Estimation of the fermentability of dietary fibre *in vitro*: a European interlaboratory study

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Five European laboratories tested a simple *in vitro* batch system for dietary fibre fermentation studies. The inoculum was composed of fresh human faeces mixed with a carbonate–phosphate buffer complex supplemented with trace elements and urea. Five dietary fibre sources (cellulose, sugarbeet fibre, soyabean fibre, maize bran and pectin) were used by each laboratory on three occasions to determine pH, residual non-starch polysaccharides (NSP) and short-chain fatty acid production during fermentation. Cellulose and maize bran degradabilities were very low (7·2 (SE 10·8) and 6·2 (SE 9·1) % respectively after 24 h), whereas pectin and soyabean fibre were highly degraded (97·4 (SE 4·4) and 91·1 (SE 3·4) % respectively after 24 h). Sugarbeet fibre exhibited an intermediate level of degradability (59·5 (SE 14·9) %). Short-chain fatty acid production was closely related to NSP degradation (r 0·99). Although each variable was ranked similarly by all laboratories, some differences occurred with respect to absolute values. However, the adaptation of donors to the experimental substrates was not an influential factor. Interlaboratory differences could be reduced either by adding less substrate during incubations or using less-diluted inocula. *In vitro* fermentations with inocula made from human faeces and from rat caecal contents gave similar results. There was a close correspondence between the data obtained in the present experiment and those previously published in *in vivo* studies in the rat using the same fibres. The *in vitro* batch system tested during the present study provides a rapid means of obtaining quantitative estimates of the fermentation and the estimation of the energy content of new sources of dietary fibre.

Dietary fibre: *In vitro* fermentation: Short-chain fatty acids

Studies of the nutritional effects of colonic fermentation of dietary fibres have been widely developed during the past decade, and *in vitro* fermentation methods have made it possible to predict some of the physiological effects of fibre (McBurney & Thompson, 1989; Adiotomre *et al.* 1990). Short-chain fatty acids (SCFA), the main end-products of dietary fibre fermentation, are extensively absorbed and metabolized. As fibre is indigestible in the small intestine, its energy content is conventionally considered to be zero, but this appears to be untrue especially when substantial quantities of fibre are fermented (Livesey, 1990). In addition, many biological effects of SCFA have been reported. Butyrate is metabolized by colonic cells (Roediger, 1982) and could be involved in the protective effect of fibre against colon cancer. SCFA influence carbohydrate and lipid metabolism (Wolever *et al.* 1992). The specific effect of each SCFA on hindgut motility has recently been reviewed

Table 1. *Summary of conditions used in the literature for batch* in vitro *fermentations*

Reference	Faeces concentration (g/l)	Substrate amounts (g/l)	Buffer*	Trace elements	Duration (h)
Vince *et al.* (1990)	250	NA	No	No	48
Perman & Modler (1982), Flick & Perman (1989)	125	50	p	No	1
Ehle *et al.* (1982)	NA	NA	No	Yes	48
Fidgor & Bianchine (1983)	NA	NA	No	No	30
McBurney *et al.* (1985)	NA	5	c	Yes	24
Tomlin *et al.* (1986), Tomlin & Read (1988)	50	5	c	No	21
Slade *et al.* (1987)	0·5 to 1·5	20	Yes	Yes	24
Patil *et al.* (1987)	50	20	No†	No	24
Rasmussen *et al.* (1987), Mortensen *et al.* (1988)	167	Up to ~ 50	No	No	72
Stevens *et al.* (1988)	1 to 1·67	NA	NA	NA	NA
Wyatt & Horn (1988)	1	20	c	Yes	168
McBurney & Thompson (1987)	13	10	c/p	Yes	24
McBurney *et al.* (1988)	60	10	c/p	Yes	24
Mortensen *et al.* (1989)	166	10	No	No	72
Mortensen *et al.* (1991)	166	10	c	No	24 or 72
McBurney & Thompson (1989)	13	10	c/p	Yes	24
Jeraci & Horvath (1989)	NA	NA	NA	NA	48
Barry *et al.* (1989), Guillon *et al.* (1992), Salvador *et al.* (1993)	250	10	c/p	Yes	24
Adiotomre *et al.* (1990)	40	10	p	Yes	24
Mortensen & Nordgaard-Andersen (1993)	166	10 to 30	c	No	24

NA, not available.
* No, no buffer; yes, buffer, composition not specified; p, phosphate buffer; c, carbonate buffer; c/p, carbonate–phosphate buffer.
† pH adjusted.

(Cherbut, 1995). Hence there are many reasons why it is important to characterize dietary fibre fermentation with respect to substrate disappearance and, especially, SCFA production.

In vivo fermentation methods allow dietary fibre digestibility to be determined but cannot predict SCFA production during fermentation in humans. It is difficult to collect human colonic contents, and the only published data have been obtained from individuals who experienced sudden death (Cummings *et al.* 1987). As SCFA are metabolized by colonic cells or the liver, their peripheral blood levels indicate little about their production. Thus, many researchers have developed *in vitro* systems to characterize fibre fermentation. Continuous or semi-continuous systems (Miller & Wolin, 1981; Mallet *et al.* 1983; Edwards *et al.* 1985; Gibson *et al.* 1988) are generally used to study the microbial mechanisms involved in fibre fermentation. When the main objective is to study the fermentative fate of various fibres, *in vitro* batch systems are generally adopted. Table 1, which summarizes the main characteristics of these methods, shows that many elements of the protocol vary from one system to another and that some systems have been modified by the same investigators. The reason why each element of a protocol has been fixed at a precise level is rarely explained. Moreover, there has been no systematic evaluation of these procedures, so the possibility of comparing the results from different centres is limited.

The purpose of the present study was to develop a standardized *in vitro* method for rapid

investigation of the fermentation of new fibre sources. Several laboratories tested a standard protocol using a panel of commercial fibre sources, and further experiments were undertaken to determine causes for discrepancies observed in results obtained by the different laboratories.

MATERIALS AND METHODS

General

Five European laboratories in Denmark, France and the United Kingdom, all previously involved in *in vitro* studies of dietary fibre fermentation, undertook these experiments. The laboratories were randomly referenced from I to V without any relation to the author list. All five tested commercial-fibre fermentation in a ring-test experiment. Specific factors (influence of substrate amounts, donor variability, influence of donor adaptation to fibre and comparison between rat and human inocula) were then studied by at least one of these laboratories.

Fibre sources

Five commercial sources of dietary fibre were used throughout the experiment. Solka-floc cellulose (crystalline cellulose, grade BW 2030) was provided by Jurgenson and Wettre Ltd (Wokingham, Berks), apple pectin ('slow set' high-methoxyl pectin) by Sanofi Bio-Industries (Paris, France), soyabean fibre ('Fibrim 2000', red) by Protein Technologies International (Corby, Northants), maize bran by Honeyville Grain Inc. (Salt Lake City, UT, USA) and sugarbeet fibre ('Betafibre') by British Sugar plc (Peterborough, Cambs). The five fibre sources were analysed for minerals, lipids, starch, total dietary fibre, and crude and soluble proteins (Table 2). The non-starch polysaccharide (NSP) content of these dietary fibre sources is presented in Table 3.

Fermentation procedure

General schedule. All experiments were conducted *in vitro* in a batch system. Fermentations were performed in vials using an inoculum made from fresh faeces collected from healthy human volunteers. The volunteers usually ingested a normal diet, presented no digestive disease and had not received antibiotics for at least 3 months. Fermentation variables were measured in vials in which fermentation was stopped at various times.

Inoculum. Faeces were collected in an insulated bottle previously warmed for about 5 min with hot tap water (approximately 65°). To eliminate O_2, the bottle was flushed for 5 min with CO_2 at a flow of 100 ml/s and faeces were then collected. When the insulated bottle was received at the laboratory, CO_2 was flushed inside. The weight of the faeces was then determined. The inoculum was produced in the insulated bottle by adding five parts of a warmed (37°) nutritive buffer to one part of faeces (v/w). The nutritive medium was made from a carbonate–phosphate buffer solution containing (g/l): $NaHCO_3$ 9·240, $Na_2HPO_4.12H_2O$ 7·125, NaCl 0·470, KCl 0·450, Na_2SO_4 0·100, $CaCl_2$ (anhydrous) 0·055, $MgCl_2$ (anhydrous) 0·047, urea 0·400, with added trace elements (10 ml of the following solution (mg/l) per litre of final solution: $FeSO_4.7H_2O$ 3680, $MnSO_4.7H_2O$ 1900, $ZnSO_4.7H_2O$ 440, $CoCl_2.6H_2O$ 120, $CuSO_4.5H_2O$ 98, $Mo_7(NH_4)_6O_{24}.4H_2O$ 17·4) according to Durand *et al.* (1988). Before use, and during preparation of the inoculum, continuous bubbling of CO_2 maintained anaerobiosis and ensured a constant pH. The slurry was mixed using a Stomacher® (Laboratory Blended, Seward Medical, London) apparatus for 2 min and then filtered through six layers of surgical gauze. The inoculum was maintained in a water bath at 37° and continuously bubbled with CO_2.

Fermentation experiments. Fermentation was conducted in 50 ml polypropylene vials (Becton Dickinson Labware, Lincoln Park, NJ, USA). Except for blanks, 100 mg (dry-matter basis) of well-homogenized experimental substrate was weighed into each vial and

Table 2. *Composition of the five dietary fibre sources (g/kg DM)*

Fibre source...	Cellulose	Maize bran	Pectin	Sugarbeet fibre	Soyabean fibre
DM (g/kg fresh wt)	926	934	875	892	888
Starch*	0	16	140	1	8
TDF†	1011	898	818	778	839
Lipids	6	22	4	3	5
Proteins	3	55	34	145	104
Minerals	1	4	13	37	43
Soluble proteins‡	3	7	21	9	23

TDF, total dietary fibre.
* Determined according to the procedure of Faisant *et al.* (1995).
† Determined according to the method of Prosky *et al.* (1992).
‡ Determined according to Vérité & Demarquilly (1978).

Table 3. *Non-starch polysaccharide content of the dietary fibre sources (g/kg DM)* measured by five European laboratories*

Laboratory	Cellulose	Maize bran	Pectin	Sugarbeet fibre	Soyabean fibre
I	820	808	611	721	782
II	1011	902	677	733	773
III	921	700	723	733	715
IV	900	783	686	606	682
V	827	801	632	658	646
Mean	916	855	644	727	778
SD	135	66	47	8	6

* Measured by the method of Englyst & Hudson (1987).

10 ml inoculum added. Air was displaced by a flow of O_2-free N_2. After the cap was screwed on, the vial was placed horizontally (time 0) in a shaking water bath or an orbital incubator. Fermentation was then performed at 37° and the results studied at 0, 6, 10 and 24 h. Two vials were used for each experimental time and for each substrate. Two blanks were used for each experimental time. At each experimental time, fermentation in corresponding vials was stopped by instantaneous freezing (dry ice).

Sample preparation. Vial contents were homogenized by mechanical mixing (Vortex), the pH was measured and samples were taken for SCFA determinations. Vials were then shaken mechanically and 40 ml absolute ethanol was added (final concentration 800 ml/l). The mixture was thoroughly stirred, kept at 0° for 30 min and then analysed or stored at −20° until carbohydrate analysis.

Experimental design

Ring-test procedure. During the ring-test experiment the five laboratories studied fermentation of the five experimental fibres added to the inoculum at 10 g/l on three different occasions. In each laboratory the same three volunteers were used each time. The inoculum was made by mixing the individual inocula made from each volunteer in equal amounts. At each experimental time, pH, residual NSP and SCFA production were measured.

Complementary experiments. The methodology used during the ring test was investigated in four complementary experiments. In each, the standard procedure (with a change in one specific point) was used, and pH and SCFA production were measured.

(1) Influence of substrate amount. We tested the influence of substrate availability on fermentation in the vials. Three laboratories studied the fermentation of pectin added at 5, 10 (standard) and 20 g/l of the inoculum.

(2) Variability between volunteers with time. Inter- and intrasubject variability were tested by one laboratory. The fermentation of one substrate (soyabean fibre) was studied on three occasions using the individual inocula of four volunteers.

(3) Influence of adaptation. The effect of adaptation of volunteers to the experimental substrates was tested by one laboratory. Two volunteers were used as donors in three states of adaptation: no adaptation or a 2-week adaptation to daily ingestion of 10 g of either pectin or sugarbeet fibre. On two occasions for each state of adaptation, the fermentation of three substrates (pectin, soyabean fibre, sugarbeet fibre) was studied using the individual inocula of each donor.

(4) Fermentation performed with rat or human inocula. In two laboratories, thirty-six rats were fed *ad lib.* for 2 weeks with a 100 g/kg fibre diet prepared according to Livesey *et al.* (1995) in which the initial fibre was replaced by a mixture in equal parts of the five fibre sources. The animals were then killed and the caecum contents collected, pooled and used to make the rat inoculum described above. Fermentation was conducted in both laboratories using rat and human inocula (four volunteers, mixed inocula). Cellulose, pectin and soyabean fibre fermentation was studied.

Analysis

Short-chain fatty acids. In all laboratories, SCFA were quantitated by similar chromatographic methods, each laboratory using its own variant (Holdeman *et al.* 1977; Jouany, 1982; Schooley *et al.* 1985; Mathers *et al.* 1990; Mortensen *et al.* 1991). Evaluation of procedures in the five laboratories was performed during a preliminary ring test on the same six aqueous standards and nine slurries from a single fermentation study.

Non-starch polysaccharides. After thawing, samples were centrifuged (10 min, 1500 *g*), supernatant fractions discarded, and 50 ml of absolute ethanol added to the pellet. After stirring, centrifugation was undertaken as indicated above, pellets were dried in a water bath or on a hotplate at about 60°. Dry residues were hydrolysed with 5 ml 12 M-H_2SO_4 (1 h, 35°). After addition of 25 ml water, vials were left for 1 h in a boiling water-bath. After hydrolysis, vials were cooled in tap water. The carbohydrate content was measured by a colorimetric method (Englyst & Hudson, 1987) using a standard solution (arabinose–glucose–galacturonic acid, 14:7:3 on a molar basis). Participating laboratories were trained in this procedure using the five dietary sources (Table 3).

Calculations

Short-chain fatty acids and residual polysaccharides in slurries. The production P_i of each SCFA was calculated as follows for each experimental time:

$$P_i = (S_i - S_0) - (B_i - B_0), \quad (1)$$

where S_i and S_0 are SCFA concentration values in vials containing substrates at time i and 0 respectively, and B_i and B_0 are SCFA concentration values for blanks at time i and 0 respectively.

For each experimental time, total SCFA production was calculated as the sum of the individual productions of acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids. When significant amounts of SCFA were produced, SCFA ratios were calculated for

acetic (C2), propionic (C3) and butyric (C4) acids and expressed as molar percentages of the sum of the production of these acids.

Calculation of residual NSP was done according to a similar principle, as described by Englyst & Cummings (1988). NSP loss was calculated as the percentage of initial NSP added that disappeared.

Statistics. All statistics were performed using Stat-View II software (Abacus Concept Inc, CA, USA) on an Apple Macintosh IIci microcomputer. ANOVA followed by the least significant difference (LSD) test were used to test the significance of treatment effects for each variable. As data obtained at different times during fermentation could not be considered to constitute independent variables, ANOVA was not performed using time as a variable.

When several laboratories were involved in a study (ring test, rat *v.* human, influence of fibre amounts), the means of the different laboratories were used as repetitions. The standard deviation within laboratories (SD_0 = repeatability) was calculated according to Daniéli (1975) as follows:

$$SD_0^2 = VCr, \tag{2}$$

where VCr is the variance component of the residual. The standard deviation among laboratories (SD_L = reproducibility) was calculated according to Daniéli (1975) as follows:

$$SD_L^2 = (VC_1 - VCr)/n, \tag{3}$$

where VC_1 is the variance component due to laboratories and n the number of occasions.

During variability experiments, SD_s was the standard deviation due to subjects and SD_0 the variability due to the occasions, calculated as described above.

When experiments were conducted in a single laboratory (adaptation, variability), the means of each replication were used as repetitions and SD values were calculated as described above.

RESULTS

Ring test

The mean characteristics of inocula at time 0 are shown in Table 4. All variables were similar within each laboratory but varied between laboratories. Differences in pH were marked even though inocula were prepared with a buffer which should have prevented such variations. Moreover, pH surprisingly varied with SCFA concentrations.

Variations of pH, NSP degradation and SCFA production during fermentation of the different experimental fibre sources are shown in Figs 1, 2 and 3 respectively. There was little or no pH decrease with cellulose, soyabean fibre or maize bran, but very rapid decreases with sugarbeet fibre and pectin. NSP degradation remained very low for cellulose and maize bran but was rapid and high with pectin and soyabean fibre. SCFA production presented similar changes, and there was a very close relationship between NSP breakdown and SCFA production (Fig. 4). The proportions of acetic, propionic and butyric acids are shown in Fig. 5. Significant differences were observed for acetic (pectin *v.* soyabean fibre; $P = 0{\cdot}0053$ at 24 h) and propionic (soyabean and sugarbeet fibre *v.* pectin; $P = 0{\cdot}0001$ at 24 h) acids. There were no significant differences in butyric acid proportions between substrates.

Individual laboratory data concerning pH, NSP degradation and SCFA production are given in Tables 5, 6 and 7 respectively. Differences between laboratories were meaningful and highly significant for all these variables. High measurement repeatability, i.e. low intralaboratory variability, was observed for pH and SCFA production, whereas interlaboratory reproducibility tended to be lower.

Unusual NSP degradation values were sometimes noted, and some negative data were

Table 4. *Characteristics of inocula used by five laboratories in a ring test of* in vitro *fermentation of the dietary fibre sources*

(Mean values and standard deviations for three experiments from each laboratory)

	pH		NSP (g/l)		SCFA (mmol/l)	
Laboratory	Mean	SD	Mean	SD	Mean	SD
I	7·5	0·3	1·7	0·7	24·6	4·5
II	7·0	0·3	5·6	0·7	15·9	5·5
III	7·4	0·2	2·0	1·0	17·4	4·3
IV	6·8	0·0	1·3	0·2	11·1	0·2
V	7·4	0·0	4·9	0·2	18·3	4·8
Mean	7·2	0·3	3·1	2·0	17·5	4·9

SCFA, short-chain fatty acids.

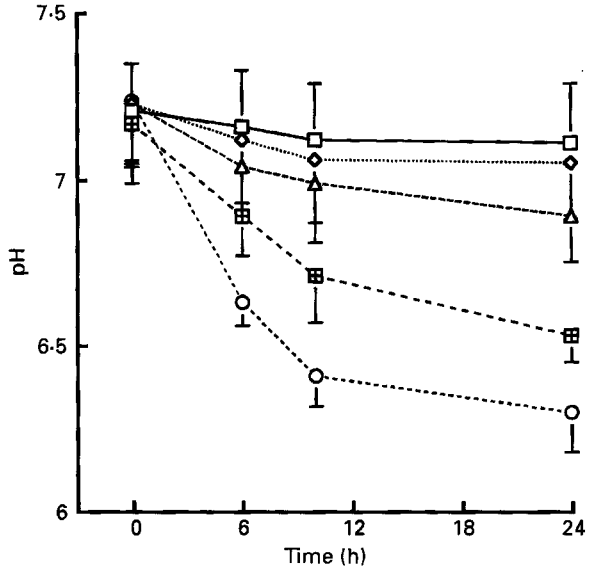

Fig. 1. Changes in pH over time during the *in vitro* fermentation of cellulose (—□—), soyabean fibre (···◇···), pectin (--○--), maize bran (--△--) and sugarbeet fibre (--⊞--) using human faecal inocula. Values are means for five laboratories, each of which made the measurements on three occasions; standard errors of the means are shown by vertical bars.

observed, especially with poorly degradable substrates. Conversely, very high degradation rates were sometimes observed for cellulose, although corresponding SCFA production was not significant. Moreover, intralaboratory reproducibility was much lower for carbohydrate determination than for pH and SCFA production. Despite interlaboratory differences for quantitative data, there was good agreement in ranking the degradation of most of the experimental substrates. Pectin was always found to be highly degraded,

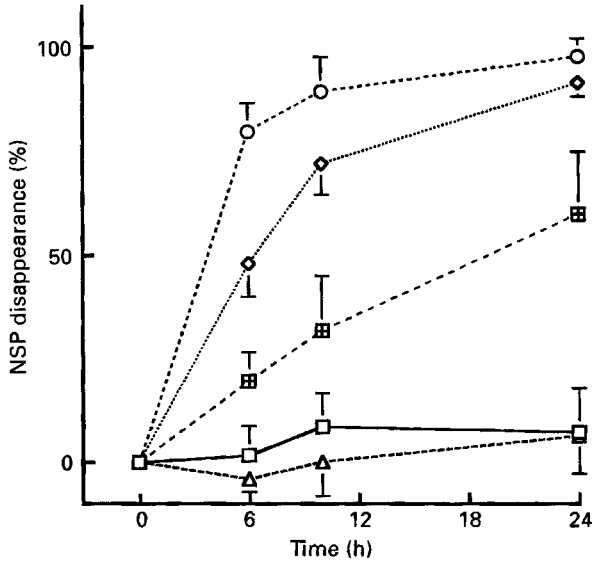

Fig. 2. Disappearance of NSP during the *in vitro* fermentation of cellulose (—□—), soyabean fibre (···◇···), pectin (--○--), maize bran (--△--) and sugarbeet fibre (--⊞--) using human faecal inocula. Values are means for five laboratories, each of which made the measurements on three occasions; standard errors of the means are shown by vertical bars.

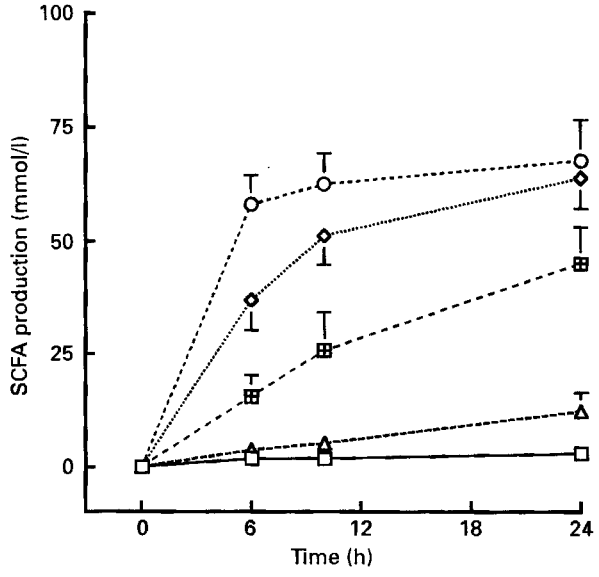

Fig. 3. Production of short-chain fatty acids (SCFA; mmol/l) during the *in vitro* fermentation of cellulose (—□—), soyabean fibre (···◇···), pectin (--○--), maize bran (--△--) and sugarbeet fibre (--⊞--) using human faecal inocula. Values are means for five laboratories, each of which made the measurements on three occasions; standard errors of the means are shown by vertical bars.

followed immediately by soyabean fibre and sugarbeet fibre. However, there was a discrepancy concerning sugarbeet fibre, which two laboratories found to be slightly degraded and the three others quite extensively degraded. Cellulose and maize bran were not, or only slightly, fermented in all laboratories.

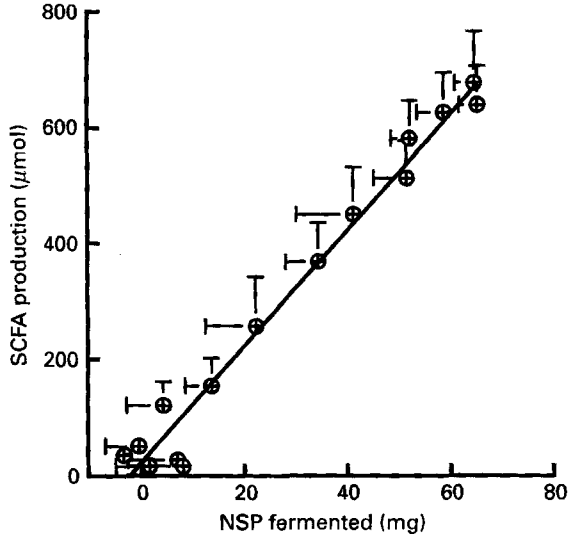

Fig. 4. Relationship between NSP degradation (mg) and short-chain fatty acid (SCFA) production (μmol) in *in vitro* fermentations of dietary fibre sources. The line is described by the equation $y = 9{\cdot}93x + 20{\cdot}8$ (r 0·988). Values are means for five laboratories, each of which made the measurements on three occasions; standard errors of the means are indicated by vertical and horizontal bars.

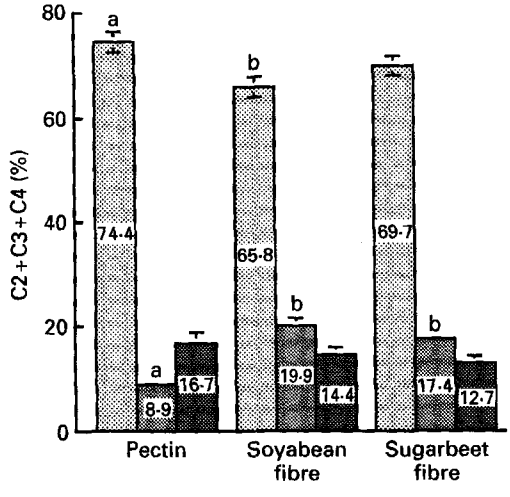

Fig. 5. Molar proportions (%) of the short-chain fatty acids acetate (C2, ⊠), propionate (C3, ▨) and butyrate (C4, ■) produced during the *in vitro* fermentation of pectin, soyabean fibre and sugarbeet fibre using human faecal inocula. Values are means for five laboratories, each of which made the measurements on three occasions; standard errors of the mean are shown by vertical bars. [a, b] Columns within a short-chain fatty acid category bearing different letters were significantly different ($P < 0{\cdot}05$).

SCFA production, despite significant interlaboratory differences, was rated in the same way by all laboratories except V which found slightly higher relative figures for soyabean fibre. SCFA molar ratios (Table 8) differed significantly between laboratories. However, despite certain discrepancies (mainly with acetate), the ranking of each SCFA according to the origin of the dietary source gave generally good agreement for all laboratories.

Table 5. *Changes in the pH* of inocula observed by five laboratories during a ring test of* in vitro *fermentation of the dietary fibre sources*

Fibre source	Time (h)	Laboratory I	II	III	IV	V	Mean	SD_L	SD_O	LSD
Pectin	6	−0·87	−0·20	−1·02	−0·20	−0·66	−0·59	0·37	0·12	0·21
	10	−1·05	−0·35	−1·37	−0·42	−0·89	−0·82	0·41	0·24	0·44
	24	−1·02	−0·53	−1·57	−0·55	−0·99	−0·93	0·42	0·15	0·28
Sugarbeet fibre	6	−0·51	−0·10	−0·50	−0·07	−0·21	−0·28	0·20	0·12	0·22
	10	−0·80	−0·17	−0·92	−0·15	−0·25	−0·46	0·36	0·14	0·25
	24	−1·00	−0·23	−0·97	−0·32	−0·68	−0·64	0·34	0·19	0·35
Soyabean fibre	6	0·00	−0·02	−0·48	−0·10	0·02	−0·12	0·20	0·12	0·22
	10	−0·03	−0·08	−0·63	−0·08	−0·03	−0·17	0·24	0·17	0·31
	24	−0·11	−0·08	−0·59	−0·05	−0·08	−0·18	0·18	0·26	0·47
Maize bran	6	−0·10	−0·02	−0·54	−0·13	−0·14	−0·19	0·20	0·08	0·14
	10	−0·09	−0·08	−0·67	−0·20	−0·18	−0·24	0·23	0·17	0·31
	24	−0·38	−0·12	−0·73	−0·23	−0·25	−0·34	0·16	0·30	0·55
Solka-floc cellulose	6	0·08	−0·13	−0·24	−0·05	0·07	−0·05	0·06	0·21	0·38
	10	0·07	−0·23	−0·34	−0·03	0·08	−0·09	0·13	0·22	0·41
	24	0·06	−0·27	−0·32	−0·05	0·08	−0·10	0·13	0·23	0·43

SD_L, standard deviation among laboratories; SD_O, standard deviation among occasions; LSD, least significant difference ($P < 0·05$).

* Variations of pH are expressed as the difference between pH at experimental times and that at zero time.

Table 6. *Percentage fibre degradability values obtained by five laboratories in a ring test of* in vitro *fermentation of the dietary fibre sources*

Fibre source	Time (h)	Laboratory I	II	III	IV	V	Mean	SD_L	SD_O	LSD
Pectin	6	104·7	66·8	70·9	83·0	71·3	79·3	13·3	13·6	24·7
	10	111·9	85·8	73·8	104·6	69·0	89·0	16·3	16·0	29·2
	24	104·9	93·2	98·6	107·3	83·0	97·4	7·5	10·9	19·8
Sugarbeet fibre	6	36·9	−2·3	29·2	11·7	22·5	19·6	14·4	9·3	16·9
	10	69·6	0·8	56·6	20·7	11·1	31·8	28·7	13·8	25·1
	24	97·8	14·4	77·2	37·6	70·7	59·5	31·5	18·3	33·4
Soyabean fibre	6	69·0	32·6	58·0	26·2	53·6	47·9	17·2	8·5	15·5
	10	95·0	55·0	80·1	56·0	72·0	71·6	14·5	14·8	26·9
	24	98·3	80·1	98·2	88·6	90·1	91·1	2·4	12·5	22·7
Maize bran	6	4·3	−2·2	−5·2	−2·0	−15·4	−4·1	1·2	12·3	22·3
	10	20·8	−4·2	11·6	−0·1	−27·5	0·1	15·8	16·1	29·4
	24	27·2	−4·6	25·0	4·3	−21·0	6·2	15·8	22·0	40·1
Solka-floc cellulose	6	−5·0	1·0	−8·3	29·4	−9·3	1·6	11·5	19·4	35·3
	10	−4·4	7·6	10·6	37·9	−9·0	8·5	14·2	20·0	36·5
	24	−14·9	10·0	7·3	45·3	−11·9	7·2	21·8	17·4	31·6

SD_L, standard deviation among laboratories; SD_O, standard deviation among occasions; LSD, least significant difference ($P < 0·05$).

Complementary experiments

Influence of substrate amount. During the course of fermentation, pH ranged between 6·67–6·97, 6·49–6·98 and 5·40–6·88 when fibre was added at levels of 5, 10 and 20 g/l respectively. Although pH values as low as 5 have already been reported, pH values with

Table 7. *Values for short-chain fatty acid production (mmol/l) obtained by five laboratories during a ring test of* in vitro *fermentation of the dietary fibre sources*

Fibre source	Time (h)	Laboratory I	II	III	IV	V	Mean	SD_L	SD_O	LSD
Pectin	6	76·4	67·4	54·1	39·5	52·5	58·0	12·7	11·2	0·2
	10	70·5	82·7	57·2	44·5	55·2	62·0	14·4	6·3	0·4
	24	76·1	96·6	67·3	46·4	52·1	67·7	19·7	5·9	0·3
Sugarbeet fibre	6	32·1	10·1	19·4	6·9	8·3	15·4	10·1	5·1	9·3
	10	53·7	17·9	36·9	6·0	13·8	25·7	18·4	8·9	16·2
	24	72·2	30·0	53·9	29·1	38·4	44·7	17·4	9·0	16·4
Soyabean fibre	6	59·7	33·1	35·1	18·3	37·5	36·7	14·9	6·7	12·5
	10	69·5	52·1	50·1	29·4	54·8	51·2	14·2	7·1	13·2
	24	78·8	75·2	63·8	41·2	59·2	63·6	13·1	13·4	24·8
Maize bran	6	4·9	3·6	5·3	1·0	4·5	3·9	1·4	3·6	6·6
	10	6·8	4·4	11·0	4·5	−1·5	5·0	3·5	4·9	9·0
	24	25·7	8·0	16·6	6·8	3·3	12·1	8·0	7·8	14·1
Solka-floc cellulose	6	2·4	1·0	1·9	0·1	3·0	1·7	1·1	2·6	4·8
	10	3·5	1·9	2·1	1·9	−1·0	1·7	0·7	2·7	5·1
	24	6·9	5·2	2·4	0·7	−0·3	3·0	2·6	2·1	4·0

SD_L, standard deviation among laboratories; SD_O, standard deviation among occasions; LSD, least significant difference ($P < 0·05$).

fibres added at the 20 g/l level were out of the generally accepted 6–7 range. Increased amounts of fibre led to greater SCFA production (Fig. 6). Additional SCFA production was low when amounts increased from 10 to 20 g/l, especially for the first fermentation periods. Increasing the amount of fibre led to a reduction of fibre degradation rate. Regardless of the fermentation stage, there were no significant modifications of the SCFA ratio between the different fibre levels.

Variability between volunteers with time. Variations in SCFA production and ratios between donors on a day-to-day basis are given in Table 9. Although there were some interdonor differences, only a few, namely those concerning the proportion of propionate produced at 6 and 10 h, were significant. The results showed that variability for each donor from one day to the next was greater than the variations observed between donors indicating that the intra- and interdonor variability are very much in the same range.

Influence of adaptation. The influence of donor adaptation on SCFA production is shown in Table 10. Irrespective of substrate tested, the type of adaptation and the time of fermentation, no influence of adaptation on fermentation was found. The relative proportions of SCFA (not reported) were not influenced by donor adaptation.

Rat v. *human inocula.* Data from the experiment conducted with rat caecal inocula *v.* human inocula are presented in Table 11. Results for all variables were similar regardless of the nature of the inoculum. No statistically significant differences were found.

DISCUSSION

In vitro fermentation of five dietary fibre sources was studied during a ring test by five groups of investigators using the same protocols on three different occasions. A complex nutritive solution, buffered with carbonate and phosphate and supplemented with trace elements and urea as a N source, was used. Carbonate is a physiological component secreted within the digestive tract, and bacteria need trace elements and N for growth. The high degradation obtained with several fibres clearly indicated that significant bacterial

Table 8. *Short-chain fatty acid profiles (mol/100 mol) observed by five laboratories in a ring test of* in vitro *fermentation of the dietary fibre sources*

Fibre	Time (h)		Laboratory I	II	III	IV	V	Mean	SD_L	SD_O	LSD
Pectin	6	C2	77·3	86·4	80·0	83·2	75·0	80·4	2·8	6·2	11·3
		C3	11·4	8·9	8·6	3·0	8·1	8·0	3·0	1·2	2·1
		C4	11·3	4·7	11·3	13·8	16·9	11·6	3·0	5·8	10·5
	10	C2	75·2	84·5	75·6	74·4	71·0	76·1	4·2	4·8	8·7
		C3	13·4	10·4	9·6	6·5	8·0	9·6	2·4	2·0	3·6
		C4	11·4	4·2	14·8	19·2	21·1	14·1	5·8	4·6	8·5
	24	C2	71·1	81·9	77·8	76·6	64·6	74·4	6·1	5·1	9·3
		C3	13·5	9·6	10·6	2·2	8·8	8·9	4·1	1·4	2·6
		C4	15·5	8·5	11·6	21·1	26·7	16·7	6·7	4·9	9·0
Sugarbeet fibre	6	C2	71·0	92·1	83·8	65·0	80·6	78·5	9·3	9·2	16·7
		C3	22·0	7·3	10·6	13·4	11·1	12·9	3·4	7·6	13·8
		C4	7·0	0·6	5·6	21·5	8·3	8·6	7·4	4·4	8·1
	10	C2	71·6	92·5	70·2	65·9	75·5	75·2	9·5	6·5	13·5
		C3	20·6	6·8	16·0	13·4	13·8	14·1	4·1	5·4	9·9
		C4	7·8	0·7	13·8	20·7	10·8	10·8	6·8	2·9	5·3
	24	C2	68·6	82·1	66·4	65·4	65·9	69·7	6·8	3·3	6·0
		C3	20·6	14·2	17·7	19·1	16·7	17·7	2·2	2·5	4·5
		C4	10·8	3·7	16·0	15·5	17·4	12·7	5·4	2·6	4·7
Soyabean fibre	6	C2	60·1	83·2	73·5	73·7	64·6	71·0	9·3	4·1	7·6
		C3	28·0	13·9	13·4	14·9	22·7	18·5	9·9	9·4	17·4
		C4	11·9	3·0	13·1	11·5	12·8	10·4	4·2	5·7	10·5
	10	C2	61·6	81·3	66·5	65·6	64·9	68·0	7·5	4·1	7·6
		C3	27·3	14·5	17·4	23·9	21·4	20·9	5·0	2·6	4·9
		C4	11·1	4·2	16·1	10·6	13·7	11·1	4·4	2·2	4·1
	24	C2	58·0	75·2	66·2	65·4	61·0	65·2	6·4	3·8	7·0
		C3	28·4	19·1	17·4	19·1	21·1	21·0	5·5	3·0	5·5
		C4	13·6	5·8	16·4	15·5	17·9	13·8	4·9	3·3	6·1

SD_L, standard deviation among laboratories; SD_O, standard deviation among occasions; LSD, least significant difference ($P < 0·05$); C2, acetate; C3, propionate; C4, butyrate.

activity was present in the slurries throughout the 24 h experiments. During preparation of faecal slurries, homogenates were filtered through gauze and a rather complex medium was used, but it remains unclear whether this procedure had any effect on the experimental results.

Although all five fibre sources contained large amounts of dietary fibre, some contaminant compounds were present in significant amounts: for example, protein and starch represented up to approximately 15% of sugarbeet fibre and pectin respectively. When fermented, proteins lead to a much lower production of SCFA than carbohydrates do (Macfarlane *et al.* 1988): their role in fermentation was hence neglected. Starch is a normal component of apples, especially in unripened fruits (Gorin *et al.* 1978), and is commonly present in apple pectin (Kravtchenko *et al.* 1992). It could have been chemically (Kravtchenko *et al.* 1992) or enzymically (Pishchiiski & Lyutskanov, 1978; Kravtchenko *et al.* 1992) removed to avoid production of SCFA from non-fibre material, but such a treatment can lead to partial destruction of the pectic fraction (Lyutskanov *et al.* 1974). Little information is available about apple starch; however, Sawai *et al.* (1978) showed that the X-ray diffraction pattern of starch from unripened apples is close to that of B type starch. As far as we know, this kind of starch is poorly degradable and is not fermented by colonic bacteria (Wyatt & Horn, 1988). Hence, fermentation of apple starch was neglected.

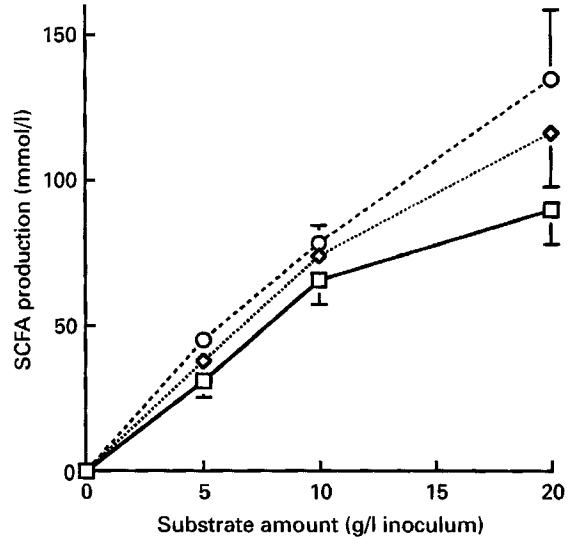

Fig. 6. Influence of the amount of substrate added to the inoculum on short-chain fatty acid (SCFA) production (mmol/l) during the course of *in vitro* fermentations using human faecal bacteria. Fermentation times were 6 (—□—), 10 (--◇--) and 24 (--○--) h. Values are means for three laboratories, with their standard errors represented by vertical bars.

Table 9. *Total short-chain fatty acid (SCFA) production (mmol/l) and molar proportions of the individual SCFA (%) produced during* in vitro *fermentation of the dietary fibre sources by faecal inocula from four different donors*

	Time (h)	Donor A	B	C	D	Mean	SD_S	SD_O	LSD
SCFA	6	52·5	28·0	40·1	32·3	38·2	7·8	12·8	24·5
	10	69·1	27·9	60·1	55·5	53·2	3·9	19·7	37·0
	24	90·0	73·6	89·6	74·6	82·0	9·1	19·3	36·4
Acetate	6	51·8	54·9	49·9	58·6	53·8	3·8	8·5	16·0
	10	55·4	51·7	50·7	58·2	54·0	0·7	5·8	11·0
	24	53·7	49·1	51·9	59·1	53·4	3·4	4·8	10·3
Propionate	6	23·3	16·8	35·5	33·2	27·2	8·4	4·0	7·6
	10	23·1	22·2	33·8	34·4	28·4	6·2	3·9	7·3
	24	24·8	27·6	32·7	23·0	27·0	3·0	5·0	10·7
Butyrate	6	24·9	28·3	14·6	8·2	19·0	7·1	10·3	19·3
	10	21·4	26·1	15·5	7·4	17·6	6·8	7·5	14·2
	24	21·6	23·3	15·4	6·7	16·8	5·8	8·3	17·9

SD_S, standard deviation among subjects; SD_O, standard deviation among occasions; LSD, least significant difference ($P < 0·05$).

The five fibre sources were selected to represent the maximal range of potential fermentabilities likely to be encountered in *in vitro* experiments. The mean degradation rates determined during the ring test are in good agreement with previously published results for cellulose (Ehle *et al.* 1982; Jeraci & Horvath, 1989), pectin (Titgemeyer *et al.* 1991), sugarbeet fibre (Titgemeyer *et al.* 1991; Guillon *et al.* 1992; Salvador *et al.* 1993), maize bran (Titgemeyer *et al.* 1991) and soyabean fibre (Titgemeyer *et al.* 1991). The low degradation of cellulose (high crystallinity) and high breakdown of pectin, soyabean fibre

Table 10. *Analysis of variance of the adaptation experiment**

Incubation time (h)	Factors	df	Total squares	Mean squares	*F*	*P*
6	Adaptation	2	10·49	5·24	0·01	0·9856
	Substrate	2	4873·76	2436·88	6·75	0·0162
	Interaction	4	254·69	63·67	0·18	0·9449
	Residual error	9	3248·15	360·91		
10	Adaptation	2	19·7	9·85	0·02	0·9809
	Substrate	2	3172·27	1586·14	3·1	0·0943
	Interaction	4	140·12	35·03	0·07	0·9899
	Residual error	9	4597·62	510·85		
24	Adaptation	2	106·05	53·03	0·29	0·7561
	Substrate	2	1074·92	537·46	2·92	0·1051
	Interaction	4	176·3	44·07	0·24	0·9088
	Residual error	9	1654·54	183·84		

* For details of the adaptation experiment, see p. 307.

Table 11. *Values for pH, total short-chain fatty acid (SCFA) production (mmol/l) and molar proportions of the individual SCFA (%) during* in vitro *fermentation of pectin and soyabean fibre by faecal inocula from rats and humans*

(Mean values and standard deviations for two laboratories)

		Time (h)	pH		SCFA		Acetate		Propionate		Butyrate	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pectin	Rat	0	6·91	0·36	—	—	—	—	—	—	—	—
		6	6·13	0·99	63·4	3·3	78·2	3·8	10·9	0·9	11·0	2·6
		24	6·57	0·40	60·9	13·6	74·9	4·8	11·8	0·6	13·4	3·9
	Human	0	6·83	0·19	—	—	—	—	—	—	—	—
		6	6·24	0·22	65·4	2·7	77·0	1·5	10·1	1·9	13·0	1·2
		24	6·28	0·10	70·2	7·2	71·8	0·5	12·1	2·1	16·2	2·6
Soyabean fibre	Rat	0	6·97	0·45	—	—	—	—	—	—	—	—
		6	5·98	0·31	49·0	14·0	63·9	2·6	21·0	0·9	15·1	3·4
		24	6·37	0·25	65·3	0·6	62·0	5·0	21·5	0·6	16·6	4·3
	Human	0	6·94	0·34	—	—	—	—	—	—	—	—
		6	6·26	0·49	64·1	32·5	64·4	4·5	19·8	3·6	15·9	0·9
		24	6·54	0·37	87·7	28·0	66·9	6·9	19·8	5·2	13·4	1·6

and sugarbeet fibre (high soluble-fibre content and pectin-rich primary cell walls) were as expected. The low rates of breakdown for maize bran were more surprising since this substrate has a very low lignin content. The limiting factor for wheat-bran degradability is thought to be the presence of branched arabinoxylans (Salvador *et al.* 1993). The fact that such highly branched compounds are found in maize bran, in which they are associated with high levels of phenolic acids (Saulnier *et al.* 1993), may account for the low fermentability of this fibre.

pH variations, when indicative of SCFA production, can be used as an index of fibre fermentation (Guillon *et al.* 1992). Surprisingly, even though soyabean fibre was highly fermented, pH during the ring test varied only slightly. Since NH_3 can be produced during fermentation of nitrogenous compounds (Vince *et al.* 1990), this may have limited the pH decrease. Although NH_3 concentrations were not measured, it is unlikely that this factor

could account for the unusual behaviour of this fibre. The amounts and solubilities of nitrogenous compounds were not very different from those measured in the other fibre sources, especially pectin, which exhibited a dramatic pH decrease. These results suggest that pH on its own should not be used as an index of fermentability.

Carbohydrate determinations in complex media led to some problems. Since bacteria contain some carbohydrates (Stephen & Cummings, 1980) which would be measured by the colorimetric procedure used in this ring test, it was important to subtract the blank value representing the carbohydrate in the inoculum at each fermentation time. Second, it was observed that faecal slurries interfered with the colorimetric reaction. Amounts of carbohydrate, when determined after addition to the inoculum, were always lower than the actual values. This problem was corrected by performing all determinations, including time 0, in slurries. However, this interference appeared to be greater with substrates such as pectin or soyabean fibre. Although care was taken to stop fermentation as soon as substrates were added in the corresponding blanks, rapid breakdown could have occurred with these highly degradable fibres. In so far as these substrates were totally degraded at the end of fermentation, this problem should only have decreased the degradability measured during the first fermentation periods. However, this may have affected the overall fermentability estimation of substrates with intermediary degradability, such as sugarbeet fibre.

With non-fermentable substrates (cellulose, maize bran), carbohydrate determinations gave some unexpected values. Problems in the quantification of crystalline cellulose often occurred. Due to its crystalline structure, this substrate resists hydrolysis (Hoebler *et al.* 1989). The occasional unexpectedly high figures for cellulose degradation suggest that the carbohydrate was hydrolysed less readily in the inoculum than in its native form.

On the whole, carbohydrate determinations were not sufficiently accurate. This imprecision was due at least in part to the high values sometimes observed with blank samples. Because the method was based on different measurements, its precision was inversely related to the relative importance of blank values. One solution could be to use more diluted inocula (McBurney & Thompson, 1987, 1989; McBurney *et al.* 1988; Adiotomre *et al.* 1990). In this case, however, the incubations would be completely non-physiological. With respect to the non-specific carbohydrate determination methods used, it would appear that a compromise needs to be found between the high fermentative activity of an inoculum obtained with low dilutions of faeces, and the low interferences obtained with high dilutions of sample. Though specific methods involving chromatographic determination of carbohydrates are more complex and thus less suited to routine use, they can provide accurate results, even with relatively concentrated inoculum (Guillon *et al.* 1992).

A very close relationship was found between NSP loss and SCFA production. Degradation has been reported for only one dietary fibre source (laminarin from seaweed) when associated SCFA production was low (Michel *et al.* 1995). In our experiments, the rate of conversion of hydrolysed NSP into SCFA was constant, irrespective of carbohydrate origin. This has been reported previously (Salvador *et al.* 1993). As SCFA determination is precise and easy to perform, this variable, which is predictive of almost all variations in substrate degradation (r^2 0·98), can be used in most experiments as an index of fibre degradation. However, it must be emphasized for future studies that the molar yield of SCFA from carbohydrate differs according to the particular end-products produced. In this respect, some discrepancies between the sum of SCFA produced and the NSP fermented could be observed when very different SCFA ratios are observed.

SCFA profiles calculated for substrates exhibiting significant degradation (pectin, soyabean fibre, sugarbeet fibre) were in close agreement with previous findings. For all

substrates, acetate was the major SCFA produced, and acetate and propionate were the two SCFA showing variability between different substrates. The highest acetate production was observed with pectin, as reported previously (Goodlad & Mathers, 1988; Titgemeyer *et al.* 1991). This may be related to the fermentation of uronic acids to acetate (Salvador *et al.* 1993). Conversely, the fact that pectin fermentation resulted in a very low proportion of propionic acid is in close agreement with previous findings *in vivo* (Englyst *et al.* 1987; Mathers & Finlayson, 1989) and *in vitro* (Goodlad & Mathers, 1988) and confirms that uronic acid metabolism is not involved in production of propionate (Salvador *et al.* 1993). Soyabean fibre fermentation was characterized by relatively high proportions of propionate, which is concordant with the findings of Levrat *et al.* (1991). There were no differences between the substrates with respect to butyrate which contributed between about 13 and 17% of SCFA products. These results contrast with those obtained when a continuous *in vitro* fermenter was used, which indicated very low butyrate production with pectin (Edwards *et al.* 1985). As significant amounts of butyrate have been quantified *in vivo*, it would appear that *in vitro* batch systems reproduce colonic fermentations at least as well as do continuous fermenters.

The procedure for the ring test gave results in good agreement with published data. Nonetheless, the use of the same procedure and substrates by laboratories accustomed to conducting such *in vitro* experiments led to differences concerning the rate of substrate degradation, the amount of SCFA produced and the relative proportion of each major SCFA. As the main potential source of variation was presumably the activities of bacteria in the faeces used for the preparation of inocula, a series of experiments analysing this variability was carried out. Interdonor variability was not significant. This is in close agreement with previous results of Mortensen *et al.* (1991) who found the intra- and interindividual variations were of the same magnitude but disagrees with the findings of McBurney & Thompson (1987). This could have been due to our use of replications on different occasions, whereas McBurney & Thompson (1987) only used duplicates made with the same faeces, which are not indicative of intradonor variability. Our experiment showed that intradonor variability was on the whole in the same range as interdonor variability. It would thus seem important to replicate experiments as well as donors.

Although large donor-related variations in activity were found between laboratories in the same country, and could also have occurred between countries because of eating habits, our experiments indicate that donor adaptation to fibre ingestion was not an influential factor. Such an adaptation effect was recently pointed out (Michel *et al.* 1996) concerning a very unusual fibre from seaweed. However, in this case, adaptation was achieved in a very short time (about 6 h), did not make products fermentable which were previously unfermentable, and changed the rate and not the extent of degradation of already fermentable fibre without altering the profile of major SCFA. For common fibres at least, the effect of the donor diet probably cannot account for interlaboratory differences. Hence, it does not seem necessary to adapt volunteers to a fibre when fermentation is to be studied *in vitro*.

Experiments conducted with rat inocula compared with human inocula confirmed these conclusions. Within a laboratory there was no difference between fermentation conducted with inocula made from caecal contents of the adapted rat, or with those made from unadapted human donors, as previously noted by Nyman *et al.* (1986). This seems to confirm that the nature of fibre during fermentation is more important than the source of the flora. Nonetheless, large differences were observed between laboratories. Since donor variability cannot account for these variations, the cause remains unclear.

Interlaboratory differences concerned the extent of fermentation in particular. These differences did not apply to highly fermentable fibres which were always extensively

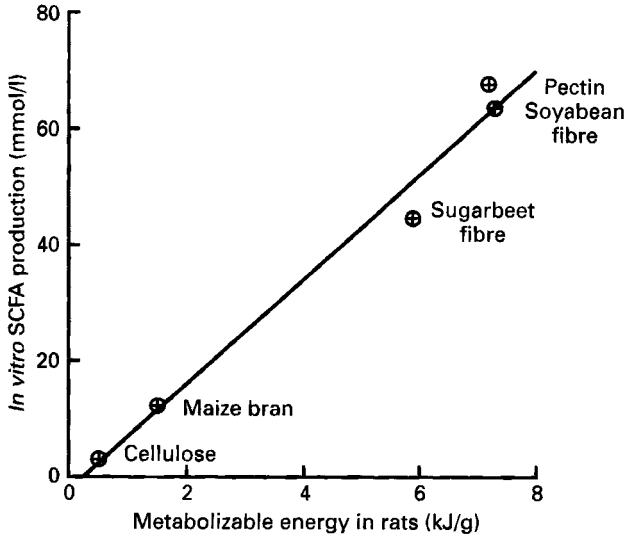

Fig. 7. Relationship between the metabolizable energy of five different fibre sources (determined in rats; Livesey *et al.* 1995) and production of short-chain fatty acids (SCFA) from the same fibre sources *in vitro*. The line is described by the equation $y = 9{\cdot}00x - 2{\cdot}12$ (r 0·991).

fermented, or to unfermentable products which were never broken down, but to fibres with intermediate fermentability such as sugarbeet fibre, which are highly degraded with high-activity inocula or poorly degraded with low-activity inocula. In so far as *in vitro* experiments are used to study the intrinsic degradability of fibre, they should be undertaken with excess degrading activity in the faecal inocula. Experiments concerned with the influence of substrate amounts demonstrated that inoculum activity was the limiting factor when substrate was added at levels higher than 10 g/l. During the ring-test experiment some laboratories may have worked with limited-activity inocula, though this question remains to be answered. A potential solution could be reached either by lowering faeces dilution or be decreasing the substrate amount from 10 to 5 g/l. In both cases, this would increase background for carbohydrate determination and indicate the need for a more specific form of carbohydrate determination than colorimetric methods.

Another difference between laboratories concerned the molar ratios of SCFA produced during fermentation. Once again, substrate ranking was the same for all laboratories. On the whole, it would appear to be difficult to obtain absolute data either for the fibre degradation rate or SCFA molar ratios. However, when only relative degradation data are needed, the methodology employed here allows accurate ranking of fibre degradability. Because some variability cannot be avoided, it would seem preferable to carry out experiments with well-referenced standard fibres. Moreover, future use of such a method should take into account the ISO Standard 5725 precision of test. Since the ranking of fibre degradation or the SCFA ratio was not modified by the length of the *in vitro* fermentation period, and in so far as only relative figures are needed, future studies could be conducted over shorter times than 24 h. However, short-term fermentations reflect the rate of fermentation, whereas extended fermentations indicate the extent of fermentation: in this respect, short and long incubations are necessary.

The same dietary fibre sources as those used here were previously tested *in vivo* in rats (Livesey *et al.* 1995). There was a very close relationship between *in vitro* (y) and *in vivo* (x) fibre degradation ($y = 0{\cdot}728x - 2{\cdot}786$, r 0·986) which indicates that there was a very close

agreement between data for both modes of measurement. NSP degradation was lower *in vitro*: we do not know how long fibres stayed in the colon of rats; if *in vitro* fermentation times had been extended to 30 or 36 h, *in vitro* fermentation figures would have been closer to those observed *in vivo*. This comparison validates the methodology tested here *in vitro*, which is accurate when rapid classification of fibre degradation by colonic bacteria is required. The relationship between *in vitro* fibre degradation and *in vivo* metabolizable energy of fibre sources is shown in Fig. 7. This relationship clearly indicates that the *in vitro* methodology which was tested during this experiment is accurate for a rapid determination of energy content of dietary fibre.

CONCLUSION

Many previous studies have used *in vitro* batch fermenters to study dietary fermentation by intestinal bacteria. Until now, none of these methodologies has been validated, which led us to undertake this first interlaboratory study. In most cases, very similar results were found during the ring test, even though some interlaboratory differences of unknown origin remain. The methodology used allows rapid, accurate study of dietary fibre fermentation, and the experimental results show a close relationship between colonic NSP degradation and SCFA production. Moreover, *in vitro* SCFA production was highly correlated with fibre energy value in rats. On the whole, the *in vitro* methodology tested allows rapid prediction of the energy content of fibre.

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REFERENCES

Adiotomre, J., Eastwood, M. A., Edwards, C. A. & Brydon, W. (1990). Dietary fibre: *in vitro* methods that anticipate nutrition and metabolic activity in humans. *American Journal of Clinical Nutrition* **52**, 128–134.

Barry, J.-L., Chourot, J.-M., Bonnet, C., Kozlowski, F. & David, A. (1989). *In vitro* fermentation of neutral monosaccharides by ruminal and human fecal microflora. *Acta Veterinaria Scandinavica* **86**, Suppl., 93–95.

Cherbut, C. (1995). Effects of short chain fatty acids on gastro-intestinal motility. In *Physiological and Clinical Aspects of Short Chain Fatty Acid Metabolism*, pp. 191–207 [J. H. Cummings, T. Sakata and J. L. Rombaud, editors]. Cambridge: Cambridge University Press.

Cummings, J. H., Pomare, E. W., Branch, W. J., Naylor, C. P. E. & Macfarlane, G. T. (1987). Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* **28**, 1221–1227.

Daniéli, P. (1975). Théorie et méthodes statistiques. Vol II: les méthodes de l'interférence statistique, pp. 121–122. Gembloux, Belgium: Les presses agronomiques de Gembloux.

Durand, M., Dumay, C., Beaumatin, P. & Morel, M.-T. (1988). Use of the rumen simulation technique (RUSITEC) to compare microbial digestion of various by-products. *Animal Feed Science and Technology* **21**, 197–204.

Edwards, C. A., Duerden, B. I. & Read, N. W. (1985). Metabolism of mixed human colonic bacteria in a continuous culture mimicking the human cecal contents. *Gastroenterology* **88**, 1903–1909.

Ehle, F. R., Jeraci, J. L., Robertson, J. B. & Van Soest, P. J. (1982). The influence of dietary fibre on digestibility, rate of passage and gastrointestinal fermentations in pigs. *Journal of Animal Science* **55**, 1071–1080.

Englyst, H. N. & Hudson, G. J. (1987). Colorimetric method for routine measurement of dietary fibre as non-starch polysaccharides. A comparison with gas-liquid chromatography. *Food Chemistry* **24**, 63–76.

Englyst, H. N. & Cummings, J. H. (1988). An improved method for the measurement of dietary fibre as the non-starch polysaccharides in plant foods. *Journal of the Association of Official Analytical Chemists* **71**, 808–814.

Englyst, H. N., Hay, S. & Macfarlane, G. T. (1987). Polysaccharide breakdown by mixed populations of human faecal bacteria. *FEMS Microbiology Ecology* **95**, 163–171.

Faisant, N., Planchot, V., Kozlowski, F., Pacouret, M.-P., Colonna, P. & Champ, M. (1995). Resistant starch determination adapted to products containing high levels of resistant starch. *Science des Aliments* **15**, 83–89.

Fidgor, S. K. & Bianchine, J. R. (1983). Caloric utilization and disposition of [^{14}C]polydextrose in man. *Journal of Agricultural and Food Chemistry* **31**, 389–393.

Flick, J. A. & Perman, J. A. (1989). Nonabsorbed carbohydrate: effect on fecal pH in methane-excreting and non-excreting individuals. *American Journal of Clinical Nutrition* **49**, 1252–1257.

Gibson, G. R., Cummings, J. H. & Macfarlane, G. T. (1988). Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulfate reduction and methanogenesis by mixed populations of human gut bacteria. *Applied and Environmental Microbiology* **54**, 2750–2755.

Goodlad, J. S. & Mathers, J. C. (1988). Effects of food carbohydrates on large intestinal fermentation in vitro. *Proceedings of the Nutrition Society* **47**, 176A.

Gorin, N., Bonisolli, F., Heidema, F. T., Klop, W. & Williams, A. A. (1978). Changes in starch content and amylase zymograms during storage of Golden delicious and Cox's orange pippin apples. *Zeischtrift für Lebensmittel-Undersuchung und -Forschung* **166**, 157–161.

Guillon, F., Barry, J.-L. & Thibault, J.-F. (1992). Effect of autoclaving sugar-beet fibre on its physico-chemical properties and its *in-vitro* degradation by human faecal flora. *Journal of the Science of Food and Agriculture* **60**, 69–79.

Hoebler, C., Barry, J.-L., David, A. & Delort-Laval, J. (1989). Rapid acid hydrolysis of plant cell wall polysaccharides and simplified quantitative determination of their neutral monosaccharides by gas-liquid chromatography. *Journal of Agricultural and Food Chemistry* **37**, 360–367.

Holdeman, L. V., Cato, E. P. & Moore, W. E. C. (eds) (1977). *Anaerobic Laboratory Manual*, 4th ed. Blacksburg, VA: VPI Anaerobe Laboratory.

Jeraci, J. L. & Horvath, P. J. (1989). *In vitro* fermentation of dietary fiber by human fecal organisms. *Animal Feed Science and Technology* **23**, 121–140.

Jouany, J.-P. (1982). Dosage des acides gras volatils (AGV) et des alcools dans les contenus digestifs, les jus d'ensilage, les cultures bactériennes et les contenus de fermenteurs anaérobies (Volatile fatty acid and alcohol determination in digestive contents, silage juices, bacterial cultures and anaerobic fermenter contents). *Science des Aliments* **2**, 131–144.

Kravtchenko, T. P., Voragen, A. G. J. & Pilnik, W. (1992). Analytical comparison of three industrial pectin preparations. *Carbohydrate Polymers* **18**, 17–25.

Levrat, M. A., Behr, S. R., Rémésy, C. & Demigné, C. (1991). Effect of soybean fiber on cecal digestion in rats previously adapted to a fiber-free diet. *Journal of Nutrition* **121**, 672–678.

Livesey, G. (1990). Energy value of unavailable carbohydrates and diets. An inquiry and analysis. *American Journal of Clinical Nutrition* **51**, 617–637.

Livesey, G., Smith, T., Eggum, B. O., Tetens, I. H., Nyman, M., Roberfroid, M., Delzenne, N., Schweizer, T. F. & Decombaz, J. (1995). Determination of digestible energy values and fermentabilities of dietary fibre supplements: a European interlaboratory study *in vivo*. *British Journal of Nutrition* **74**, 289–302.

Lyutskanov, N., Pishtiiskii, I. & Krachanov, K. (1974). Enzymic purification of apple pectin. In *Pervyi simposium Proizvodstvo i primeneie fermentnykh preparatov v pishchevoi promyshlennosti izhivotnovodstve*, 726–727 [G. Klyushnik, V. Rzhendovski, E. Rembovski, N. Nestorov, A. Grozdanov, P. Konishev, G. Uzunov, S. Chara, T. Angelov, I. Grigorov and M. Angelova, editors]. USSR: Koordinatsionnyi Tseutr po Problem.

McBurney, M. I., Horvath, P. J., Jeraci, J. L. & Van Soest, P. J. (1985). Effect of *in vitro* fermentation using human faecal inoculum on the water-holding capacity of dietary fibre. *British Journal of Nutrition* **53**, 17–24.

McBurney, M. I. & Thompson, L. U. (1987). Effect of faecal inoculum on *in vitro* fermentation variables. *British Journal of Nutrition* **58**, 233–243.

McBurney, M. I. & Thompson, L. U. (1989). Effect of human faecal donor on *in vitro* fermentation variables. *Scandinavian Journal of Nutrition* **24**, 359–367.

McBurney, M. I., Thompson, L. U., Cuff, D. J. & Jenkins, D. J. A. (1988). Comparison of ileal effluents, dietary fibers, and whole foods in predicting the physiological importance of colonic fermentation. *American Journal of Gastroenterology* **83**, 536–540.

Macfarlane, G. T., Allison, C., Gibson, S. A. W. & Cummings, J. H. (1988). Contribution of the microflora to proteolysis in the human large intestine. *Journal of Applied Bacteriology* **64**, 37–46.

Mallett, A. K., Bearne, C. A. & Rowland, I. R. (1983). Metabolic activity and enzyme induction in rat fecal microflora maintained in continuous culture. *Applied and Environmental Microbiology* **46**, 591–595.

Mathers, J. C., Fernandez, F., Hill, M. J., McCarthy, P. T., Shearer, M. J. & Oxley, A. (1990). Dietary modification of potential vitamin K supply from enteric bacterial menaquinones in rats. *British Journal of Nutrition* **63**, 639–652.

Mathers, J. C. & Finlayson, H. J. (1989). Manipulation of rat caecal metabolism by incubating Avoparin and pectin in the diet. *Proceedings of the Nutrition Society* **48**, 139A.

Michel, C., Lahaye, M., Bonnet, C., Mabeau, S. & Barry, J.-L. (1996). *In vitro* fermentation by human faecal bacteria of total and purified dietary fibres from brown seaweeds. *British Journal of Nutrition* (In the Press).

Miller, T. M. & Wolin, M. J. (1981). Fermentation by the large intestine microbial community in an *in vitro* semicontinuous culture system. *Applied and Environmental Microbiology* **42**, 400–407.

Mortensen, P. B., Heghøj, J., Rannen, T., Rasmussen, H. S. & Holtug, L. (1989). Short-chain fatty acids in bowel contents after intestinal surgery. *Gastroenterology* **97**, 1090–1096.

Mortensen, P. B., Holtug, K. & Rasmussen, H. S. (1988). Short-chain fatty acid production from mono- and disaccharides in a fecal incubation system: implications for colonic fermentation of dietary fiber in humans. *Journal of Nutrition* **118**, 321–325.

Mortensen, P. B., Hove, H., Clausen, M. R. & Holtug, K. (1991). Fermentation to short-chain fatty acids and lactate in human faecal batch cultures. *Scandinavian Journal of Gastroenterology* **26**, 1285–1294.

Mortensen, P. B. & Nordgaard-Andersen, I. (1993). The dependence of the *in vitro* fermentation of dietary fibre to short-chain fatty acids on the contents of soluble non-starch polysaccharides. *Scandinavian Journal of Gastroenterology* **28**, 418–422.

Nyman, M., Asp, N. G., Cummings, J. H. & Wiggins, H. (1986). Fermentation of dietary fibre in the intestinal tract: comparison between man and rat. *British Journal of Nutrition* **55**, 487–496.

Patil, D. H., Westaby, D., Mahida, Y. R., Palmer, K. R., Rees, R., Clark, M. L., Dawson, A. M. & Silk, D. B. A. (1987). Comparative modes of action of lactitol and lactulose in the treatment of hepatic encephalopathy. *Gut* **28**, 255–259.

Perman, J. A. & Modler, S. (1982). Glycoproteins as substrates for production of hydrogen and methane by colonic bacterial flora. *Gastroenterology* **83**, 388–393.

Pishchiiski, I. & Lyutskanov, N. (1978). Improving the purity of apple pectin by use of amylolytic preparations. *Nauchni-Trudovev-Vissh-Institut-po-Khranitelna-i-Vkusova-Promyshlennost* **25**, 347–350.

Prosky, L., Asp, N. G., Schweizer, T., De Vries, J. W. & Furda, I. (1992). Determination of insoluble and soluble dietary fiber in foods and food products: collaborative study. *Journal of the Association of Official Analytical Chemists* **75**, 360–367.

Rasmussen, H. S., Holtug, K., Andersen, J. R., Krag, E. & Mortensen, P. B. (1987). The influence of ispaghula husk and lactulose on the *in vivo* and the *in vitro* production capacity of short-chain fatty acids in humans. *Scandinavian Journal of Gastroenterology* **22**, 406–410.

Roediger, W. E. W. (1982). Utilisation of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology* **83**, 424–429.

Salvador, V., Cherbut, C., Barry, J.-L., Bertrand, D., Bonnet, C. & Delort-Laval, J. (1993). Sugar composition of dietary fibre and short-chain fatty acid production during *in vitro* fermentation by human bacteria. *British Journal of Nutrition* **70**, 189–197.

Saulnier, L., Mestres, C., Doublier, J.-L., Roger, P. & Thibault, J.-F. (1993). Studies of polysaccharides solubilized during alkaline cooking of maize kernels. *Journal of Cereal Science* **17**, 267–276.

Sawai, K., Sandonsuge, Y., Izuiyama, Y. & Okagawa, Y. (1978). Biochemical studies on apple fruits. III. Some physical properties of starch granules observed through an X-ray diffractometer and a microscope. *Bulletin of the Faculty of Agriculture, Hirosaki University* **29**, 1–9.

Schooley, D. L., Kubiak, F. M. & Evans, J. V. (1985). Capillary gas chromatographic analysis of volatile and non-volatile organic acids from biological samples as the t-butylmethylsilyl derivatives. *Journal of Chromatographic Science* **23**, 385–390.

Slade, A. P., Wyatt, G. M., Bayliss, C. E. & Waites, W. M. (1987). Comparison of populations of human faecal bacteria before and after *in vitro* incubation with plant cell wall substrates. *Journal of Applied Bacteriology* **62**, 231–240.

Stephen, A. M. & Cummings, J. H. (1980). The microbial contribution to human faecal mass. *Journal of Medical Microbiology* **13**, 45–56.

Stevens, J. H., Selvendran, R. R., Bayliss, C. E. & Turner, R. (1988). Degradation of cell wall material of apple and wheat bran by faecal bacteria *in vitro*. *Journal of the Science of Food and Agriculture* **44**, 151–166.

Titgemeyer, E. C., Bourquin, L. D., Fahey, G. C. & Garleb, K. A. (1991). Fermentability of various fiber sources by human fecal bacteria *in vitro*. *American Journal of Clinical Nutrition* **53**, 1418–1424.

Tomlin, J. & Read, N. W. (1988). The relation between bacterial degradation of viscous polysaccharides and stool output in human beings. *British Journal of Nutrition* **60**, 467–475.

Tomlin, J., Read, N. W., Edwards, C. A. & Duerden, B. I. (1986). The degradation of guar gum by faecal incubation system. *British Journal of Nutrition* **55**, 481–486.

Vérité, R. & Demarquilly, C. (1978). Qualité des matières azotées des aliments pour ruminants (Quality of crude proteins in feeds for ruminants). In *La Vache Laitière*, pp. 143–157. Versailles: INRA Publications.

Vince, A. J., McNeil, N. I., Wager, J. D. & Wrong, O. M. (1990). The effect of lactulose, pectin, arabinogalactan and cellulose on the production of organic acids and metabolism of ammonia by intestinal bacteria in a faecal incubation system. *British Journal of Nutrition* **63**, 17–26.

Wolever, T. M. S., Spadafora, P. & Ashuis, H. (1992). Interaction between colonic acetate and propionate in humans. *American Journal of Clinical Nutrition* **53**, 681–687.

Wyatt, G. M. & Horn, N. (1988). Fermentation of resistant starches by human and rat intestinal bacteria. *Journal of the Science of Food and Agriculture* **44**, 281–288.

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