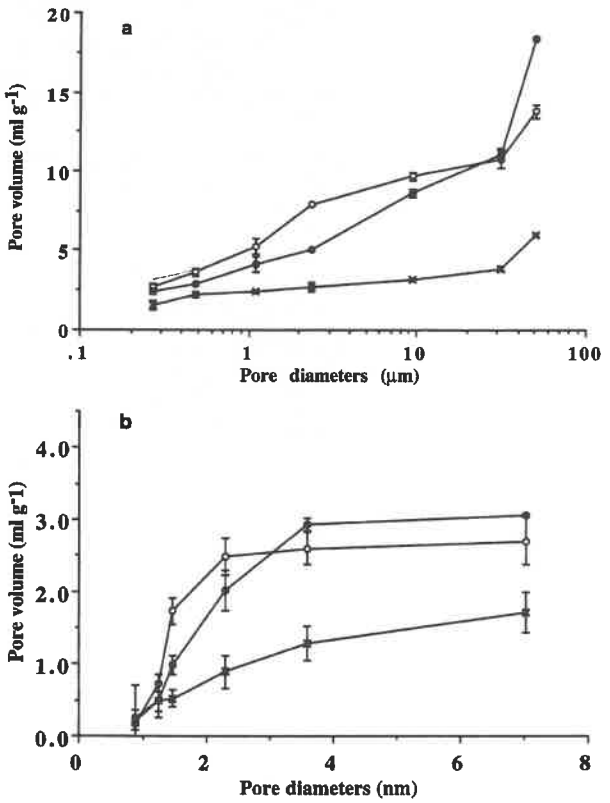

Fig. 4. Pore size distribution for P (– ∘ –), PH− (– • –), PS+ (– × –). (a) Macroporosity, pore size estimation by suction pressure; (b) Microporosity, pore size estimation by solute exclusion.

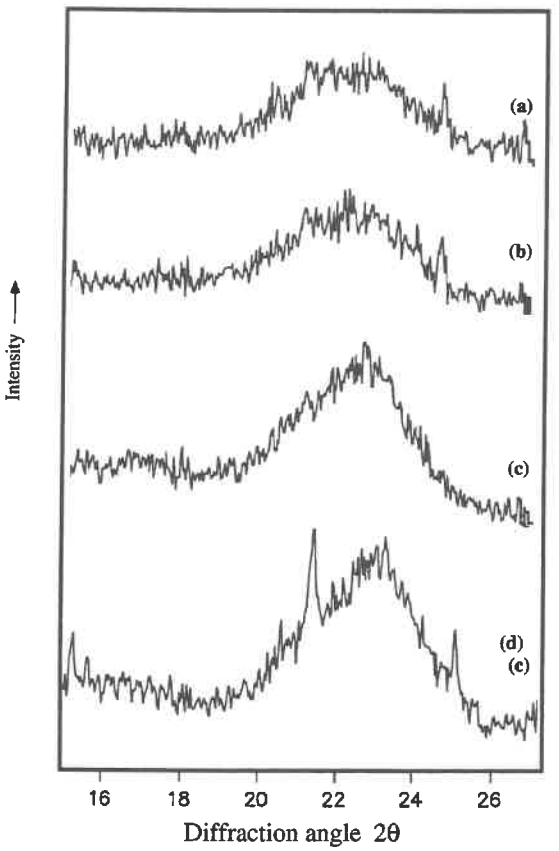

Fig. 5. X-ray diffraction patterns of sugar-beet fibre (a) P and corresponding residues: (b) PH−; (c) PS−; (d) PS+.

was typical of amorphous products. The % crystallinity values were 27% and 22% for respectively P and PH−. A slight peak at 2ϕ near 22.5° was observed on the spectra of the treated fibres, indicating the presence of crystalline cellulose. The peak height was amplified in both samples PS− and PS+ with no noticeable difference in the diffraction pattern. The % of crystallinity values were 33% and 46% for, respectively, PS+ and PS−. Therefore, modifications in cellulose crystallinity were ascribed to the enrichment of cellulose within the treated fibres and to changes in the crystallinity of the cellulose due to physical treatments such as drying.

3.7. In vitro fermentability

The fermentability coefficients of the neutral and acid sugars are given in Table 5. Fermentation of raw fibres led to a rapid and extensive depletion of all the sugars in agreement with previous work (Guillon et al., 1992; Auffret et al., 1993). The fermentation was most active during the first 12 h with galacturonic acid the most rapidly fermented sugars. Its fermentation is nearly complete after 12 h; i.e. the fermentation coefficient at 12 h is similar to 24 h. Neutral sugars depletion was slower since these sugars contained both the less fermentable cellulose as well as the more readily degradable sugars associated with pectic polysaccharides (arabinans, arabinogalactans).

If any differences were found between the raw and the treated fibres they were observed during the initial stages (6 h) of fermentation. Washing following by drying had no apparent effect on fermentability. Fibres dried at high temperature after chemical extraction (CS+ and PS+) were more slowly fermented and to a lesser extent than the corresponding fibres submitted to soft drying (CS− and PS−). Both neutral sugars and uronic acid were involved. Extracted fibre dried by solvent exchange (PS−) were rapidly fermented compared to extracted and freeze-dried fibre (CS−). This difference was mainly ascribed to the difference in the fermentation pattern of the neutral sugar fraction. As glucose was the major neutral sugar in both samples (83% and 76% in, respectively, PS−and CS−), we can assume that this difference reflected differences in cellulose degradation. In P, PS−, and PS+ fibres, the % of disappearance of glucose varied from 32 to 62% at 6 h of incubation and from 81 to 70% at 24 h of incubation, the highest values being obtained for PH− and PS−. In absolute terms for uronic acids, it appeared that 94–98% of the uronic acid present in raw samples were lost as a result of 24 h fermentation or chemical extraction plus 24 h fermentation. This indicates that even after drastic heat treatment as a result drying accessibility of this component for fermentation was not deeply affected. Thus when major structural modifications occured to the fibre as a result of the more severe physical drying treatments then fermentability was reduced mainly in terms of disappearance of neutral

Table 5
Fermentability coefficient of main sugars of sugar-beet fibre samples at 6, 12, 24 h of incubation with faecal bacteria

Fibres	Hours	C	C+	CS−	CS+	P	PH−	PS−	PS+
Neutral sugars	6 h	50 (± 2)*	43 (± 5)	21 (± 4)	19 (± 2)	35 (± 7)	63 (± 3)	58 (± 3)	33 (± 9)
	12 h	66 (± 2)	62 (± 2)	44 (± 7)	29 (± 12)	66 (± 4)	81 (± 2)	81 (± 0)	51 (± 3)
	24 h	81 (± 1)	78 (± 1)	70 (± 4)	37 (± 12)	81 (± 1)	83 (± 1)	87 (± 1)	66 (± 3)
Uronic acids	6 h	70 (± 4)	73 (± 5)	78 (± 5)	58 (± 6)	61 (± 3)	89 (± 6)	63 (± 13)	55 (± 8)
	12 h	90 (± 1)	90 (± 1)	83 (± 2)	71 (± 6)	86 (± 2)	93 (± 1)	75 (± 0)	67 (± 4)
	24 h	94 (± 1)	90 (± 1)	76 (± 5)	93 (± 1)	94 (± 0)	94 (± 0)	84 (± 0)	75 (± 4)
Total sugars	6 h	55 (± 1)	52 (± 5)	28 (± 3)	24 (± 2)	43 (± 5)	69 (± 4)	58 (± 2)	35 (± 8)
	12 h	73 (± 2)	70 (± 1)	48 (± 7)	33 (± 12)	72 (± 3)	84 (± 1)	81 (± 0)	53 (± 3)
	24 h	84 (± 1)	83 (± 1)	72 (± 3)	41 (± 12)	85 (± 1)	88 (± 1)	87 (± 1)	67 (± 3)

*(SEM).

sugars, but modification of substrate through chemical extraction alone had less effect on fermentability.

3.8. Relationship between porostiy data and fermentability

Linear multiple regression analysis was used to model the relationship between the influence of porosity on the fermentation profile of the different sample (Table 6). A strong relationship was found both regarding the total pore volume accessible to enzymes and the total pore volume accessible to bacteria ($r = 0.88$ and 0.87, respectively). However, a lower correlation was found between the fermentability and micropore volume only accessible to enzymes ($r = 0.69$). This would be consistent with microbial polysaccharides activity being physically associated with the bacteria present.

4. Discussion

Much effort has been devoted to understanding the factors involved in the control of fermentation of dietary fibre and its importance in health. Fermentation can be described as a two step phenomenon: the degradation of polysaccharides into sugars and their subsequent metabolisation by bacteria to SCFA. Thus the rate of hydrolysis of polysaccharides will limit the availability of sugars for SCFA production. Previous studies (Mortensen et al., 1988; Barry et al., 1989) using pure sugars or oligosaccharides have demonstrated that the nature of sugars can influence the amount and the profile of SCFA produced but other studies (Salvador et al., 1993), have failed to account for the variation in SCFA production just on the basis of the monosaccharide composition of dietary fibres. Chemical composition alone cannot adequately reflect the complexity and heterogeneity of the fibre matrix with respect to structure involving interactions of polysaccharides within cell walls and structural features of the constituent polysaccharides. Each may play an important role in the sensitivity of fibre to microbial attack. In our study, sugar-beet fibres with similar chemical composition exhibited different rates of fermentation illustrating the importance of fibre structure in fermentation. Although chemical extraction resulted in differences in composition between samples it did allow the importance of pectic polysaccharides in the control of porosity to be determined. It was also possible to compare the properties of lignocellulosics from vegetables with those from forages. This confirmed the importance of pectic polysaccharides in the cell wall and also that the behaviour of lignocellulosics is similar irrespective of sample. In the past, it has been thought that crystallinity was an over riding factor in determining sensitivity of cellulosic materials to hydrolysis by enzymes complexes (Sasaki et al., 1979; Fan et al., 1980; Ryu et al., 1982; Lee et al., 1983). Several investigators (Puri, 1984; Sinitsyn et al., 1991; Ramos et al., 1993) have demonstrated that other physical properties of cellulose such as its degree of polymerisation, its allomorphic form and accessibility for enzymes may also be important whilst, for some authors (Grethlein, 1985; Grous et al., 1986), the surface area available to the cellulases apparently had the greatest impact. The influence of crystallinity on the cellulose attack by complex microflora is still debated. But whilst it may be important for the degradability of forage lignocellulosics, in fruits, vegetables and most cereal foods cellulose is usually present in an amorphous form. To some investigators, the crystallinity of the cellulose does not influence its fermentation by ruminal microflora (Buléon and Bertrand, 1982; Weimer et al., 1990) because of the synergistic activities of the microflora included bacterial species but also fungi and protozoa. How this relates to conditions in the human large intestine remains unclear as the cellulolytic activities of human microflora are poorly documented. In the case of sugar-beet fibre, we have shown that chemical extractions followed by harsh drying of samples enriched in cellulose resulted in some changes in crystallinity (from 32% to 46% for PS^- and PS^+ respectively) and in a decrease in the cellulose degradability (from 91 to 70% at 24 h of fermentation, which suggests that crystallinity of cellusose may be a limiting factor to fermentation in the human.

To degrade polysaccharides, microbial glycosidases must have access to their substrates within the cell walls. One factor important for the control of accessibility is particle size (Dehority and Johnson, 1961; Gharpuray et al., 1983).

Table 6
Correlation of pore accessibilities with the fermentability by multiple linear regression analysis ($P < 0.05$; $n = 6$)

Fermentability coefficient of total sugars = *A*/incubation hour + *B*/pore volume accessible to bacteria or enzymes + constant	Pore volume accessible to bacteria (pore diameter > 1 μm) [ml g^{-1}]	Pore volume accessible to enzymes (pore diameter > 5 nm) [ml g^{-1}]
A/incubation hour	$A = -211$	$A = -211$
B/pore volume accessible	$B = -159$	$B = -195$
Constant	104	104
r	0.88	0.87

Fermentation data were first fitted by a simple linear regression model in which variables were Time (T) of fermentation and Porosity (P). Various variable changes were tested until the best fit was found. Then, fermentation data were fitted with a multilinear regression model (Mendenhall and Sincich, 1992) including both time (*T*) and porosity (*P*) as variables, changed according to the best fit found with the simple regression model. Stat-View II software (Abacus concept Inc., CA, USA) on an Apple Macintosh PowerPC microcomputer was used.

Decreasing particle size increases the external surface area and so increases the area exposed to bacteria. The impact of decreasing particle size on fermentation is more marked when the external surface is the major portion of accessible surface as in a compact lignocellulosic or cellulosic materials with poor swelling capacity (Weimer et al., 1990 Gama et al., 1994). However, in the case of sugar beet fibres, average particle size varied from 500 to 150 μm between the raw fibres C and P, but no marked differences were observed in their fermentability. This suggests that sugar beet fibres have a considerable available pore volume, as might be expected from a microporous cellular solid.

Most of the data available on porosity of plant cell wall materials arise from studies on the cellulose–cellulase systems or lignocellulosic material–ruminal bacteria (Stone and Scallan, 1968; Grethlein et al., 1984; Lin et al., 1985; Grous et al., 1986; Lin et al., 1987; Gama et al., 1994) and focussed on the microporosity which is the size domain of enzymes. In this study, we have estimated both macro and microporosity of sugar beet fibre by combining two methods, measurement of the water absorption capacity at different suction pressures and measurement of solute exclusion of probe molecules. These methods have their limitations; both are indirect methods and the values obtained depends on the shape of the pores. The former requires artificial pressures and removal of water results in a 'shrinking' of the matrix while the latter is dependent on the shape and reactivity of the molecule probes. Also, the range 0.4 mm–6.4 nm of the pore regime is not explored. Although from the results obtained there is apparently little change in porosity within this range. However, the methods used have the advantage over other methods (Hiçsasmaz and Clayton, 1992) in that they measure the porosity in a wet form in a hydrated environment which corresponds to conditions existing during fermentation.

The total micropore volume of raw sugar-beet fibres (P and C) was higher than that reported for cellulosic and lignocellulosic materials (Lin et al., 1987; Gama et al., 1994). The effects of drying were observed on both macroporosity and microporosity, but were more pronounced when pectic material was removed. The macropores seemed to be affected more than the micropores. Microscopic examination suggested that collapse of the cell walls occurred during drastic drying process. These results reinforce the important role of pectins in maintaining fibre and cell wall structure, as both matrix embedding the cellulose framework within cell-walls, and to cement adjacent cells in immature tissue and ensure cohesiveness within the tissue. Carpita and Gibeaut (1993) have already highlighted the role of pectins in controlling pore size distribution in primary cell walls. Our results suggests an important role for the gross porosity. Moreover, our data illustrate how important is the processing history of dietary fibre with respect to the modification of the physicochemical properties and its sensitivity to microbial degradation. The prediction from the regression analysis of the substrate fermentability through the pore volume accessible to bacteria indicated the predominant importance of larger pores compared with micropores in the control of fermentability of fibres. This strengthens the idea that most of the cell wall polysaccharide degrading activities produced by bacteria are organised multi-enzyme-complexes, which remain associated with bacteria (MacFarlane et al., 1991; Salyers and Leedle, 1983). This is emphasied from consideration of surface availability, for example, assuming a regular cylindrical network of pores, and a pore volume of 1 ml g^{-1} at 1 μm pore diameter and the surface area is about 4 $m^2 g^{-1}$ and at 5 nm it is about 800 $m^2 g^{-1}$. Thus, there is potentially a very large surface area available to enzyme attack compared to microbial attack and consequently it is less likely that surface area:enzyme ratio will be limiting during fermentation compared to the surface area:bacteria ratio. Similarly a reduction in pore volume will have a greater impact on surface availability for bacteria compared to surface availability for enzymes, as predicted from the regression model of the experimental data.

Such conclusions agree with those from studies using on purified celluloses and cornstalk residues fermented by rumen fluids (Lin et al., 1985; Weimer et al., 1990). Although there are probably significant differences in the bacterial species present in the colon compared with the rumen and the nature of consumed plant cell wall material is very different both in constitution and structure, (Stevani, 1990), it can be presumed that the microbial degradation of substrates are governed by the similar factors. With regard to dietary fibres in general, it is probable then that the surface area available for bacteria is a major factor involved in the control of fermentation. When not limited by surface availability function, other parameters, such as crystallinity of cellulose and the chemical structure of polysaccharides will become more important factors.

The current study has identified the importance of matrix porosity in controlling the fermentation behaviour of fibre; results were obtained using relatively imprecise techniques and this has focussed the need to adapt and apply more precise physicochemical techniques to the study of porosity in non ligno-cellulosic cell wall materials. In particular there is a need to identify more precisely how constituent polysaccharides control cell wall porosity and the events involved in modifying porosity by chemical extraction, e.g. polysaccharide swelling or particular collapse of the wall structure.

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