Fermentation of non-starch polysaccharides in mixed diets and single fibre sources: comparative studies in human subjects and *in vitro*

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(Received 2 September 1997 – Revised 19 February 1998 – Accepted 17 March 1998)

The present study investigated whether the extent of fermentation of NSP in human subjects could be predicted by an *in vitro* batch system. Fibre sources studied were five mixed diets containing different amounts and types of fibre and three single fibre sources (citrus fibre concentrate, coarse and fine wholemeal rye bread). Fermentation in human subjects was determined in balance experiments in women who were also donors of the faecal inocula. *In vitro* fermentations were performed with fibre residues prepared from duplicates of the fibre-containing foods consumed during the balance trials. Fermentation of total NSP *in vivo* was between 65·8 and 88·6 % for the mixed diets and 54·4, 58·0 and 96·9 % for the coarse and fine wholemeal rye breads and the citrus fibre concentrate respectively. For the mixed diets and the citrus fibre concentrate, mean differences between the extent of NSP degradation after 24 h *in vitro* incubation and that *in vivo* were between –0·7 and 5·0 %. Differences were significant for one diet ($P < 0·05$). For the wholemeal rye breads, the fermentation *in vitro* exceeded that *in vivo* significantly, but the magnitude of the difference in each case was small and without physiological importance. Particle size of breads had no influence on the extent of NSP degradation. These results indicate that the *in vitro* batch system used could provide quantitative data on the fermentation *in vivo* of NSP in mixed diets and some single fibre sources. An *in vitro* incubation time of 24 h was sufficient to mimic the NSP degradation *in vivo*.

Fermentation: Non-starch polysaccharides: *In vitro* batch system

NSP are the predominant components of dietary fibre (DF). Their susceptibility to bacterial fermentation is of major significance for the actions of DF in the large intestine. Fermentation of NSP affects their structural properties and water-holding capacity and thereby their effect on stool weight. Fibres that are extensively degraded have a lower faecal bulking capacity than more resistant types of fibre (Stephen & Cummings, 1980). This is especially the case if the fermentation is rapid and occurs mainly in the proximal colon (Edwards *et al.* 1990). The short-chain fatty acids (SCFA) acetate, propionate and butyrate formed from bacterial NSP breakdown are efficiently absorbed. Besides their contribution to the body's energy supply (McNeil, 1984), they have specific metabolic functions. Butyrate, for example, is the preferred fuel of colonic epithelial cells (Roediger, 1982) and is thought to contribute to the protective role of fibre against large-bowel cancer (Bingham, 1990).

Fermentability of NSP and production of SCFA can be studied rather easily with *in vitro* batch systems utilizing faecal bacteria. These *in vitro* systems have several advantages over time-consuming and expensive human fermentation studies. *In vitro* systems are inexpensive, different NSP sources can be fermented at the same time and they can be used for screening of new fibre sources not yet permitted for human consumption. *In vitro* studies using rat faecal inocula suggest that the energy values of NSP may be predicted by their fermentability *in vitro* (Barry *et al.* 1995).

However, in a ring test on the *in vitro* fermentation of several purified and semi-purified fibres which were chosen to cover a wide range of fermentability, the extent of substrate degradation differed markedly between laboratories. The greatest differences were observed for fibre with intermediate fermentability (Barry *et al.* 1995). The reasons for these discrepancies are difficult to establish. If *in vitro* fermentation is to be used for quantitative purposes, for example for the estimation of energy values of NSP for food labelling purposes, it will be necessary for the same degree of NSP degradation to occur *in vitro* and *in vivo*. Therefore, the fermentability of NSP obtained *in vitro* should be validated using human balance studies.

Abbreviations: DF, dietary fibre; SCFA, short-chain fatty acids.

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Fibre sources such as those studied by Barry *et al.* (1995) have specific properties and may be used for special purposes as food ingredients. However, normal human diets consist of mixtures of different fibres derived from natural or manufactured foods, which are ingested at various intake levels. Processing of foods such as heating or grinding may modify the physicochemical properties of DF and thereby affect the susceptibility of NSP to bacterial fermentation (Guillon *et al.* 1996). At present, there is not much information about whether *in vitro* systems are able to predict the fermentability, in human subjects, of NSP contained in mixed diets and in fibre sources that are processed in different ways.

The objective of the present study was to determine whether the extent of fermentation in human subjects of NSP in mixed diets, and in single fibre sources contained in these diets, could be predicted quantitatively by an *in vitro* batch system. The diets studied differed in the amounts and proportions of cereal, fruit and vegetable fibres they contained. The single fibre sources investigated were wholemeal rye breads of two different particle sizes and a citrus fibre concentrate. Fermentation *in vivo* was determined in balance experiments; fermentation *in vitro* was performed by an established batch system (Goering & Van Soest, 1970) using human faecal inocula. The subjects participating in the balance trials were also donors of the faecal inocula.

Experimental: human balance studies

Subjects

A total of seventeen healthy, free-living female students aged 22–31 years took part in the balance experiments. The subjects were highly motivated students in nutritional sciences, who were interested in the objectives of the study. They had not taken antibiotics for at least 6 weeks before the beginning of the experiments. Informed written consent was obtained from all volunteers. The study was approved by the Ethics Committee of the Medical Faculty of the University of Kiel.

Study plan

Two studies with a total of five experimental periods were performed. Study 1 included two experimental periods of 28 d each; periods were separated from each other by 28 d. The low-fibre control diet and the high-fibre diet were consumed in a crossover design. Study 2 included three experimental periods during which the low-fibre control diet and two high-fibre diets were eaten. Subjects were randomly assigned to start on one of these diets and then followed a different sequence of diets. Each study period lasted 22 d; periods were separated from each other by 21 d. During the studies, subjects had a controlled food intake that maintained their body weight in the range ± 1 kg of their starting weight. The subjects had lunch together in the institute; foods for all other meals were prepackaged and eaten at home. To ensure compliance, three of the authors (MD, EW, WF) ate lunch with the subjects and talked with them about potential problems arising during the study.

Diets

All food consumed during the experiments was provided by the institute and was weighed to the nearest gram. In each study, two DF-containing menus of similar composition were given in rotation during each experimental period. All subjects consumed the same amounts of these foods. Additional fibre-free foods were provided in amounts that covered individual energy requirements, but were kept constant for each subject during the experimental periods.

Study 1. When the high-fibre diet was consumed, 35 g of a citrus fibre concentrate (CitroFibre DF-50, Citrosuco S/A, Matao, Brazil) providing 20·2 g NSP was added to bread, 'Quark' (a kind of cottage cheese), 'Dickmilch'(fermented milk), mashed potatoes, soup and juice. During the low-fibre diet these foods were consumed without added citrus fibre (Table 1). The low-fibre control diet and the high-fibre diet provided 19·4 and 40·2 g NSP/d, 36·6 and 69·4 % of which was derived from fruits, vegetables and potatoes.

Study 2. The diets consisted of a low-fibre control diet and two high-fibre diets, which differed in the type of bread they contained (Table 1). During the low-fibre control diet, 200 g low-fibre bread and 75 g cake were eaten daily. When the high-fibre diets were consumed, low-fibre bread and cake were replaced by 350 g coarse or 377 g fine wholemeal rye bread (high-fibre diets containing coarse and fine wholemeal rye bread respectively). Daily intakes of NSP were 16·7 (SE 0·3), 38·2 (SE 0·3) and 37·7 (SE 0·3) g, when the low-fibre diet and the high-fibre diets containing coarse or fine wholemeal rye bread respectively, were consumed. Cereal foods contributed 60·1, 82·5 and 83·0 % respectively, to total NSP intake on these diets. Low-fibre bread was bought in a single batch in an amount sufficient for all experimental periods and stored at −20° until consumed. Wholemeal rye breads were baked from one single batch of rye milled to two different particle sizes (coarse bread: 50 % of particles > 2 mm, 90 % > 1 mm; fine bread: 99 % of particles < 0·5 mm, 58 % < 0·2 mm) (Federal Institute of Grain, Potato and Fat Research, Detmold, Germany) and kept frozen until used.

Balance technique

Balances were performed during the last 7 d of each experimental period. Duplicates of all foods consumed were weighed, homogenized and freeze-dried. Acid brilliant green (E142, provided by H. Schulz, Dragoco, Holzminden, Germany) in a gelatin capsule was given as a faecal marker at the beginning and end of each collection period. Faeces were collected quantitatively from the appearance of the first marker until the appearance of the second marker. Faeces were collected separately in plastic pots that were immediately transferred to the laboratory, weighed and frozen. After each balance period, faeces obtained from a given subject were combined, thawed, homogenized and one sample was freeze-dried.

Experimental: *in vitro* fermentation studies

General procedure

Study 1. *In-vitro* fermentations were carried out 3–4 months after the balance experiments when subjects were consuming

Table 1. Dietary fibre sources consumed (g/d) during the balance experiments*

Study 1			Study 2			
					High-fibre diets containing:	
Food	Low-fibre control diet	High-fibre diet	Food	Low-fibre control diet	coarse whole-meal rye bread	fine whole-meal rye bread
Wheat/rye mixed bread†	300	300	Wheat mixed bread	200	-	-
Tomato/chicken soup†‡	210	210	Cake	75	-	-
Mashed potatoes†	205	230§	Coarse wholemeal rye bread	-	350	-
Cucumber/Chinese leaves‡	150/40	150/40	Fine wholemeal rye bread	-	-	377
Strawberries/oranges‡	150	150	Potatoes	100	100	100
'Quark'/'Dickmilch'†‡	118	155§	Salad/cucumber‡	60/150	60/150	60/150
Orange juice/maracuja juice†‡	500	500	Orange/strawberries‡	150/150	150/150	150/150
Marmalade/honey‡	40/20	40/20	Marmalade/honey‡	40/20	40/20	40/20

* Each subject consumed the same amounts of these foods daily during the low-fibre and the high-fibre diet periods respectively. Substrates for the *in vitro* fermentation were prepared from these foods as described on p. 255.
† Foods were enriched with citrus fibre during the high-fibre diet period.
‡ Foods were consumed in rotation for 4 weeks (study 1) or 3 weeks (study 2) in each experimental period.
§ Weight of foods was increased during the high-fibre diet period because additional water was added.

their habitual diets. Seven of the ten subjects who took part in the balance experiments participated in the *in vitro* trials; three subjects had finished their studies and had left the university. Subjects had not taken antibiotics during the time between the balance trials and the *in vitro* fermentations. From each subject, one faecal sample was obtained and prepared as described later.

Study 2. *In vitro* fermentations were performed between day 17 and day 21 of each experimental period. During each of the experimental periods one faecal sample from each subject was prepared for the *in vitro* trials.

Fermentation substrates

DF residues obtained from a freeze-dried mixture of the fibre-containing foods in the experimental diets (Table 1), the citrus fibre concentrate and the coarse and fine wholemeal breads were used as substrates for the *in vitro* fermentations. With the exception of the substrates containing coarse bread, the freeze-dried foods were milled through a 0·5 mm screen. For the diet containing coarse wholemeal bread, bread was dried separately from the other foods and crumbled by hand to a particle size resembling that of the coarse meal used for bread making. Bread and the other freeze-dried ground foods were mixed together before the preparation of the DF residues. Coarse bread alone was also comminuted to the particle size of the coarse meal. DF residues were prepared according to a gravimetric method for DF analysis (Prosky *et al.* 1985) and freeze-dried. Filtration was performed using Fibretec E filtration apparatus (Tecator, Höganäs, Sweden). For each diet and the fibre concentrate respectively, DF residues were prepared in an amount sufficient for all incubations and kept in a desiccator until used.

Faecal inoculum

Freshly passed faeces were immediately homogenized for 3 min with four times their weight of distilled water, filtered through four layers of cheesecloth to remove particles and immediately used as inoculum. Inoculum preparation was performed under a constant flow of CO_2.

Fermentations

Study 1. Portions of each faecal homogenate obtained from each subject were incubated with the DF residues obtained from the low-fibre diet, the high-fibre diet and the citrus fibre concentrate respectively. Incubations were performed for 0 and 24 h. Flasks without added substrate as blanks were also incubated for 0 and 24 h with each inoculum. All incubations were performed in duplicate.

Study 2. In each experimental period, portions of each faecal homogenate obtained from the individual subjects were incubated with the DF residues prepared from the fibre sources eaten by these subjects during this period. Incubations were performed for 0, 24 and 48 h. Flasks without added substrate as blanks were also incubated for each time point with each inoculum source. All incubations were performed in duplicate.

Fermentations were conducted in 125 ml Erlenmeyer flasks using the technique and the solutions as described by Goering & Van Soest (1970). The basic components of this system include substrate, culture medium, reducing solution and faecal inoculum. The culture medium contained (per litre): 2·5 g trypticase peptone, 1·0 g NH_4HCO_3, 8·75 g $NaHCO_3$, 1·43 g Na_2HPO_4 anhydrous, 1·55 g KH_2PO_4 anhydrous, 0·15 g $MgSO_4 . 7H_2O$, 1·25 mg resazurin, 16·5 mg $CaCl_2 . 2H_2O$, 12·5 mg $MnCl_2 . 4H_2O$, 1·25 mg $CoCl_2 . 4H_2O$, and 10·0 mg $FeCl_3 . 6H_2O$. The reducing solution contained (per litre): 6·25 g cysteine –HCl, 1·6 g NaOH, and 6·25 g $Na_2S.9H_2O$. Culture medium (32 ml) was added to 400 mg substrate (DF residue) 12–24 h before the start of the incubation to ensure complete hydration of the samples. Flasks were sealed with Parafilm and stored in the refrigerator to limit the possibility of microbial growth. At 1–2 h before inoculation, the bottles were placed in a 37° shaking water bath, reducing solution (1·6 ml) was added and then flasks were sealed with rubber stoppers. Stoppers were fitted with three openings (an inlet tube, a Bunsen valve, and a gassing tube connected to a common manifold as described by Goering & Van Soest (1970). The manifold was connected to a supply of CO_2, and flasks were bubbled with CO_2. Into each

flask 8 ml of faecal suspension was injected through the inlet tube, and incubations were performed under a steady stream of CO_2 (3–4 ml/min). Flasks were gently shaken (shaking rate: 50/min). Fermentation was stopped by adding 1 ml ethylmercurithiosalicylate (10 g/l). Residual NSP was determined in the freeze-dried contents of the flasks.

Chemical analyses

Freeze-dried samples of food and faeces were milled through a 0·5 mm screen. DM content of food and faeces was determined by drying the freeze-dried samples at 105° for 8 h. N was assayed by a micro-Kjeldahl method. Protein was calculated as N × 6·25. Starch in faeces, fermentation substrates and residues was determined by the method of Björck *et al.* (1986). Neutral NSP monomers in foods, faeces and in the *in vitro* fermentation substrates and residues were determined by GLC as alditol acetates by the Uppsala method C (Theander & Westerlund, 1986) using 1-methylimidazole as a catalyst for the derivatization of NSP monomers. Corrections for hydrolytic losses and detector response were made by performing the analyses with known sugar standards. Uronic acids were measured in the acidic hydrolysate according to the method of Englyst *et al.* (1982). Total NSP was calculated as the sum of neutral sugars and uronic acids. NSP constituents and total NSP were expressed as polysaccharides (weight of monomers × 0·9).

Calculations

The fermentation of NSP *in vivo* was estimated as the apparent digestibility of NSP, i.e. the difference between dietary intake and faecal excretion, expressed as percentage of intake. The fermentation of the additional NSP (citrus fibre, coarse or fine wholemeal rye bread) during the high-fibre diet periods was calculated from the difference between intake and excretion of NSP during the high-fibre (HF) diet and the corresponding low-fibre (LF) control diet as:

$$\text{fermentation (\%)} = [[(NSP_{\text{intake HF-diet}} - NSP_{\text{intake LF-diet}}) - (NSP_{\text{excretion HF-diet}} - NSP_{\text{excretion LF-diet}})]/ (NSP_{\text{intake HF-diet}} - NSP_{\text{intake LF-diet}})] \times 100.$$

The fermentation of NSP *in vitro* was calculated as follows:

$$\text{fermentation (\%)} = [[(NSP_0 - B_0) - (NSP_i - B_i)]/ (NSP_0 - B_0)] \times 100,$$

where NSP_0 and NSP_i are the NSP contents in flasks containing substrates at time 0 and i respectively, and B_0 and B_i are NSP contents in blank flasks at time 0 and i respectively.

For each subject differences between the fermentation of NSP *in vitro* and *in vivo* were calculated as percentage fermentation *in vitro* − percentage fermentation *in vivo*. In study 1, only the subjects (*n* 7) participating in the balance trials as well as in the *in vitro* fermentations were taken into account with the calculations.

Statistical analyses

The comparison *in vitro v. in vivo* was done as a paired comparison by analysing the differences between the fermentation of NSP *in vitro* and *in vivo* for the same subject, DF source and time. Data were analysed as a crossover design with subjects as the random factor, and period (of the balance experiments), diets and in study 2, fermentation time (24 and 48 h) as fixed factors. For the single fibre sources (citrus fibre concentrate, coarse and fine wholemeal rye bread), differences between *in vivo* and *in vitro* fermentation were analysed separately without the factors period and diet, because the fermentation of these fibres *in vivo* was obtained by differences between the fermentation on the high-fibre and the corresponding low-fibre diet (see p. 256). Least squares means for the differences were estimated by mixed model analysis and tested by *t* test to see whether they were significantly different from zero. The statistical analysis was done using the procedure MIXED of SAS Release 6.12 (Statistical Analysis Systems Institute Inc., Cary, NC, USA). Differences were regarded as significant at $P < 0·05$.

Results

The compositions of the DF residues used as substrates for the *in vitro* fermentations are shown in Table 2. Total NSP accounted for 45·0–70·7 % of the substrates. In addition, substrates contained variable amounts of protein and some starch resistant to the degradation by termamyl/amyloglucosidase (*EC* 3.2.1.3). The pattern of NSP constituent sugars depended on the foods the residues were prepared from. Fruit and vegetable fibre sources (study 1: high-fibre diet and citrus fibre concentrate) were especially rich in uronic acids (i.e. pectin) and glucose (i.e. cellulose). In the residues in which cereal fibre sources predominated (study 1: low-fibre diet, study 2: all fibre sources), the most abundant constituent sugars were pentoses (arabinose and xylose) and glucose. Compared with the NSP monomers ingested during the balance experiments, the preparation of the residues had no major effect on the chemical composition of the NSP.

Study 1

Fermentation *in vivo* of total NSP in all subjects (*n* 10) was 77·4 (SD 6·3), 88·5 (SD 2·6) and 98·7 (SD 6·3) % for the low-fibre diet, the high-fibre diet and the citrus fibre concentrate respectively. The extent of bacterial NSP degradation as measured in the balance experiments (*in vivo*) in the subjects acting as inoculum donors (*n* 7) and *in vitro* is presented in Table 3. Total NSP and all NSP monomers present in the low-fibre diet and the high-fibre diet were fermented to more than 70 % *in vivo* as well as *in vitro*. NSP in the citrus fibre concentrate was nearly completely degraded. The differences between *in vivo* and *in vitro* fermentations are given in Table 4. Differences between *in vivo* and *in vitro* fermentation of total NSP were small (5·0, 2·6 and −0·2 % for the low-fibre diet, the high-fibre diet and the citrus fibre concentrate respectively), and were

Table 2. Composition of the dietary fibre (DF) residues used as substrates for the *in vitro* fermentations and contribution of NSP constituent sugars to total NSP in the DF-residues and the experimental diets (*in vivo*)*

	Study 1			Study 2				
					High-fibre diets containing:		Wholemeal rye breads:	
	Low-fibre diet	High-fibre diet	Citrus fibre concentrate	Low-fibre diet	coarse wholemeal rye bread	fine wholemeal rye bread	coarse	fine
Composition of DF residues (g/kg DM)								
Total NSP	450	571	685	478	634	654	621	707
Resistant starch	54	23	0	80	106	54	105	46
Protein	345	230	153	295	170	25	140	90
NSP constituent sugars (g/100 g total NSP)								
Arabinose								
in vitro	18·9	15·2	13·0	18·0	20·2	21·7	23·8	25·0
in vivo	14·4	13·7	14·9	13·3	18·6	19·0	20·9	22·0
Xylose								
in vitro	25·1	11·4	3·1	23·8	31·5	33·6	38·5	42·1
in vivo	21·6	11·4	3·5	19·3	31·5	32·4	36·3	37·8
Mannose								
in vitro	6·9	4·4	2·6	7·1	3·3	3·8	3·7	3·8
in vivo	4·6	3·6	3·6	6·0	3·4	3·2	3·3	2·4
Galactose								
in vitro	8·0	8·1	7·2	8·4	5·2	6·1	3·1	3·4
in vivo	10·8	8·9	6·5	10·2	6·0	6·1	4·4	4·9
Glucose								
in vitro	28·4	29·2	26·4	28·0	29·3	24·6	28·5	23·2
in vivo	31·4	28·7	30·8	34·3	31·5	30·2	30·8	29·3
Uronic acids								
in vitro	12·7	31·7	47·7	14·6	10·4	10·1	2·3	2·4
in vivo	17·3	33·8	40·7	16·6	8·9	9·1	4·4	3·7

* Dietary fibre residues were prepared from the fibre sources presented in Table 1. For details of the preparation of the residues, see p. 255.

only significant ($P < 0·05$) for the low-fibre diet, mostly due to differences in the degradation of NSP-glucose which was 11·1 % higher *in vitro* ($P < 0·05$). Where, for the other NSP constituent saccharides, significant differences were found, they were either small (e.g. for arabinose in the low- and high-fibre diets) or concerned monomers present in low concentrations (e.g. xylose in the citrus fibre concentrate, mannose and galactose in the low-fibre diet).

Study 2

The extent of NSP fermentation as measured in the balance experiments (*in vivo*) and *in vitro* after 24 and 48 h incubation is shown in Table 5. Fermentability of these fibres was between 54·1 and 86·3 %. The differences between *in vivo* and *in vitro* fermentations are given in Table 4. After 24 h *in vitro* fermentation, the breakdown of total NSP in the mixed diets agreed very well with the fermentation of these NSP measured in the balance trials. For the main constituent sugars (pentoses and glucose), the fermentability of arabinose in the low-fibre diet was slightly, but significantly higher ($P < 0·01$) *in vitro* than *in vivo* and that of glucose in the high-fibre diet containing fine wholemeal bread was approximately 10 % lower *in vitro*

Table 3. Study 1. Extent of fermentation (%) of NSP in mixed diets and citrus fibre concentrate as determined in human balance experiments (*in vivo*) and *in vitro**†

(Mean values with their standard deviations for seven subjects)

	Arabinose		Xylose		Mannose		Galactose		Glucose		Uronic acids		Total NSP	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fermentation *in vivo*														
Low-fibre diet	70·7	5·0	77·1	5·6	85·6	5·0	76·5	4·5	71·7	13·8	90·5	3·2	77·7	6·1
High-fibre diet	86·4	3·4	80·1	6·3	89·0	3·7	83·7	4·0	83·2	6·6	95·6	1·3	88·6	3·2
Citrus fibre concentrate	98·9	2·9	100·0	0·0	94·3	6·1	92·2	6·1	92·0	9·0	97·3	1·3	96·9	4·0
Fermentation *in vitro*														
Low-fibre diet	76·6	1·9	80·2	2·6	92·0	2·4	83·0	1·4	82·4	4·5	91·1	4·2	82·5	1·1
High-fibre diet	90·1	1·6	84·2	3·7	91·7	3·2	85·2	1·9	90·0	5·3	97·7	1·3	91·4	1·6
Citrus fibre concentrate	97·9	1·1	89·6	5·6	90·0	5·8	89·3	1·3	94·8	2·1	99·4	0·3	96·7	0·8

* For details of the determination of NSP fermentation *in vivo*, see p. 254 and p. 256.
† For details of the determination of NSP fermentation *in vitro*, see pp. 254–256.

Table 4. Differences (%) between the fermentation of NSP in human balance experiments (*in vivo*) and *in vitro*†‡ (Least squares means with their standard errors for seven subjects)

	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acids	Total NSP
Study 1							
Mixed diets							
Low-fibre diet	5·9**	3·3	6·5*	6·5**	11·1*	0·6	5·0*
High-fibre diet	3·6	3·8	2·6	1·6	6·3	2·1	2·6
SE	1·6	2·5	2·1	1·5	4·6	1·2	1·9
Citrus fibre concentrate	−1·0	−10·4**	−4·4	−2·9	2·8	2·1**	−0·2
SE	0·9	2·1	3·4	2·5	2·9	0·5	1·4
Study 2							
Mixed diets: 24 h *in vitro* fermentation							
Low-fibre diet	4·3**	3·0	3·6	6·8**	−7·2	2·6	1·0
High-fibre diet, coarse bread	−0·6	0·6	0·2	5·4*	−3·6	5·6*	−0·7
High-fibre diet, fine bread	0·3	−0·2	4·2*	6·6**	−10·3*	6·5**	−1·1
Mixed diets: 48 h *in vitro* fermentation							
Low-fibre diet	5·2**	4·4*	7·8**	9·0**	6·9	5·0*	6·3*
High-fibre diet, coarse bread	3·9*	6·0**	8·4**	8·8**	9·8	5·0*	7·0**
High-fibre diet, fine bread	2·2	1·8	9·7**	10·8**	0·4	7·2**	3·2
SE	1·5	1·9	2·1	2·0	4·9	2·1	2·2
Coarse wholemeal rye bread							
24 h *in vitro* fermentation	7·5**	5·8	36·6**	23·7*	12·6	n.c.	8·8*
48 h *in vitro* fermentation	7·1**	8·8*	42·4**	25·5*	19·9*	n.c.	12·6**
SE	2·1	2·7	9·3	8·6	6·0		3·1
Fine wholemeal rye bread							
24 h *in vitro* fermentation	9·9**	6·4**	50·9*	17·0**	2·4	n.c.	8·1**
48 h *in vitro* fermentation	9·6**	6·1**	53·0*	23·1**	7·5*	n.c.	9·5**
SE	2·0	1·5	16·3	3·2	2·8		1·3

n.c., not calculated.
Differences were significantly different from zero: * $P < 0·05$; ** $P < 0·01$.
† For the calculation of the differences between the fermentation *in vivo* and *in vitro*, see p. 256.
‡ For the statistical analysis of the differences see p. 256.

than *in vivo* ($P < 0·05$). After 48 h *in vitro* fermentation, the breakdown of NSP present in the low-fibre diet and the high-fibre diet containing coarse bread was significantly higher ($P < 0·05$ and $P < 0·01$ respectively) than that *in vivo* due to a higher breakdown of all NSP constituent sugars *in vitro*. For the diet containing the fine wholemeal bread, however, differences between *in vivo* fermentation and 48 h *in vitro* fermentation were small and not significant with the exception of the minor NSP components (mannose, galactose and uronic acid), which were more fermentable *in vitro*. For the coarse as well as the fine wholemeal bread, the fermentation of total NSP and most of their constituent saccharides was significantly higher *in vitro* than *in vivo* for the two incubation times; differences were higher after 48 h than after 24 h *in vitro* fermentation.

Discussion

We investigated, in young women in two studies, whether the extent of fermentation *in vivo*, of NSP contained in five mixed diets and three single fibre sources, could be predicted quantitatively by *in vitro* fermentation. The fibre sources studied contained variable proportions of NSP from fruits, vegetables and cereal foods and covered a range of fermentability *in vivo* between 54 and 98 %. For two of the diets and the wholemeal breads, the influence of the particle size on NSP degradation was studied. The experimental periods, during which *in vivo* fermentation was determined, were arranged independently from the menstrual cycle of the subjects. In previous investigations no changes were detected in individual transit time which could be related to the stage of menstrual cycle (Wyman *et al.* 1978; Marlett *et al.* 1981). Hence, cycle-related effects on the fermentation *in vivo* seem to be rather unlikely.

Large differences have been found in the fermentation of NSP between human subjects eating the same diets (Southgate & Durnin, 1970; Bach Knudsen *et al.* 1994). Several investigators have also reported differences in the capacity of individual faecal inocula to ferment a given substrate *in vitro* (McBurney & Thompson, 1989; Bourquin *et al.* 1993). In the present work, the faecal inocula were obtained from subjects who also participated in the *in vivo* experiments, but two different study protocols were used. In study 1, the *in vitro* experiments were performed subsequent to the *in vivo* studies, i.e. subjects were eating their habitual diets when providing faecal inocula, and different fibre sources were fermented by portions of one inoculum per subject. As such, the *in vitro* fermentations in study 1 agreed with the results of previous *in vitro* investigations (e.g. McBurney & Thompson, 1989; Bourquin *et al.* 1993; Barry *et al.* 1995). The validity of this experimental procedure is supported by results of McBurney & Thompson (1987) who reported that faeces collected on different occasions from the same subject yielded very similar *in vitro* fermentation results. In study 1, *in vitro* fermentations were repeated in four subjects when they consumed the citrus fibre concentrate for 2 weeks in addition to their usual diets. The adaptation to citrus fibre had no effect on the degradation of NSP *in vitro* (results not shown). Previous studies also showed no effect on *in vitro* fermentation results of the adaptation of

Table 5. Study 2. Extent of fermentation (%) of the NSP in mixed diets and in wholemeal rye breads as determined in human balance experiments (*in vivo*) and *in vitro* after 24 and 48 h incubation*†

(Mean values with their standard deviations for seven subjects)

	Arabinose		Xylose		Mannose		Galactose		Glucose		Uronic acids		Total NSP	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fermentation *in vivo*														
Low-fibre diet	79·8	3·9	82·2	5·1	85·9	5·1	74·2	6·7	75·8	13·4	90·4	5·6	80·5	7·1
High-fibre diet, coarse bread	59·7	3·1	71·3	7·3	76·6	9·4	58·9	3·1	59·1	19·7	82·4	5·7	65·8	9·3
High-fibre diet, fine bread	58·9	3·2	73·9	3·4	75·8	11·5	60·0	4·6	64·3	11·3	80·9	5·2	68·0	5·2
Coarse bread	50·7	4·1	67·3	8·4	41·0	29·7	11·5	18·6	44·1	25·8	n.c.		54·1	11·8
Fine bread	49·5	4·5	71·0	3·8	30·3	51·0	21·1	8·2	52·3	12·3	n.c.		57·8	5·0
Fermentation *in vitro*, 24 h														
Low-fibre diet	83·9	2·6	85·1	3·9	89·2	5·2	81·2	5·5	68·2	21·1	93·0	3·6	81·3	8·0
High-fibre diet, coarse bread	59·1	4·3	72·0	6·4	77·1	9·1	64·5	2·9	55·9	21·8	88·1	3·4	65·3	10·2
High fibre diet, fine bread	59·1	2·8	73·7	1·7	80·1	11·8	66·2	3·4	54·0	20·2	87·8	2·4	66·9	5·5
Coarse bread	58·2	4·1	73·1	4·1	77·7	7·9	35·2	3·4	56·6	16·4	41·6	10·0	62·8	7·4
Fine bread	59·4	2·2	77·4	1·6	81·1	10·3	38·1	5·0	54·7	16·5	53·0	8·1	65·9	4·6
Fermentation *in vitro*, 48 h														
Low-fibre diet	84·8	3·0	86·5	3·0	93·3	3·5	83·4	3·5	82·3	14·4	95·4	3·0	86·5	5·7
High-fibre diet, coarse bread	63·6	4·5	77·4	2·9	85·2	7·7	67·8	3·2	69·3	17·0	87·4	1·9	72·9	7·1
High-fibre diet, fine bread	61·1	4·3	75·6	2·9	85·6	7·6	70·5	4·7	64·7	12·7	88·1	3·4	71·1	5·2
Coarse bread	57·9	5·2	76·1	3·0	83·5	7·4	37·0	8·4	63·9	19·8	48·7	8·0	66·6	7·9
Fine bread	59·1	1·8	77·1	1·5	83·3	8·4	44·2	4·5	59·8	11·0	59·0	15·8	67·3	3·5

n.c., not calculated.
* For details of the determination of NSP fermentation *in vivo*, see p. 254 and p. 256.
† For details of the determination of NSP fermentation *in vitro*, see pp. 254–256.

inoculum donors to the cereal fibres under investigation (Daniel *et al.* 1997). In study 2, *in vivo* and *in vitro* experiments were performed simultaneously, and the fibres fermented *in vitro* were derived from the fibre sources actually consumed. So it is likely that in study 1 as well as in study 2 the capacity of the individual microflora was the same *in vivo* and *in vitro*, provided that the inoculum preparation had no major effect on the microbial activity. Previous investigators used very different concentrations of faecal inocula which ranged between 3–15 g faeces/l (McBurney & Thompson, 1987; Titgemeyer *et al.* 1991; Bourquin *et al.* 1992) and 160–250 g faeces/l (Guillon *et al.* 1992; Barry *et al.* 1995). In the present investigations, at concentrations of 40 g faeces/l (Adiotomre *et al.* 1990) inocula had an activity sufficient to mimic *in vivo* fermentability of the NSP sources studied.

For the highly fermentable fibre sources investigated in study 1, an *in vitro* fermentation time of 24 h was sufficient to mimic the extent of fermentation *in vivo*. This was not only the case for uronic acids (i.e. pectic material) which were almost completely fermented *in vivo* and *in vitro*, but also for NSP-glucose, i.e. mainly cellulose, which in the low-fibre diet was even more degraded (11·1 %) *in vitro* than *in vivo*. Bourquin *et al.* (1993) who determined the fermentation *in vitro* of substrates prepared from several kinds of vegetables also found after 24 h a degradation of uronic acids of greater than 90 %, but a considerably lower fermentation of NSP-glucose (42·1 and 67·3 %). However, there are no direct comparisons available to indicate to what degree these fibre sources would have been degraded in the colon of the inoculum donors.

In study 2, with the exception of the low-fibre diet, less-fermentable fibre sources were investigated (i.e. mainly cereal fibres containing, in addition to endosperm fibre, also lignified fibre from the outer hulls of rye). Because the fermentation of these fibres may require longer times (Van Soest *et al.* 1982), *in vitro* fermentations were performed for 24 and 48 h. These *in vitro* fermentation times were in the range of the colonic transit times of healthy women, which were on average 38·8 (SE 2·9) h (Metcalf *et al.* 1987). For the three mixed diets, the extent of NSP breakdown *in vitro* after 24 h agreed very well with the degradation measured in the balance experiments. In the fine and coarse wholemeal rye breads, NSP breakdown after 24 h was higher *in vitro* than *in vivo*, but these differences, although significant, were small (approximately 8 %) and certainly of no physiological importance. In a previous study, for three mixed diets containing NSP fermented *in vivo* between 59 and 84 %, differences between the fermentation *in vitro* after 24 h and that *in vivo* were also relatively small (−4·9 to 8·8 %), although they were significant for two diets. In the same study, the fermentation of rather resistant NSP present in a barley fibre concentrate, however, was considerably higher (19·7 %) *in vitro* than *in vivo* (Daniel *et al.* 1997).

Differences between *in vivo* and *in vitro* fermentability have several causes. For the *in vitro* fermentation substrates, available nutrients were enzymically hydrolysed and separated. Compared with the NSP ingested, the preparation of the DF residues had no major effect on the chemical composition of the NSP. However, in natural fibre sources the structural properties of the three-dimensional cell walls may be more important for the fermentability than the chemical components *per se* (Guillon *et al.* 1992). Due to freeze-drying and the predigestion procedure, changes in the cell wall structure, such as macro- and microporosity, could have occurred leading to increased susceptibility for faecal bacteria (Lebet *et al.* 1996). If such effects occurred, they should have greater consequences for rather resistant types of NSP (e.g. NSP from outer layers of cereal grains), than for those that are easy to ferment (e.g. fruit and vegetable fibres). Our findings that the less fermentable NSP were

degraded to a higher extent *in vitro* than *in vivo*, in contrast to the more fermentable fibres, agree with these assumptions.

Differences between *in vivo* and *in vitro* fermentation may also be due to the difficulty in estimating accurately the fermentation of fibre supplements *in vivo*. Normally, supplements are added to diets that already contain NSP, the fermentation of which cannot be distinguished from the fermentation of the additional fibre. As in previous studies (Wisker *et al.* 1997), the fermentation *in vivo* of NSP in the citrus fibre concentrate and the wholemeal breads was calculated from differences in NSP intake and excretion during the high-fibre diet and the corresponding low-fibre diet periods, i.e. basal fermentation was subtracted. Animal studies indicate that NSP supplements do not interfere with the fermentation of NSP in the basal diet (Goodlad & Mathers, 1991; Key & Mathers, 1993), and therefore this procedure appears to be valid. However, the disadvantage of this procedure is that it can lead to errors in the calculation of NSP fermentation when small changes in basal fibre excretions occur due to real changes and/or analytical errors. Accordingly, deviations from true fermentability may be large with minor NSP components, especially when parts of the excreted amounts could be host-derived, as may be the case for galactose and mannose (Hoskins & Boulding, 1981; Longland & Low, 1988). In the present study, such errors could have caused the large differences between *in vivo* and *in vitro* fermentation of mannose and galactose in the wholemeal breads. However, mannose and galactose were only minor constituents in these fibre sources and had no great impact on the digestibility of total NSP.

Consistent with results obtained *in vivo* (Wisker *et al.* 1996), the fermentation *in vitro* of NSP in the wholemeal breads and the diets containing these breads was not affected by the particle size of the breads, although the particle size is directly related to the surface area that is accessible to the colonic bacteria. However, the effect of the particle size on *in vitro* NSP breakdown is inconsistent. Grinding increased the rate and extent of the fermentation of pea fibre *in vitro*, but had a much smaller effect on the fermentability of sugarbeet fibre (Guillon *et al.* 1996). Hence, particle size *per se* may not be important for the fermentation of all types of fibre, but seems to have a greater effect for fibre sources that have a low internal pore volume and a low affinity for water, such as pea fibre (Guillon *et al.* 1996).

Previous investigators have used *in vitro* fermentation times of up to 48 h and longer in order to determine the potential fermentability of resistant fibre sources (Titgemeyer *et al*. 1991; Bourquin *et al*. 1992; Monsma & Marlett, 1996). Our present studies showed that *in vitro* fermentation times of 48 h led to significant overestimations of *in vivo* NSP fermentation and therefore seem to be unnecessary. In addition, in a static culture system bacterial composition and activity will change over time and end-products of bacterial metabolism can accumulate and reach bacteriostatic concentrations after long incubation times (Edwards & Rowland, 1992). Changes in bacterial metabolism during *in vitro* fermentation and substrate limitation may also influence the amounts and proportions of SCFA and gases resulting from NSP breakdown (McBurney & Thompson, 1987). However, it is not known how far SCFA concentrations and proportions obtained at any time *in vitro* will be the same as those in the large intestine where fermentation conditions are more complex and where the SCFA produced are rapidly absorbed (Ruppin *et al*. 1988). In static *in vitro* systems, SCFA accumulate and may undergo further reactions. Therefore, most authors agree that *in vitro* fermentations in static systems should not last longer than 24 h (Edwards & Rowland, 1992).

In conclusion this study indicates that the *in vitro* batch system used can provide quantitative data on the extent of fermentation in the human colon of NSP in mixed diets containing variable amounts and proportions of fruit, vegetable and cereal NSP and of single fibre sources such as highly fermentable fibre concentrates. Although the degradation of NSP in the wholemeal breads was significantly higher *in vitro* than *in vivo*, the magnitude of these differences was small and of no physiological importance. An *in vitro* incubation time of 24 h was sufficient to mimic the degree of NSP degradation *in vivo*.

Acknowledgements

Special appreciation goes to the volunteers for their excellent cooperation. We are grateful to K. Golz and A. Richardsen for preparing the diets and to W. Seibel and J. Brümmer, Federal Institute of Grain, Potato and Fat Research, Detmold, Germany, for the supply of the wholemeal rye breads. Some of the foods were kindly provided by Union Deutsche Lebensmittelwerke, Hamburg, Germany. We thank A. Susenbeth and S. Wolffram, Institute of Animal Nutrition, University of Kiel, for the opportunity to perform parts of the chemical analyses in their laboratories.

References

Adiotomre J, Eastwood MA, Edwards CA & Brydon WG (1990) Dietary fiber: in vitro methods that anticipate nutrition and metabolic activity in humans. *American Journal of Clinical Nutrition* **52**, 128–134.

Bach Knudsen KE, Wisker E, Daniel M, Feldheim W & Eggum BO (1994) Digestibility of energy, protein, fat and non-starch polysaccharides in mixed diets: comparative studies between man and the rat. *British Journal of Nutrition* **71**, 471–487.

Barry J-L, Hoebler C, Macfarlane GT, Macfarlane S, Mathers JC, Reed KA, Mortensen PB, Nordgaard I, Rowland IR & Rumney CJ (1995) Estimation of the fermentability of dietary fibre *in vitro*: a European interlaboratory study. *British Journal of Nutrition* **74**, 303–322.

Bingham S (1990) Mechanisms and epidemiological evidence relating dietary fibre (non-starch polysaccharides) and starch to protection against large bowel cancer. *Proceedings of the Nutrition Society* **49**, 153–171.

Björck I, Nyman M, Pedersen B, Siljeström M, Asp N-G & Eggum BO (1986) On the digestibility of starch in wheat bread - Studies in vitro and in vivo. *Journal of Cereal Science* **4**, 1–11.

Bourquin LD, Titgemeyer EC, Garleb KA & Fahey GC (1992) Short-chain fatty acid production and fiber degradation by human colonic bacteria: effects of substrate and cell wall fractionation procedures. *Journal of Nutrition* **122**, 1508–1520.

Bourquin LD, Titgemeyer EC & Fahey GC (1993) Vegetable fiber

fermentation by human fecal bacteria: cell wall polysaccharide disappearance and short-chain fatty acid production during in vitro fermentation and water-holding capacity of unfermented residues. *Journal of Nutrition* **123**, 860–869.

Daniel M, Wisker E, Rave G & Feldheim W (1997) Fermentation in human subjects of nonstarch polysaccharides in mixed diets, but not in a barley fiber concentrate, could be predicted by in vitro fermentation using human fecal inocula. *Journal of Nutrition* **127**, 1981–1988.

Edwards CA, Bowen J & Eastwood M (1990) The effect of isolated complex carbohydrate on caecal and faecal short chain fatty acids and stool output in the rat. In *Dietary Fibre. Chemical and Biological Aspects*, pp. 273–276 [DAT Southgate, K Waldron, IT Johnson and GR Fenwick, editors]. Cambridge, Royal Society of Chemistry.

Edwards CA & Rowland IR (1992) Bacterial fermentation in the colon and its measurement. In *Dietary Fibre–A Component of Food. Nutritional Function in Health and Disease*, pp. 119–136 [TF Schweizer and CA Edwards, editors]. London: Springer.

Englyst HN, Wiggins HS & Cummings JH (1982) Determination of non-starch polysaccharides in plant foods by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst* **107**, 307–318.

Goering HK & Van Soest PJ (1970) *Forage Fiber Analyses. Agriculture Handbook* no. 379. Washington, DC: United States Department of Agriculture, U.S. Government Printing Office.

Goodlad JS & Mathers JC (1991) Digestion by pigs of non-starch polysaccharides in wheat and raw peas (*Pisum sativum*) fed in mixed diets. *British Journal of Nutrition* **65**, 259–270.

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 bacteria. *Journal of the Science of Food and Agriculture* **60**, 69–79.

Guillon F, Cloutour F & Barry J-L (1996) Dietary fibre: relations between intrinsic characteristics and fermentation pattern. In *Dietary Fibre and Fermentation in the Human Colon. Proceedings of the COST Action 92 Workshop*, pp. 110–115 [Y Mälkki and JH Cummings, editors]. Brussels: Office for Official Publications of the European Communities.

Hoskins LC & Boulding ET (1981) Mucin degradation in human colonic ecosystem. Evidence for the existence and role of bacterial subpopulations producing glycosidases as extracellular enzymes. *Journal of Clinical Investigation* **67**, 163–172.

Key FB & Mathers JC (1993) Complex carbohydrate digestion and large bowel fermentation in rats given wholemeal bread and haricot beans (*Phaseolus vulgaris*) fed in mixed diets. *British Journal of Nutrition* **69**, 497–509.

Lebet V, Jörger F, Arrigoni E, Wenk C & Amadò R (1996) Influence of a simulated digestion procedure on in vitro colonic fermentation patterns. In *Dietary Fibre and Fermentation in the Human Colon. Proceedings of the COST Action 92 Workshop*, pp. 101–109 [Y Mälkki and JH Cummings, editors]. Brussels: Office for Official Publications of the European Communities.

Longland AC & Low AG (1988) Digestion of diets containing molassed or plain sugar-beet pulp by growing pigs. *Animal Feed Science and Technology* **23**, 67–78.

McBurney MI & Thompson LU (1987) Effect of human faecal inoculum on in vitro fermentation variables. *British Journal of Nutrition* **58**, 233–243.

McBurney MI & Thompson LU (1989) Effect of human faecal donor on in vitro fermentation variables. *Scandinavian Journal of Gastroenterology* **24**, 359–367.

McNeil NI (1984) The contribution of the large intestine to energy supplies in humans. *American Journal of Clinical Nutrition* **39**, 338–342.

Marlett JA, Slavin MS & Brauer PM (1981) Comparison of dye and pellet gastrointestinal transit time during controlled diets differing in protein and fiber levels. *Digestive Diseases and Sciences* **26**, 208–213.

Metcalf AM, Phillips SF, Zinsmeister AR, MacCarthy RL, Beart RW & Wolff BG (1987) Simplified assessment of segmental colonic transit. *Gastroenterology* **92**, 40–47.

Monsma DJ & Marlett JA (1996) Fermentation of carbohydrate in rat ileal excreta is enhanced with cecal inocula compared with fecal inocula. *Journal of Nutrition* **126**, 554–563.

Prosky L, Asp N-G, Furda I, DeVries JH, Schweizer TF & Harland B (1985) Determination of total dietary fiber in foods and food products: collaborative study. *Journal of the Association of Official Analytical Chemists* **68**, 677–679.

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

Ruppin H, Bar-Meir S, Soergel KH, Wood CM & Schmitt MG Jr (1980) Absorption of short-chain fatty acids by the colon. *Gastroenterology* **78**, 1500–1507.

Southgate DAT & Durnin JVGA (1970) Calorie conversion factors: an experimental reassessment of the factors used in the calculation of the energy value of human diets. *British Journal of Nutrition* **24**, 517–535.

Stephen AM & Cummings JH (1980) Mechanism of action of dietary fibre in the human colon. *Nature* **284**, 283–284.

Theander O & Westerlund EA (1986) Studies on dietary fiber. 3. Improved procedure for analysis of dietary fiber. *Journal of Agricultural and Food Chemistry* **34**, 330–336.

Titgemeyer EC, Bourquin LD, Fahey GC & Garleb KA (1991) Fermentability of various fiber sources by human fecal bacteria in vitro. *American Journal of Clinical Nutrition* **53**, 1418–1424.

Van Soest PJ, Jeraci J, Foose T, Wrick K & Ehle F (1982) Comparative fermentation of fibre in man and other animals. In *Fibre in Human and Animal Nutrition*, pp. 75–80 [G Wallace and L Bell, editors]. Palmerston North: The Royal Society of New Zealand.

Wisker E, Bach Knudsen KE, Daniel M, Eggum BO & Feldheim W (1997) Energy values of non-starch polysaccharides: comparative studies in humans and rats. *Journal of Nutrition* **127**, 108–116.

Wisker E, Daniel M & Feldheim W (1996) Particle size of wholemeal rye bread does not affect the digestibility of macro-nutrients and non-starch polysaccharides and the energy value of dietary fibre in humans. *Journal of the Science of Food and Agriculture* **70**, 327–333.

Wyman JB, Heaton KW, Manning AP & Wicks ACB (1978) Variability of colonic function in healthy subjects. *Gut* **19**, 146–150.