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Content of short-chain fatty acids in the hindgut of rats fed processed bean (*Phaseolus vulgaris)* flours varying in distribution and content of indigestible carbohydrates

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Red kidney beans (*Phaseolus vulgaris*) processed to differ in distribution and content of indigestible carbohydrates were used to study hindgut fermentability and production of short-chain fatty acids (SCFA). Bean flours with low or high content of resistant starch (RS), mainly raw and physically-inaccessible starch, were obtained by milling the beans before or after boiling. Flours containing retrograded starch and with a high or low content of oligosaccharides were prepared by autoclaving followed by freeze-drying with or without the boiling water. Six diets were prepared from these flours yielding a total concentration of indigestible carbohydrates of 90 or 120 g/kg (dry weight basis). The total fermentability of the indigestible carbohydrates was high with all diets (80–87 %). Raw and physically-inaccessible starch was more readily fermented than retrograded starch (97–99 % *v.* 86–95 %; $P < 0{\cdot}05$). Non-starch glucans were fermented to a lesser extent than RS, but the fermentability was higher ($P < 0{\cdot}05$) in the case of autoclaved (50–54 %) than boiled beans (37–41 %). The distribution between acetic, propionic and butyric acid in the caecum was similar for all diets, with a comparatively high percentage of butyric acid (approximately 18). However, with diets containing the high amounts of RS, the butyric acid concentration was significantly higher in the distal colon than in the proximal colon ($P = 0{\cdot}009$ and $P = 0{\cdot}047$ for the high- and low-level diets respectively), whereas it remained constant, or decreased along the colon in the case of the other diets. Furthermore, the two diets richest in RS also promoted the highest percentages of butyric acid in the distal colon (24 and 17 *v.* 12 and 12–16 for the high- and low-level diets respectively).

Short-chain fatty acids: Red kidney beans: Indigestible carbohydrates

Short-chain fatty acids (SCFA; acetic, propionic and butyric acid) formed during bacterial fermentation of carbohydrates in the colon, have been suggested to have specific physiological effects. Thus, butyric acid, the main energy substrate for the colonocytes (Roediger, 1982), may play a role in the prevention and treatment of various colonic diseases (McIntyre *et al.* 1993; Hague *et al.* 1995). In colonic tumour cell lines, butyrate has been shown to inhibit growth (Whitehead *et al.* 1986), differentiation (Siavoshian *et al.* 2000) and to induce apoptosis (Hague & Paraskeva, 1995). Further, patients with distal ulcerative colitis given rectal infusions with SCFA mixtures (Breuer *et al.* 1991; Vernia *et al.* 1995) or butyrate alone (Scheppach *et al.* 1992) improved, regarding symptoms and/or, histological and endoscopic scores. However, lower concentrations of SCFA or butyrate enemas had no effect on diversion colitis (Guillemot *et al.* 1991) or distal ulcerative colitis (Steinhart *et al.* 1996). Propionic acid is discussed as beneficial in relation to lipid metabolism (Venter *et al.* 1990; Wright *et al.* 1990; Todesco *et al.* 1991). It has thus been proposed that propionate may lower plasma cholesterol concentrations by inhibiting hepatic cholesterogenesis (Chen *et al.* 1984). However, the results from studies examining the effects of propionic acid on cholesterol metabolism are not consistent. Both lack of cholesterol lowering (Bach Knudsen & Canibe, 1993) and increased plasma cholesterol levels (Beaulieu & McBurney, 1992) have been observed in pigs caecally infused with propionate. In human subjects however, the synthesis of cholesterol from acetate decreased when propionate was infused rectally (Wolever *et al.* 1991).

Although not all studies are indicative of colonic or metabolic benefits, the possible health-promoting effect of

Abbreviations: DCF, damaged-cell flour; dwb, dry weight basis; HOF, high-oligosaccharide flour; ICF, intact-cell flour; LOF, low-oligosaccharide flour; NDO, non-digestible oligosaccharides; RS, resistant starch; SCFA, short-chain fatty acids.

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SCFA has generated interest in the pattern formed from different carbohydrate sources. The content and distribution of SCFA are dependent on the microflora in the intestinal tract (Macfarlane & Cummings, 1995) and the carbohydrate substrate (Cummings & Englyst, 1987; Brighenti *et al.* 1989). Indigestible carbohydrates reaching the colon are mainly resistant starch (RS), NSP and non-digestible oligosaccharides (NDO). Studies *in vivo* in pigs (Brown *et al.* 1997; Martin *et al.* 1998; Bird *et al.* 2000) and human subjects (Phillips *et al.* 1995) have shown that RS yields different proportions of butyrate on fermentation, depending on the origin of the RS substrates. Other substrates (e.g. pectin) have been shown to give high proportions of acetic acid in rats, while guar gum yielded high proportions of propionic acid (Brighenti *et al.* 1989; Berggren *et al.* 1993).

Up to now, SCFA patterns formed from fermentation have been studied mainly using single substrates. Few studies are at hand regarding the potential impact of the nature of the substrate, or whether or not the substrate is one of a mixture, which is more relevant to the human diet (Hara *et al.* 1994; Campbell *et al.* 1997). Previously, it was shown that the percentage of butyric acid in the rat caecum was 18–21 with diets composed of a mixed source of indigestible carbohydrates (pea (*Pisum sativum*) fibre, oat bran and pectin; AM Berggren, IME Björck and EMGL Nyman, unpublished results), which was considerably higher than when the same carbohydrates were tested as single substrates: pea fibre (11 %), oat bran (9 %) and pectin (7 %; Berggren *et al.* 1993). Other researchers have also demonstrated a synergistic effect of a combination of carbohydrates on butyrate yield. Thus, Topping *et al.* (1985) reported that a mixture of gum arabic and cellulose was more efficient in generating butyric acid in the rat than the individual substrates. Furthermore, fermentation of the pericarp and aleurone in wheat bran generated a higher mass of butyrate in the rat than the individual fractions (Cheng *et al.* 1987).

In the present study rats were used as a model for fermentation in man, and the content of SCFA were measured at different sites along the hindgut. Although rats are caecum fermenters, the degree of fermentation of a variety of dietary fibres has been shown to correlate well between rat and man (Nyman *et al.* 1986). Further, there are similarities in fermentability and SCFA pattern when comparing *in vitro* fermentation data for different dietary fibres, using rat and human faecal inocula (Barry *et al.* 1995).

The purpose of the present investigation was to study the fermentability and hindgut SCFA content and distribution in rats fed bean-flour diets. The red kidney beans (*Phaseolus vulgaris* L.) were processed to yield flours varying in distribution of indigestible carbohydrates (i.e. RS, NSP and NDO) and botanical microstructure.

Materials and methods

Materials

The raw material used was red kidney beans from AB Risenta (Stockholm, Sweden). One batch of beans was processed in different ways in order to obtain four precooked flours, as follows. Two of the bean flours varied with respect to the integrity of the cells and either contained more or less intact cells (intact-cell flour; ICF), or was completely devoid of cell structure (damaged-cell flour; DCF). The two other materials were autoclaved and either freeze-dried with the boiling water included to retain NDO (high-oligosaccharide flour; HOF) or drained to partly remove soluble components such as NDO (low-oligosaccharide flour; LOF).

Processing of the flours

ICF was prepared in following manner. Beans were soaked in twice their weight of water for 20 min at room temperature and drained. Then the soaked seeds were boiled for 70 min in a water-bath, with seed–water being 1:3 (w/v). The beans, along with the boiling water, were freeze-dried and ground to pass a 1 mm screen in a Cyclotec 1093 mill (Tecator AB, Höganäs, Sweden). The resulting flour was kept in a desiccator until used.

DCF was obtained from beans that were soaked in twice their weight of water for 20 min at room temperature and drained. The soaked seeds were ground to pass a 1 mm screen in a Cyclotec 1093 mill. This rough treatment resulted in breakage of the cell walls in the beans. The milled beans were boiled for 70 min in water-bath, with seed–water being 1:6 (w/v). The beans, together with the boiling water, were freeze-dried and then again ground to pass a 1 mm screen in a Cyclotec 1093 mill. The resulting flour was kept in a desiccator until used.

HOF was obtained in following manner. Unsoaked beans were autoclaved for 20 min (69 kPa), with seed–water being 1:5 (w/v). Then the beans, along with the cooking water (containing NDO), were freeze-dried and ground to pass a 1 mm screen in a Cyclotec 1093 mill. The resulting flour was kept in a desiccator until used.

LOF was prepared by first soaking the beans in water (1:3, w/v) for 12 h at room temperature and then draining them. The soaked beans were autoclaved for 20 min (69 kPa), with seed–water being 1:5 (w/v). The autoclaved beans were drained to remove the extracted oligosacharides, freeze-dried and ground to pass a 1 mm screen in a Cyclotec 1093 mill. The resulting flour was kept in a desiccator until used.

Animals and experimental diets

Male Wistar rats (B & K Universal, Stockholm, Sweden) with an initial weight of 79 ± 5 g were divided into groups, seven per diet, and housed individually in metabolism cages that minimised the risk of coprophagy (Berggren *et al.* 1993). The feed intake was restricted to 12 g (dry weight basis; dwb)/d. After an adaptation period of 7 d, faeces were collected daily during a 5 d balance period. Faeces were kept at −20°C and then freeze-dried and milled before analysis. On the final day of the balance experiment, the animals were killed with CO_2, and caecum and proximal and distal colons were removed. The pH of the caecal contents was measured before transferring the gastro-intestinal contents to a freezer. The animal experiment was

approved by the Ethics Committee for Animal Studies at Lund University.

To study the potential impact of differences in the distribution of various kinds of indigestible carbohydrate on hindgut SCFA production, diets were prepared from the various bean flours. The diets were prepared to give two levels of indigestible carbohydrates, about 90 g/kg (dwb) and 120 g/kg (dwb) respectively (Table 1).

Six bean-flour diets were prepared of which four (prepared from ICF, DCF, HOF and LOF) contained the low concentration of indigestible carbohydrates (approximately 90 g/kg; dwb). Further, raffinose (30 g/kg; dwb) was added to one of the bean flours, LOF, yielding a diet with a proportionally higher content of oligosaccharides, and a final concentration of indigestible carbohydrates of 120 g/kg (dwb). This diet was compared with a diet containing ICF at a higher concentration of indigestible carbohydrates (approximately 120 g/kg; dwb; Table 1). In addition to the protein present in the beans, casein (Sigma Chemical Company, St Louis, MO, USA) was added to give a final level of 120 g/kg (dwb). The DM content of the diets was adjusted with wheat starch, a starch source that can be expected to be completely digested and absorbed in the rat small intestine (Björck *et al.* 1987), and thus not contribute to hindgut fermentation.

Analytical methods

Scanning electron microscopy. The bean flours were studied at cell level with scanning electron microscopy. Flours were mounted on Al specimen stubs with double-sided sticky tape, coated with Au–Pd in a Polaron E5500 diodsputter (Polaron Range, East Grinstead, UK), and examined in a JEOL T330 Scanning electron microscope (JEOL, Tokyo, Japan), operated at 10 kV.

NSP. The content of soluble and insoluble dietary fibre in the bean material was analysed gravimetrically in principle according to Asp *et al.* (1983). The composition of the isolated dietary fibre was analysed by GLC for the neutral sugars as their alditol acetates and by a spectrophotometric method for the uronic acids (Theander *et al.* 1995). The dietary fibre values were corrected for the amount of total starch present in the isolated fibre residue and referred to as NSP. The content of NSP in faeces was analysed as for the raw materials but without the gravimetric step.

Starch. Total starch content in the raw materials (bean flours and gravimetric dietary fibre residues) and in faeces from the rats was analysed as liberated glucose after solubilisation in KOH and enzymic treatment with a thermostable α-amylase (Termamyl 300L DX; Novo Nordisk A/S, Copenhagen, Denmark) and amyloglucosidase (Björck & Siljeström, 1992) with one modification. Thus, the sample was suspended in 10 ml 0·1 M-phosphate buffer (pH 6·0) instead of water, in the first incubation step.

The amount of RS in the bean flours was analysed using an *in vitro* model (Åkerberg *et al.* 1998) Six human subjects chewed glass beads and rinsed their mouth with 5 ml water, and thereafter the saliva was pooled. Five ml of pooled saliva was transferred to a beaker containing the test product and water. The pH was adjusted to 1·5 and pepsin was added. Thereafter the samples were incubated at 37°C for 30 min. The pH was adjusted to 5·0 after addition of pancreatin and amyloglucosidase. The suspension was incubated for 16 h at 40°C, and undigested starch was precipitated with ethanol and analysed as above (Björck &

Table 1. Composition (g/kg; dry weight basis) and distribution (%) of various types of indigestible carbohydrates in test diets containing four flours prepared from red kidney bean (*Phaseolus vulgaris*) by different procedures†

	High-level diets (120 g/kg)		Low-level diets (90 g/kg)			
	ICF	LOF + raffinose	ICF	DCF	HOF	LOF
Ingredients (g/kg)						
Bean flour	332	332	251	332	332	332
Raffinose	–	30	–	–	–	–
Casein	38	35	58	39	35	35
Basal diet mixture*	209	209	209	209	209	209
Wheat starch	421	394	482	420	424	424
Indigestible carbohydrate composition						
Total amount (g/kg)	123	120	93	86	88	89
RS (%)	42	13	42	16	16	17
Soluble NSP (%)	11	20	11	18	27	27
Insoluble NSP (%)	34	35	34	48	39	46
NDO (%)	13	32	13	18	18	10

DCF, damaged-cell flour; HOF, high-oligosaccharide flour; ICF, intact-cell flour; LOF, low-oligosaccharide flour; NDO, non-digestible oligosaccharides; RS, resistant starch.

* Contained (g/kg): 100 sucrose, 50 maize oil, 1·2 DL-methionine (Sigma Chemical Company, St Louis, MO, USA), 2 choline chloride (Aldrich-Chemie, Steinheim, Germany), 48 mineral mixture (containing (g/kg): $CuSO_4.5H_2O$ 0·37, $ZnSO_4.7H_2O$ 1·4, KH_2PO_4 332·1, $NaH_2PO_4.2H_2O$ 171·8, $CaCO_3$ 324·4, KI 0·068, $MgSO_4$ 57·2, $FeSO_4.7H_2O$ 7·7, $MnSO_4.H_2O$ 3·4, $CoCl.6H_2O$ 0·020, NaCl 101·7), 8 vitamin mixture (containing (g/kg): menadione 0·62, thiamine hydrochloride 2·5, riboflavin 2·5, pyridoxine hydrochloride 1·25, calcium pantothenate 6·25, nicotinic acid 6·25, folic acid 0·25, inositol 12·5, *p*-aminobenzoic acid 1·25, biotin 0·05, cyanocobalamin 0·00375, vitamin A 0·19, vitamin D 0·000613, vitamin E 25, maize starch 941·25).

† For details of procedures, see p. 380.

Siljeström, 1992). Pooled saliva was used instead of an initial chewing of the sample since the product was not realistic food item but a dry flour. The analysis was performed in triplicate.

Total starch detected in faeces, when corrected for free glucose analysed with a glucose oxidase/peroxidase reagent, was regarded as resistant (Björck & Siljeström, 1992). The analysis was preformed in duplicate. The amount of free glucose in faeces was very small and less than 12% of the total α-glucan in faeces was glucose.

In vitro rate of starch hydrolysis. The *in vitro* rate of starch hydrolysis in ICF and DCF was measured according to Granfeldt *et al.* (1992). The rate of appearance of starch degradation products was analysed in the dialysate following sequential incubation with pooled saliva, pepsin and α-amylase. The analysis was preformed in triplicate.

Low-molecular-weight carbohydrates. The content of raffinose, stachyose and verbascose in the bean flours and in representative samples of the rat faeces, was analysed according to Nygaard Johansen *et al.* (1996). Low-molecular-weight carbohydrates were extracted from 1 g sample in boiling water for 30 min and quantified by high-performance anion-exchange chromatography with pulsed ampereometric detection, using a Dionex DX 500 chromatography system Dionex, Sunnyvale, CA, USA. The analytical column was a CarboPac PA 10 (4 × 250 mm) (Dionex) and a PA 10 Guard column (4 × 50 mm) was also installed. Arabinose was used as internal standard (5·0 mg/l). Before injection, the samples were cleaned by filtering through Millex®-HV filters (0·45 μm; Millipore, Bedford, MA, USA) and on Guard-A (Dionex) to remove peptides. Water and 300 mM-NaOH were used as eluents with a flow rate of 1·0 ml/min.

Protein. The amount of N in the bean flours was determined by the Kjeldahl method (Association of Official Analytical Chemists, 1984). The amount of protein was calculated as N × 6·25.

Short-chain fatty acids. A GLC method was used to analyse the SCFA (formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic and heptanoic acid) and succinic acid in caecal and colonic contents (Richardson *et al.* 1989). The intestinal content was homogenised

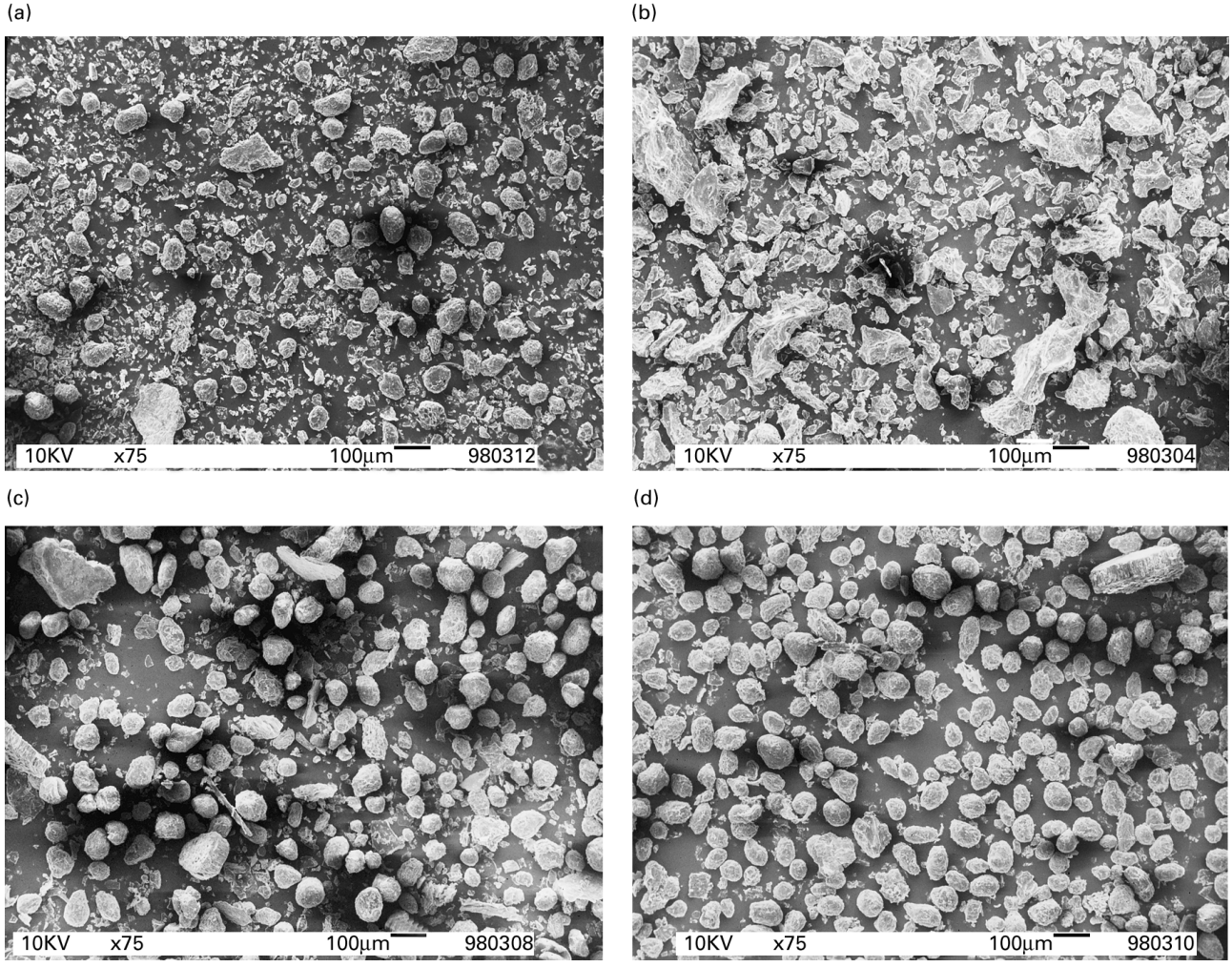

Fig. 1. Scanning electron micrographs of four flours prepared by different procedures from red kidney bean (*Phaseolus vulgaris*). (a) Intact-cell flour, (b) damaged-cell flour, (c) high-oligosaccharide flour, (d) low-oligosaccharide flour. For details of procedures, see pp. 380–383. magnification ×75.

(Polytron®; Kinematica, Luzern, Switzerland) with 2-ethylbutyric acid (internal standard). HCl was added to protonise the SCFA, which were then extracted with diethylether and silylated with *n*-(tert-butyldimethylsilyl)-*n*-methyltrifluoroacetamide (MTBSTFA; Sigma Chemical Company). The samples were allowed to stand for 24 h to complete derivatization before injection on a HP-5 column (Hewlett-Packard Corp., Wilmington, DE, USA).

Calculations and statistical evaluation

Total fermentability of the indigestible carbohydrates was calculated as the amount of carbohydrates excreted in faeces divided by the ingested amount of indigestible carbohydrates.

The caecal concentration of SCFA were corrected for the small differences in feed intake and extrapolated to a complete intake of the diet.

All statistical analyses were performed with the Minitab® statistical software package version 12.2 (Minitab Inc, State College, PA, USA). The means were analysed by ANOVA using the general linear model procedure according to Minitab®. Significance of difference between the means was determined by multiple comparison of Tukey's. The level of significance was $P < 0{\cdot}05$.

Results

Scanning electron microscopy of the bean flours

Scanning electron microscopy indicated that the ICF contained structures (75–100 μm) corresponding to intact cells (Fig. 1a). In contrast, the DCF was essentially devoid of such structures (Fig. 1b), suggesting that the milling of raw beans resulted in an extensive cell damage. The autoclaved bean flours HOF (Fig. 1c) and LOF (Fig. 1d) seemed to contain mainly intact cells.

In vitro starch hydrolysis

The course of *in vitro* starch hydrolysis for ICF and DCF is shown in Fig. 2. The rate of hydrolysis was considerably lower for ICF than for DCF, and after 180 min the starch in ICF was 30 % hydrolysed, compared with 54 % in DCF. The rate of *in vitro* starch hydrolysis was not measured in HOF and LOF.

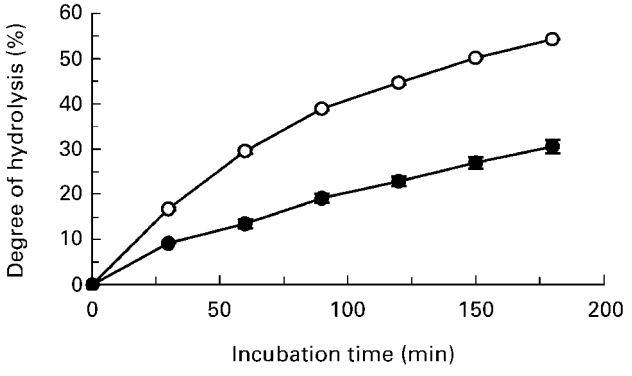

Fig. 2. *In vitro* hydrolysis of starch in intact-cell flour (●) and damaged-cell flour (○) prepared by different procedures from red kidney bean (*Phaseolus vulgaris*). For details of procedures, see p. 380.

Table 2. Content of carbohydrates (g/kg; dwb) in the four flours prepared by different procedures from red kidney bean (*Phaseolus vulgaris*)†

Bean flour…	ICF	DCF	HOF	LOF
Total starch	421[a]	411[b]	405[b]	435[c]
RS	154[a]	42[b]	42[b]	47[b]
Total NSP: Soluble	42[a]	46[b]	71[c]	71[c]
Insoluble	126[a]	125[a]	104[bc]	125[ac]
Rhamnose: Soluble	1[a]	1[a]	4[b]	4[b]
Insoluble	5[a]	5[a]	2[b]	2[b]
Fucose: Soluble	2[a]	1[a]	4[b]	4[b]
Insouble	4[a]	4[a]	1[b]	1[b]
Arabinose: Soluble	14[a]	18[b]	17[b]	18[b]
Insoluble	30[a]	27[ab]	26[b]	29[ab]
Xylose: Soluble	5[a]	6[b]	10[c]	10[c]
Insoluble	15[a]	15[a]	11[b]	13[c]
Mannose: Soluble	0[a]	0[a]	6[b]	6[b]
Insoluble	5[a]	5[a]	1[b]	1[b]
Galactose: Soluble	7[a]	7[a]	10[b]	10[b]
Insoluble	8[a]	9[a]	4[b]	4[b]
Glucose: Soluble	1[a]	1[a]	7[b]	7[b]
Insoluble	43[a]	45[a]	45[a]	57[a]
Uronic acids: Soluble	12[a]	12[a]	13[a]	12[a]
Insoluble	16[a]	17[ac]	14[b]	18[c]
Total NDO	47[a]	46[a]	47[a]	26[b]
Raffinose	2[a]	2[a]	2[a]	1[b]
Stachyose	43[a]	42[a]	42[a]	24[b]
Verbascose	2[a]	2[a]	3[c]	1[d]

ICF, intact-cell flour; DCF, damaged-cell flour; HOF, high-oligosaccharide flour; LOF, low-oligosaccharide flour; dwb, dry weight basis; RS, resistant starch; NDO, non-digestible oligosaccharides.

[a,b,c]Mean values (n 3) in the same row with different superscript letters were significantly different ($P < 0{\cdot}01$).

† For details of procedures, see p. 380.

Content of indigestible carbohydrates in the bean flours

The content (g/kg; dwb) of RS, soluble and insoluble NSP and NDO (raffinose, stachyose and verbascose) in the bean flours is listed in Table 2.

The amount of RS was considerably higher in the ICF than in the other three bean flours. The total amount of NSP was similar for ICF, DCF and HOF (on average 171 g/kg; dwb), whereas that of LOF was somewhat higher (196 g/kg; dwb). The apparently higher NSP content in LOF was due to a loss of DM, mainly NDO and other low-molecular-weight carbohydrates, when the beans were drained. Of the total NSP, approximately 26 % was soluble in the boiled-bean flours (ICF and DCF) *v.* about 38 % in the autoclaved flours (HOF and LOF). The main constituents in the soluble-NSP fraction were arabinose, uronic acids and some galactose. The insoluble NSP consisted mainly of glucose and arabinose, but considerable amounts of uronic acids, galactose and xylose were also detected. The overall composition of NSP was similar in the two boiled-bean flours (ICF and DCF). A significantly higher amount ($P = 0{\cdot}0001$) of soluble arabinans was, however found for DCF. With autoclaving there was a solubilization of all

NSP components, except for those containing uronic acids and arabinose.

The content of NDO was between 26 and 47 g/kg in the different flours. The main component in all flours was stachyose, comprising about 91 % total NDO. The LOF (the flour for which the beans had been drained following autoclaving) contained 56 % of the NDO originally present.

Distribution of indigestible carbohydrates in the test diets

High-level diets (120 g indigestible carbohydrates/kg). RS formed a higher percentage of the indigestible carbohydrates in the ICF diet (42) compared with LOF + raffinose diet (13; Table 1). This diet instead contained more NDO (32 % *v.* 13 %) and soluble NSP (20 % *v.* 11 %). Both diets had similar amounts of insoluble NSP (34 % indigestible carbohydrates).

Low-level diets (90 g indigestible carbohydrates/kg). A higher percentage of the indigestible carbohydrates was RS in the ICF diet (42 %) than in the other diets (16–17). DCF and HOF diets had a similar composition of indigestible carbohydrates, both with comparatively high percentages of NDO (18) and NSP (66). However, the amount of soluble NSP was higher in the HOF diet. Further, microstructure of the two diets differed, with HOF displaying an apparently intact cell structure (Fig. 1(c)) and DCF a disrupted cell structure (Fig. 1(b)). The LOF diet had the lowest amount of NDO, 10 % total indigestible carbohydrates compared with 13–18 % total indigestible carbohydrates in the other diets. The differences in the contents of RS and NDO in the four diets resulted in various contents of NSP. Thus, the percentage of NSP was lower in ICF (45) compared with the other test diets (66–73).

Fermentability in the rat hindgut

The fermentability of the indigestible carbohydrates in the beans was generally high, and averaged 80–87 % (Table 3). However, the different constituents were fermented to different extents.

High-level diets (120 g indigestible carbohydrate/kg). RS was fermented to a higher extent in the ICF diet than in the diet LOF + raffinose (only 3 % was excreted compared with 14 % in the diet LOF + raffinose). Of total NSP about 26 % was excreted, and arabinose-containing polysaccharides were fermented to the highest extent in both diets, only about 7 % being recovered in faeces. The other main components were less fermented and of the polymers containing xylose and uronic acids; 14–21 % of the ingested amounts were excreted in faeces, with no differences between diets. Interestingly, the excretion of NSP-glucose in faeces was significantly higher ($P = 0{\cdot}037$) with the ICF (61 %) than with the LOF + raffinose diet (46 %). No NDO was detected in the faeces, and these substances were therefore assumed to be completely fermented.

Low-level diets (90 g indigestible carbohydrates/kg). RS in the ICF diet was almost completely fermented, with only 1 % being recovered in faeces, which was significantly lower ($P = 0{\cdot}0004$) than that with the LOF diet, where 9 % was recovered. RS in the DCF diet was also almost completely fermented, and <3 % of the intake was excreted.

The fermentability of total NSP was similar to that with the high-level diets. Thus, approximately 25 % of the total NSP was excreted in all groups. Furthermore, arabinose-containing polysaccharides were fermented to a high extent in all diets (mean of 5 % was excreted), whereas xylose- and uronic acid-containing polysaccharides were somewhat less

Table 3. Faecal recovery of indigestible carbohydrates (% intake) in rats given test diets containing flours prepared by different procedures from red kidney bean (*Phaseolus vulgaris*)†

(Values are means with their standard errors for seven rats per diet)

	High-level diets (120 g/kg; dwb)				Low-level diets (90 g/kg; dwb)							
	ICF		LOF + raffinose		ICF		DCF		HOF		LOF	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
RS‡	3	1	14*	6	1^{a}	1	3^{a}	1	5^{ab}	1	9^{b}	2
NSP: Total	27	2	25	2	26^{a}	1	26^{a}	2	23^{a}	2	25^{a}	2
Rhamnose	32	2	32	2	35^{a}	3	26^{a}	2	26^{a}	1	23^{a}	1
Fucose	12	1	12	1	12^{a}	1	13^{a}	1	11^{a}	1	11^{a}	1
Arabinose	7	1	6	1	6^{a}	1	5^{a}	1	4^{a}	1	6^{a}	1
Xylose	18	3	14	2	18^{a}	1	13^{a}	2	13^{a}	2	15^{a}	2
Mannose	32	2	12***	1	31^{a}	2	14^{b}	1	9^{c}	1	10^{bc}	1
Galactose	15	1	23*	3	17^{a}	1	16^{a}	1	15^{a}	1	16^{a}	1
Glucose	61	6	46*	4	59^{ab}	3	63^{a}	4	48^{b}	4	50^{ab}	4
Uronic acids	19	2	21	3	17^{a}	2	17^{a}	2	19^{a}	2	23^{a}	3
NDO	−§		−§		−§		−§		−§		−§	
Total	13	1	15	2	13	1	17	2	16	1	20	2

ICF, intact-cell flour; LOF, low-oligosaccharide flour; DCF, damaged-cell flour; HOF, high-oligosaccharide flour; NDO, non-digestible oligosaccharides; RS, resistant starch.

^{a,b,c}Mean values in the same row with different superscript letters were significantly different ($P < 0{\cdot}05$).

Mean values were significantly different from those for diet ICF: * $P < 0{\cdot}05$, *** $P < 0{\cdot}001$.

† For details of diets and procedures, see Table 1 and p. 380.

‡ Faecal recovery of RS was calculated as RS in faeces:RS intake.

§ Not calculated as no NDO was detected in faeces.

fermented, and between 13 and 23 % of the ingested amounts were excreted. NSP-glucans were more resistant and 48–63 % were excreted. Less NSP-glucans appeared to be fermented with the ICF (41 %) and DCF (37 %) diets than with the HOF and LOF diets (52 and 50 % respectively). However, only the differences between the DCF and the HOF diets were significant ($P = 0{\cdot}049$).

Caecal pH

The pH in the caecum varied between 6·7 and 7·1 (mean 6·9), with no significant differences among test diets.

Distribution of short-chain fatty acids in rat hindgut

The total concentration and distribution of SCFA throughout the hindgut is shown in Table 4. The main SCFA formed were acetic, propionic and butyric acid, and 92 % of the SCFA could be attributed to these acids.

High-level diets (120 g indigestible carbohydrate/kg). The distribution between acetic, propionic and butyric acids in caecal contents was on average 71, 12 and 17 % respectively, with no significant difference between the two diets (Table 4). In the proximal colon the percentage of acetic acid was higher and those of propionic and butyric acids were lower (80, 8 and 12 % respectively).

In the distal colon, some differences between the diets were obtained. The ICF diet generated a significantly higher percentage of butyric acid (24) than the LOF + raffinose diet (12; $P = 0{\cdot}0005$).

Low-level diets (90 g indigestible carbohydrate/kg). In the caecum acetic, propionic and butyric acids were present in a molar ratio of 69:13:18, which was similar to those for the two high-level diets. The percentage of propionic acid was significantly ($P = 0{\cdot}017$) higher with the ICF diet (15) than with the LOF diet (11). No other differences between the diets were obtained.

In proximal colon acetic, propionic and butyric acids were present in a molar ratio of 77:10:13, with no significant differences among test diets.

The percentages of acetic, propionic and butyric acids in the distal colon were on average 76, 9 and 15 respectively.

Table 4. The total concentration of short-chain fatty acids (mmol/kg wet content) and the relative percentages of acetic, propionic and butyric acid in different parts of the hindgut of rats given test diets containing flours prepared by different procedures from red kidney bean (*Phaseolus vulgaris*)†

(Values are means with their standard errors for seven rats per diet)

	Acetic acid (%)		Propionic acid (%)		Butyric acid (%)		Total concentration (mmol/kg)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
High-level diets (120 g/kg; dwb)								
Caecum								
ICF	68	2	13	1	19	2	79	4
LOF+raffinose	74	2	12	3	14	3	85	8
Proximal colon								
ICF	78	3	8	2	14	1	65	6
LOF+raffinose	81	1	8	1	11	2	79	7
Distal colon								
ICF	66	2	10	1	24	2	74	8
LOF+raffinose	79***	2	9	2	12***	2	78	9
Low-level diets (90 g/kg; dwb)								
Caecum								
ICF	69[a]	2	15[a]	2	16[a]	2	73[a]	7
DCF	68[a]	3	13[ab]	1	19[a]	3	72[a]	6
HOF	70[a]	2	12[ab]	1	18[a]	2	81[a]	10
LOF	70[a]	3	11[b]	1	19[a]	3	87[a]	16
Proximal colon								
ICF	79[a]	3	10[a]	2	10[a]	1	56[a]	8
DCF	76[a]	2	10[a]	1	14[a]	2	63[a]	7
HOF	77[a]	1	10[a]	1	13[a]	1	53[a]	8
LOF	77[a]	2	9[a]	1	14[a]	3	64[a]	8
Distal colon								
ICF	73[a]	3	10[a]	2	17[a]	2	81[a]	7
DCF	74[a]	1	10[a]	1	16[a]	1	81[a]	8
HOF	77[a]	3	8[a]	2	15[a]	1	61[a]	7
LOF	79[a]	2	9[a]	2	12[a]	2	78[a]	6

ICF, intact-cell flour; LOF, low-oligosaccharide flour; DCF, damaged-cell flour; HOF, high-oligosaccharide flour.

[a,b]Mean values along the hindgut and within the same part of the hindgut with different superscript letters were significantly different ($P < 0{\cdot}05$).

Mean values along the hindgut and within the same part of the hindgut (i.e. caecum, proximal colon or distal colon) were significantly different from the corresponding values for diet ICF: *** $P < 0{\cdot}001$.

† For details of diets and procedures, see Table 1 and p. 380.

ICF yielded the highest percentage of butyric acid (17), but was not significantly different from the other low-level diet.

Concentration of short-chain fatty acids along the hindgut

The concentration of acetic acid was similar throughout the hindgut with most diets; the only exception was the HOF diet, for which the concentration was higher ($P = 0{\cdot}034$) in the caecum than in the proximal colon. The concentration of propionic acid tended to be lower in the colon than in the caecum. This gradient was significant in the proximal colon ($P < 0{\cdot}05$) for all diets, except for diets LOF + raffinose and ICF at the lower level. For the HOF diet the concentration of propionic acid was also significantly lower ($P = 0{\cdot}0004$) in the distal colon than in the caecum.

The concentration of butyric acid along the hindgut varied for different diets (Fig. 3). Thus, in the case of DCF and LOF + raffinose diet the concentration of butyric acid was similar along the hindgut, and with the HOF and LOF diet it decreased in the distal colon compared with the caecum ($P < 0{\cdot}05$). Interestingly, for the diets containing ICF, the butyric acid concentration was higher in the distal colon compared with the proximal colon ($P = 0{\cdot}009$ for ICF at the higher level and $P = 0{\cdot}047$ for ICF at the lower level). This increased concentration of butyric acid in the distal colon was found only for these two diets, both containing considerably more RS (Table 1) than the other diets.

Discussion

Evaluation of the bean flours

The limited availability of starch *in vitro* and the high content of RS in ICF were probably attributed to the physical encapsulation of starch granules within cell walls in the ICF. Physically-inaccessible starch entrapped within intact plant cells are defined as RS_1 by Englyst *et al.* (1992). As legumes are characterised by a relatively high amylose content (Hoover & Sosulski, 1991), and the fact that the beans in ICF were boiled using a small amount of water (beans–water, 1:3, w/v), some of the RS may be partially ungelatinised starch, i.e. RS_2 (Englyst *et al.* 1992). The starch in DCF, with its disrupted cells, was more easily degraded when exposed to the α-amylase, and this flour contained a lower content of RS.

The LOF and HOF consisted of beans that were autoclaved in excess of water (beans–water, 1:5, w/v). Treatments at high temperature and subsequent cooling have previously been shown to result in retrograded starch, mainly retrograded amylose (Björck *et al.* 1987). According to the scanning electron microscopy analyses, HOF and LOF contained a relatively high proportion of intact cells, but yet displayed a lower content of RS than ICF. This finding might be explained by an increased porosity of the cell walls as a result of autoclaving, thus facilitating amylase penetration.

The higher percentage of soluble NSP in the autoclaved flours (HOF and LOF; 38) compared with the boiled flours (ICF and DCF; 26) was probably the result of the more extensive processing conditions during autoclaving (higher temperature and pressure), leading to cleavage of glycosidic linkages and disassociation of polysaccharide chains (Svanberg *et al.* 1995, 1997).

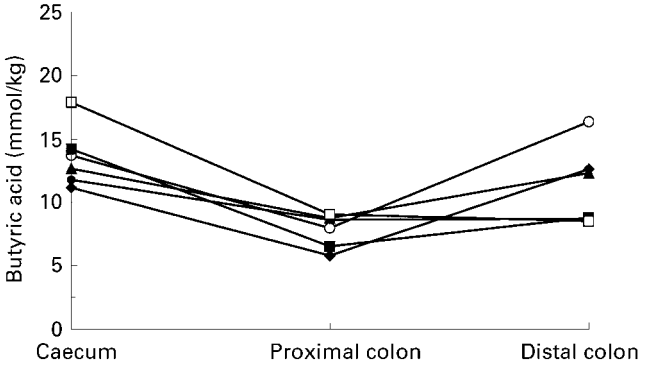

Fig. 3. Concentration of butyric acid (mmol/kg wet contents) along the hindgut of rats given test diets containing flours prepared by different procedures from red kidney bean (*Phaseolus vulgaris*) at either approximately 120 g/kg ((○), intact-cell flour; (●), low-oligosaccharide flour + raffinose), or approximately 90 g/kg ((◆), intact-cell flour; (▲), damaged-cell flour; (■), high-oligosaccharide flour; (□), low-oligosaccharide flour). For details of diets and procedures, see Table 1 and p. 380.

Fermentability in the rat hindgut

A higher fermentability of RS in the ICF diet (97) compared with the LOF + raffinose diet (86) was found in the high-level diets, and may be explained by the presence of different forms of RS in the two diets. The present finding suggests that the microflora digests RS_1 and RS_2 in ICF more easily than RS_3 in LOF. This conclusion is supported by the results with the low-level diets, which showed that RS in the ICF diet was almost quantitatively fermented (99 %). This explanation seems plausible from a physical point of view, since the RS_1 type of starch may contain an important fraction of enzyme-susceptible starch, once released from the botanical cell in the hindgut. In accordance with these findings, Schulz *et al.* (1993) found a higher fermentability of RS_2 (91 %) than RS_3 (64 %) in rats. However, in the present study the variation in fermentability of RS within a group was relatively high. Similar results have been obtained in rats given cooked haricot beans (*Phaseolus vulgaris*) by Key & Mathers (1993). A marked variation has also been reported in human subjects, indicating a high variability in the capacity of the individual colonic microflora to metabolise RS (Cummings *et al.* 1996).

NSP-glucans were fermented to a lower extent (37–54 %) than that reported for other heat-treated vegetables such as cooked carrots and microwaved green beans (approximately 70 %; Nyman *et al.* 1991; Svanberg *et al.* 1999). It has been suggested that RS exerts a sparing effect on NSP on fermentation in the colon, which means that bacteria prefer to ferment RS than to ferment NSP (Shetty & Kurpad, 1986; Phillips *et al.* 1995; Cummings *et al.* 1997). In the present study all diets contained RS, which may explain the comparatively low fermentability of the NSP-glucans.

The excretion of NSP-glucose in faeces was significantly higher ($P = 0{\cdot}037$) with the ICF diet (61 %) than with the

LOF + raffinose diet (46 %). An explanation could be that the beans in the diet LOF + raffinose had been autoclaved, which may have affected the NSP, making them more easily fermented (Björck *et al.* 1984; Nyman *et al.* 1991). The tendency to a lower faecal excretion of NSP-glucans with the HOF and LOF diets compared with the ICF and DCF diets may also be due to the fact that HOF and LOF were prepared by autoclaving the beans. The possibility that autoclaving increased the fermentability was further established by the finding that although DCF and HOF have a very similar composition with respect to indigestible carbohydrates, the HOF diet was fermented more due to the higher solubility of the NSP after autoclaving.

Distribution of short-chain fatty acids in rat hindgut

In the rat caecum, all diets yielded comparatively high percentages of butyric acid (approximately 18) compared with other substrates tested, such as pectin (7) and guar gum (11; Berggren *et al.* 1993). *In vitro* fermentation of red kidney beans has also been reported to yield high percentages of butyric acid (20–22; McBurney & Thompson, 1987, 1989). However, it must be also taken into account that available carbohydrates (primarily starch) and protein that would normally be digested in the upper intestinal tract were available for fermentation in these studies. Fermentation of soyabean fibre *in vitro* led to higher proportions of butyrate than other substrates such as oat fibre, sugarbeet fibre and pea fibre (Titgemeyer *et al.* 1991). Relatively high percentages of butyric acid in the caecum were also observed when legumes (e.g. haricot beans 18, peas 15 %) were fed to rats (Goodlad & Mathers, 1990; Key & Mathers, 1995). Beans contain a complex mixture of indigestible carbohydrates, and it has been suggested that a mixture of different indigestible carbohydrates favour a higher butyrate yield (Topping *et al.* 1985). Further, in the present study all diets contained NDO and RS which may favour the production of butyric acid in the rat caecum. Both soyabean oligosaccharides, such as raffinose, stachyose and verbascose (Kapadia *et al.* 1995), and RS (Phillips *et al.* 1995) have been shown to yield high faecal levels of butyrate on fermentation in human subjects.

In the distal colon the ICF diet generated a significantly higher percentage of butyric acid (24) than the LOF + raffinose diet (12; $P < 0{\cdot}001$). The ICF diet contained a considerably higher amount of RS (42 % total indigestible carbohydrates) compared with the LOF + raffinose diet (13 % total indigestible carbohydrates). Raffinose has also been shown to yield high amounts of butyrate in the caecum of rats (Berggren *et al.* 1993). Raffinose, however, is readily fermented (Livesey, 1992; Berggren *et al.* 1993; Ferguson & Jones, 2000), probably in the upper part of the hindgut, which might explain the low butyrate yield in the distal colon in the case of the LOF + raffinose diet.

Both diets (ICF at low- and high-levels) containing RS_1 and RS_2 promoted an increased butyric acid concentration in the distal colon compared with diets containing higher proportions of NDO and NSP. The increase in butyric acid concentration in the distal colon was more prominent with the diet having the highest amount of RS (i.e. ICF containing 120 g indigestible carbohydrates/kg). Interestingly, this diet also contained approximately the same levels of NSP and NDO as two of the diets with a low content of RS (DCF and HOF diets). The major difference between these diets was in fact the content of RS, suggesting that this form of starch contributes to the difference in butyric acid production in the distal colon. Thus, RS from the ICF seemed to be fermented mainly in the lower part of the colon. We found previously that the butyric acid concentration increased in the distal colon when a mixed diet containing high amounts of RS from potatoes and peas was fed to rats (AM Berggren, IME Björck and EMGL Nyman, unpublished results). This finding is interesting, as butyric acid has been suggested to be effective in the treatment of distal ulcerative colitis (Scheppach *et al.* 1992). Furthermore, the majority of colonic cancer tumours occur in the distal part of the colon in both human subjects (Bufill, 1990) and experimentally-induced rodent cancer models (Holt *et al.* 1996).

Further research is needed to investigate the production of butyric acid and other SCFA when various types of RS are present in combinations with other indigestible carbohydrates. In relation to colonic diseases it will be particularly interesting to study the possibility of increasing not only the butyric acid level, but also more specifically the distal yield, since diseases of the colonic epithelium most frequently occur at this site.

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