Sugar composition of dietary fibre and short-chain fatty acid production during *in vitro* fermentation by human bacteria

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The aim of the present study was to assess the relationship between the disappearance of dietary fibre sugars and the production of individual short-chain fatty acids (SCFA). The bacterial degradation of five dietary fibres whose sugars were quantified was investigated *in vitro* using a human faecal inoculum. Involvement of the main fibre sugars in SCFA production was evaluated by a stepwise multiple linear regression. The results show first that the nature and chiefly the associations between the fibre sugars were key variables in the fermentability. Second, the nature and the amounts of SCFA produced were closely related to the *in vitro* fermentation of the main sugars available: uronic acids seemed to be principally involved in the production of acetic acid whereas the production of propionic acid could be promoted by the fermentation of glucose and, to a lesser extent, by that of xylose and arabinose. Xylose tended to have a greater impact than uronic acids and glucose on the production of butyric acid. Thus, it would be possible to predict which SCFA could be specifically produced during the fermentation of a fibre, as far as the chemical composition and structure of this fibre are known.

Dietary fibre: Sugars: Fermentation: Short-chain fatty acids

Fermentative breakdown of dietary fibre in the colon produces gases and short-chain fatty acids (SCFA) and influences the physiological and metabolic functions of animals and humans (Sakata, 1987; Nishina & Freedland, 1990; Cherbut *et al.* 1991; Titgemeyer *et al.* 1991). Many studies indicate that the rate and extent of fibre fermentation depend on their botanical origin, chemical composition and physico-chemical properties (Van Soest *et al.* 1983; McBurney *et al.* 1985; McBurney & Thompson, 1989, 1990).

Dietary fibre is composed of complex arrangements of sugars. When isolated, monomers are specifically utilized *in vitro* by the colonic bacteria (Barry *et al.* 1989) and their metabolism results in different production patterns of SCFA (Mortensen *et al.* 1988). As a whole, these results suggest that it is largely the sugar composition of fibre which controls the fermentation process. However, the architecture of the cell wall and the linkages between monomers may change the sugar fermentability and turn the metabolism products into specific SCFA (Titgemeyer *et al.* 1991). When they are arranged in complex structures in the plant cell wall the sugars are broken down through mechanisms which are still unknown.

In the present work the bacterial degradation of fibre sugars was followed using a system of fermentation *in vitro* with human faecal bacteria, previously described and validated (Barry *et al.* 1989; Auffret *et al.* 1991). Moreover, the relationship between the disappearance of sugars and the production of individual SCFA was assessed.

* For reprints.

MATERIALS AND METHODS

Chemical composition of dietary fibre

Five fibres were prepared from wheat bran, sugar beet, maize, pea hulls and cocoa by mechanical processes (grinding, air-classification, sieving and drying) and ground to the same average particle size (0·06 (SEM 0·02) mm). Their composition is presented in Table 1. Native wheat bran and maize were prepared by amylase (*EC* 3.2.1.1; 3.2.1.3) digestion to remove starch (Prosky *et al.* 1988). The neutral and acidic sugars of the fibres were determined by gas–liquid chromatography (Hoebler *et al.* 1989) and colorimetry (Thibault, 1979) respectively after the five fibres and their *in vitro* fermentation residues were hydrolysed at 35° for 1 h in H_2SO_4 (720 g/kg).

In vitro *fermentation*

Fresh faeces were collected from healthy subjects accustomed to eating an unspecified Western diet. The faecal inocula were mixed (1:3, w/v) with a CO_2-saturated nutritive buffer (Table 2) and then filtered through six gauzes to remove non-digested materials. Faecal contents, solutions and containers were kept under a constant flow of CO_2 during inoculum preparation. Two experiments were performed. For each experiment two batches of each fibre were prepared by mixing 400 mg fibre with 20 ml human faecal inoculum in a N_2 atmosphere. A control batch containing only 20 ml faecal inoculum was also prepared and incubated for 24 h. At time zero the batches were put into a water-bath at 40°. The time course of fermentation was followed by measuring total gas production each hour. The fermentations were stopped at 6, 12 and 24 h of incubation by adding 0·1 ml $HgCl_2$ (100 g/l). In the batches removed for analysis the pH of the medium was immediately measured and bottles were rinsed with 20 ml distilled water. The mixture was centrifuged for 10 min at 5000 ***g***. Supernatants were analysed for SCFA by gas–liquid chromatography (Jouany, 1982) and the pellets were analysed for cell wall sugars as described earlier.

Calculations and statistical analysis

The fermentability of each sugar (S), i.e. the rate of S disappearance, was calculated as follows: initial amount of S = amount of S in the fibre + amount of S in the inoculum, amount of digested S = initial amount of S − amount of S in the residue,

$$\text{S fermentability (\%)} = 100 \times (\text{amount of S digested/initial amount of S}).$$

The monosaccharides quantified in both native and fermented fibres were expressed as polymers (0·9 × weight of monosaccharide). To evaluate the involvement of the main fibre sugars in SCFA production a multiple linear regression was performed by using a stepwise regression procedure (Statview™ SE+ Graphics, 1987–1988 Abacus Concepts, Inc., California, USA). The four predictive variables considered were the amounts of arabinose, xylose, glucose and uronic acid which disappeared during the fermentation. They were tested in three equations corresponding to the production (mmol/l) of acetic, propionic and butyric acids respectively. This stepwise regression started with no variables in the equation of SCFA production. The variable with the highest partial correlation was inserted in the model if its partial F value exceeded the level defined to enter, which corresponded to a probability level of 5%. After the insertion of a variable, any variable currently in the model was examined for removal based on whether its probability was less than the level defined to remove ($P > 0{\cdot}05$). Additional variables were selected or discarded from the equation in the same way. This procedure continued until no variables currently in the equation could be removed and the variable with the highest partial correlation not

Table 1. *Chemical composition (% of dry matter) of fibre material**

Source of fibre	Ash	Crude protein	Starch	TF	SF	Lignin (Klason)
Wheat bran	6·1	15·1	22·9	52·7	9·2	10·9
Sugar beet	4·4	8·7	1·5	75·5	14·0	5·4
Maize	1·8	3·2	14·3	66·2	2·6	6·7
Pea	2·4	3·0	1·2	88·1	4·9	2·3
Cocoa	9·2	14·2	0·0	81·1	18·8	29·1

TF, total fibre; SF, soluble fibre.
* For details of procedures, see p. 190.

Table 2. *Composition (g/l) of the nutritive buffer mixed with the faecal inocula (1:3 w/v) to prepare the fermentation medium**

Component	
$NaHCO_3$	9·240
$Na_2HPO_4.12H_2O$	7·125
NaCl	0·470
KCl	0·450
Na_2SO_4	0·100
$CaCl_2$	0·055
$MgCl_2$	0·047
Urea	0·400
$FeSO_4.7H_2O$ (mg/l)	36·800
$MnSO_4.7H_2O$ (mg/l)	19·000
$ZnSO_4.7H_2O$ (mg/l)	4·400
$CoCl_2.6H_2O$ (mg/l)	1·200
$CuSO_4.5H_2O$ (mg/l)	0·980
$Mo_7(NH_4)_6O_{24}.4H_2O$ (mg/l)	0·174

* For details of procedures, see p. 190.

in the equation failed the test to enter. The results are expressed as the final equation variables, i.e. the constant, the multiple correlation coefficient (R), the partial correlation coefficient (r) and the F value of the entered variables. The higher the coefficient (r) assigned to each sugar the more this sugar was needed to produce SCFA. The higher the F value attributed to each sugar the more significantly this sugar was predictive for SCFA production.

RESULTS

The five fibres were all predominantly composed of arabinose, xylose, glucose and uronic acids (Table 3). Wheat bran and maize were characterized by high contents of arabinose and xylose, a relatively high content of glucose and a low content of uronic acids. On the contrary, sugar beet, pea and cocoa were rich in uronic acids but differed in their main neutral sugar composition; arabinose and glucose were predominant in sugar beet, glucose and xylose in pea, and glucose in cocoa.

The fermentability of the total sugars *v.* time was used to classify the five fibres from the most to the least degraded as follows: sugar beet, cocoa, wheat bran, pea and maize (Fig. 1). Sugar beet was the most rapidly (46·1 (range 43·6–48·7) % at 6 h), and completely (81·9

Table 3. *Sugar composition (% initial dry matter) of fibres**

Source of fibre	Rha	Ara	Xyl	Man	Gal	Glc	Total NS	Uron
Wheat bran	0·1	7·6	12·5	0·8	0·8	8·5	30·3	1·9
Sugar beet	1·7	17·8	1·5	1·3	4·5	22·7	49·6	19·4
Maize	0·2	10·7	16·7	0·9	3·1	12·2	43·8	3·2
Pea	0·7	3·6	11·6	0·2	1·1	50·2	67·6	14·7
Cocoa	0·8	1·8	1·8	2·6	2·9	15·8	25·7	12·9

Rha, rhamnose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, cellulosic glucose; NS, neutral sugars; Uron, uronic acids.

* Starch of wheat bran and maize is previously enzymically removed. For details of procedures, see p. 190.

(range 81·9–82·0)% at 24 h) degraded, whereas maize was least fermented (11·0 (range 6·2–15·8)% at 24 h). Cocoa, wheat bran and pea were only partly broken down after 24 h of incubation, 49·1 (range 47·3–50·9)%, 43·7 (range 42·5–44·8)% and 28·7 (range 25·2–32·2)% respectively. The fermentation of pea began much more slowly than that of cocoa and wheat bran, 8·5 (range 8·3–8·8)% compared with 34·4 (range 34·2–34·7)% and 35·0 (range 28·3–41·6)% respectively after 6 h incubation.

The respective fermentabilities of the main sugars of the five fibres are represented in Fig. 2. The disappearance of uronic acids always began rapidly and immediately in all fibres. After 6 h it continued increasing sharply for sugar beet and slightly for cocoa and pea, whereas a plateau was rapidly reached for wheat bran and maize. By contrast, xylose was only slightly degraded except in wheat bran in which this sugar was more fermented than uronic acids. Arabinose in sugar beet, cocoa and pea also largely disappeared, whereas it was almost undegraded in wheat bran and maize. Glucose was only slightly broken down in pea and very poorly fermented in cocoa, maize and wheat bran. Its degradation was high in sugar beet but began more slowly than that of uronic acids and arabinose.

SCFA production was significantly correlated with pH values (R 0·93, $P = 0·0001$), gas production (R 0·92, $P = 0·0001$) and sugar fermentability (R 0·91, $P = 0·0001$). The production of the main SCFA, i.e. acetic, propionic and butyric acids, was different for the five fibres as shown in Fig. 3. Acetic acid was the major product in the medium with respect to the rate of fibre degradation, whatever the fibre. The incubation of maize, cocoa and pea produced similar concentrations of propionic acid, whereas this acid accumulated in greater amounts during the fermentation of sugar beet and wheat bran. The production of butyric acid was also higher from sugar beet and wheat bran than from cocoa, pea and maize.

With regard to the stepwise multiple linear regression performed to evaluate the involvement of four sugars, i.e. arabinose, xylose, glucose and uronic acids, in SCFA production, the variables of the final regression equation for acetic, propionic and butyric acids are presented in Table 4. For all three equations R was high. Among the four sugars taken into account, only uronic acids, xylose and glucose were inserted in the final equation of the production of acetic acid. Both r and F values of xylose and glucose were similar, but those of uronic acids were higher; the latter, therefore, provided the best predictor. Regarding the production of propionic acid, only glucose, xylose and arabinose were selected in the final equation; glucose showed slightly higher r and F values than arabinose or xylose. In the production of butyric acid, only xylose, glucose and uronic acids entered the final equation but both r and F values associated with xylose were higher than those of glucose and uronic acids. As a whole, the respective F values showed that the amounts of

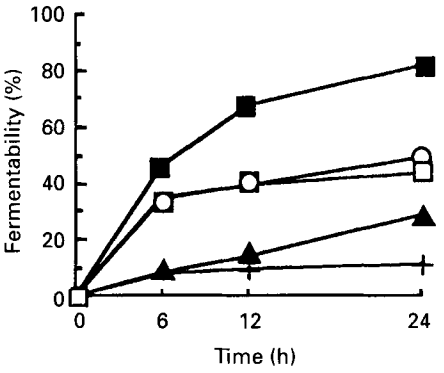

Fig. 1. *In vitro* fermentability of the total sugars of five fibres: wheat bran (□), sugar beet (■), maize (+), pea (▲) and cocoa (○). Values are the mean of two experiments. Starch of wheat bran and maize has been enzymically removed.

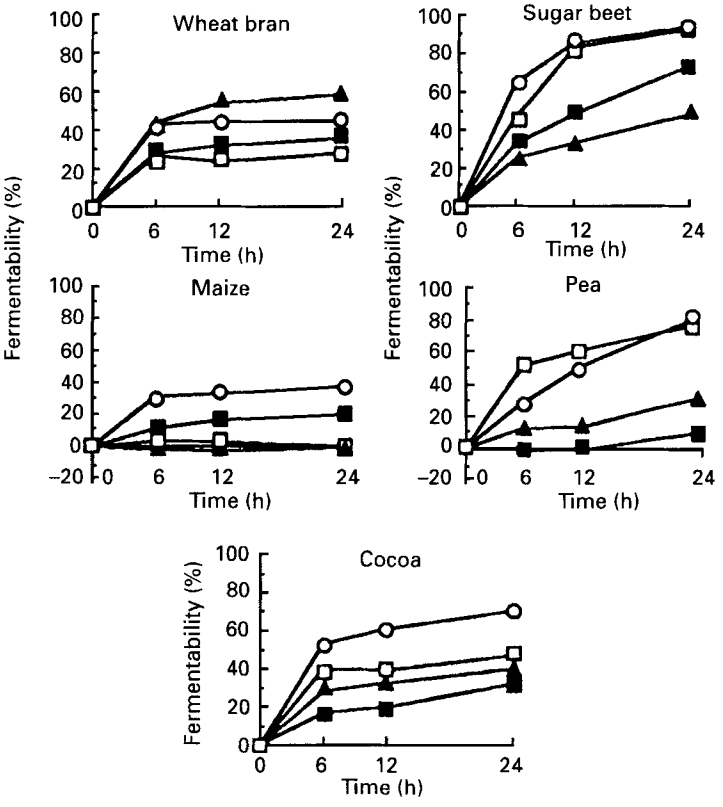

Fig. 2. *In vitro* fermentability of the main sugars of five fibres: arabinose (□), xylose (▲), glucose (■) and uronic acids (○). Values are the mean of two experiments. Starch of wheat bran and maize has been enzymically removed.

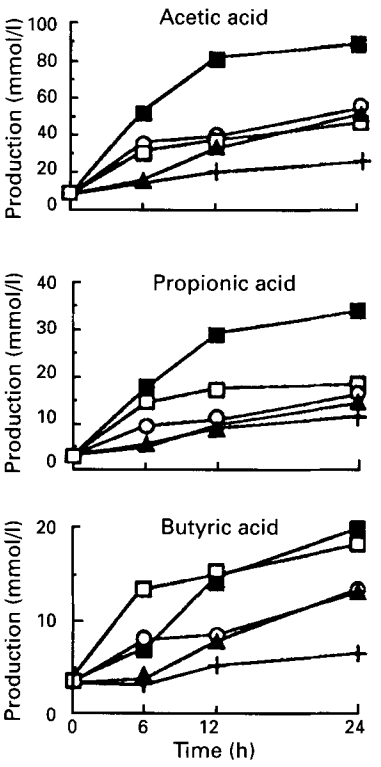

Fig. 3. Production of short-chain fatty acids (acetic, propionic and butyric acids) during the *in vitro* incubation of five fibres: wheat bran (□), sugar beet (■), maize (+), pea (▲) and cocoa (○). Values are the mean of two experiments. Different scales are used for each acid.

Table 4. *Variables of the multiple linear regression final equations**

(Values in parentheses are the partial *F* values assigned to sugars; *n* 30 ($P < 0.05$), e.g. acetic acid = 12·07 + 0·33 xylose + 0·33 glucose + 0·72 uronic acids)

	r		
	Acetic acid	Propionic acid	Butyric acid
Dietary fibre sugars:			
Arabinose	NS	0·16 (7·6)	NS
Xylose	0·33 (9·2)	0·11 (6·8)	0·23 (42·8)
Glucose	0·33 (12·1)	0·22 (16·9)	0·08 (6·6)
Uronic acids	0·72 (51·0)	NS	0·11 (11·1)
Constant	12·07	6·51	2·51
Multiple *R*	0·94	0·92	0·88

r, partial correlation coefficient; NS, not significant.
* For details of procedures, see p. 190.

fermented sugars only accounted for about 32% in the production of propionic acid whereas they accounted for about 72 and 61% respectively in the production of acetic and butyric acids.

DISCUSSION

The results show that fibre sugars were not equally utilized by human bacteria during *in vitro* fermentation in terms of rate and extent of degradation together with nature of SCFA produced from these sugars. The chemical nature and the physical arrangement of the sugars in the fibre matrix controlled the rate and extent of degradation, together with the nature of the SCFA produced form these sugars. Uronic acids of sugar beet, cocoa and pea fibres were highly fermented. This is in agreement with numerous results which report that pectins and uronic acids are extensively degraded by colonic bacteria (Van Soest *et al.* 1983; Stevens & Selvendran, 1988; Graham *et al.* 1989; Cherbut *et al.* 1991). However, the present results conflict somewhat with those of Nyman *et al.* (1990) which show a low faecal digestibility of uronic acids of carrot, pea and Brussels sprout in rats. Nevertheless, the thermal processing followed by a long frozen storage applied to these vegetables could have altered the cell wall structure thus changing fermentability patterns (Plat *et al.* 1991; Guillon *et al.* 1992). In the present study, when compared with the uronic acids of sugar beet and cocoa those of pea were degraded to a lesser extent and much more slowly. Pea fibres are known to contain pectic substances but are also rich in acidic xylans and cellulose (Titgemeyer *et al.* 1991). Linkages between uronic acids and xylose could explain the low fermentability of both sugars in pea. Indeed, resistance of xylose in polymeric and monomeric forms against fermentation is observed in both *in vivo* (Graham *et al.* 1989) and *in vitro* (Barry *et al.* 1989) studies respectively. In agreement with these observations, the present results show that xylose was only slightly degraded in all the fibres except wheat bran, as previously reported (Stevens & Selvendran, 1988). This preferential degradation of the xylose in wheat bran would be due to its arrangement in the cell wall. The hemicelluloses of wheat bran are composed of two fractions (Brillouet & Mercier, 1981). The major fraction is essentially composed of linear xylans which are preferentially degraded, whereas the minor fraction, mainly composed of highly branched arabinoxylans, are only slightly hydrolysed by bacterial enzymes. These highly-branched arrangements of arabinoxylans would impede the fermentation of arabinose (Salyers *et al.* 1978; Brillouet & Mercier, 1981), which could explain the low fermentability of arabinose in wheat bran and maize in our study. By contrast, the degradation of arabinose was probably favoured by its combination with uronic acids of the pectic substances in sugar beet fibres.

Glucose from cellulose in the five fibres was poorly used by bacteria, in agreement with most fermentation or digestibility studies (McBurney & Thompson, 1989; Nyman *et al.* 1990; Goodlad & Mathers, 1991). The crystalline structure of cellulose (Kerley *et al.* 1988) and its linkage with antibacterial substances such as lignin in wheat bran (Stevens & Selvendran, 1988) and tannins in cocoa (Brownlee *et al.* 1990) could explain the low fermentability of glucose. Moreover, the degradation of glucose in the present study always began when acidic sugars had almost totally disappeared. Similar observations are reported in previous studies about the synergistic effects of pectinolytic and cellulolytic enzymes on the degradation of vegetable cell wall (Stevens *et al.* 1988; Thibault & Rouau, 1990).

Thus, fermentation of the respective sugars of the fibres depended on their linkages with each other and on the architecture of the cell wall. These structural arrangements were key variables governing the availability of sugars and might consequently be involved in SCFA production. In fact, the fermentation of the five fibres differed significantly in the production of total and individual SCFA, in agreement with the results of McBurney & Thompson (1989). The production rates of individual SCFA were closely correlated with the fermentabilities of the sugars. We observed the highest concentrations of acetic acid during the fermentation of sugar beet, cocoa and pea, which were the richest in uronic acids, in agreement with *in vitro* studies showing that uronic acids and pectins induce acetic

acid formation (McBurney & Thompson, 1987; Mortensen *et al.* 1988; Titgemeyer *et al.* 1991). Indeed, the percentage disappearance of uronic acids was the best predictor of acetic acid concentration. However, the stepwise regression variables calculated (Table 3) show that xylose and glucose were less involved in this production. Mortensen *et al.* (1988) reported that the degradation of pentoses leads to the production of propionic acid. Our results support this finding since fermentabilities of both arabinose and xylose were correlated with the concentration of propionic acid. Glucose was more predictive of the production of propionic acid than pentoses. This result is in accordance with that of Barry *et al.* (1989) who observed that degradation of monomeric glucose greatly influences the production of propionic acid *in vitro*. The fermentation of wheat bran and sugar beet, in which xylose and uronic acids respectively were the most fermented sugars, led to a higher production of butyric acid than with the other fibres. Results of the stepwise regression suggest that xylose was the most suitable of the fibre sugars for the production of butyric acid *in vitro*, whereas glucose and uronic acids were less involved. This is in accordance with the findings of Cheng *et al.* (1987) who showed that arabinoxylans and β-glucans in wheat bran are effective in generating butyric acid, and with Mortensen *et al.* (1988) who observed that butyric acid concentration was higher in assays incubated with uronic acid derivatives. The predictive F values allowed propionic acid to be considered apart from other SCFA since its formation was less predicted by sugar disappearance compared with acetic and butyric acids. This could be related to the different metabolic pathways. Propionic acid formation involves the production of succinate as an intermediate whereas both acetic and butyric acids directly result from pyruvate via acetyl coenzyme A (Miller & Wolin, 1979). Such a relationship would be interesting to investigate.

The present study suggests first that fibre sugars behaved *in vitro* in a particular way unconnected with their own fermentability but depending on their associations with the other sugars. The fermentability of fibre sugars is, therefore, mainly based on the chemical structure of fibres. Second, the production of individual SCFA is controlled by the nature and the quantity of the fibre sugars available for the fermentation. It might, thus, be possible to predict which SCFA would be produced during the fermentation of a fibre, as far as the chemical structure of this fibre was already known.

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