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Effects of dietary fibre-rich juice colloids from apple pomace extraction juices on intestinal fermentation products and microbiota in rats

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Effects of colloids isolated from apple pomace extraction juices (so-called B-juices) produced by enzymic liquefaction on food intake, body and faecal weights, short-chain fatty acid (SCFA) profile and selected intestinal microbiota were investigated in rats. Ten male Wistar rats per group were fed diets without any apple dietary fibre (DF) (control) or supplement with 5 % B-juice colloids or an alcohol-insoluble substance (AIS) from apples for 6 weeks. Rats fed with apple DF (5 % B-juice colloids or AIS) gained less weight than control rats ($P < 0{\cdot}05$). B-juice colloids did not affect food intake, whereas feeding AIS resulted in a 10 % higher food consumption than in control rats. Both juice colloids and AIS increased the weight of caecal contents in rats and lowered luminal pH values ($P < 0{\cdot}05$). In addition, SCFA concentrations and total yields were also raised ($P < 0{\cdot}05$) in caecum of these rats indicating good fermentability of apple substrates by gut microflora. Distinctly higher concentrations of acetate and propionate were found in intestinal contents of juice colloid-fed rats ($P < 0{\cdot}05$), whereas AIS also increased butyrate yield. Changes in microbiota due to apple DF in diets were restricted in the caecum to the *Eubacterium rectale* cluster (AIS; $P < 0{\cdot}05$) and in faeces to the *Bacteroidaceae* (juice colloids and AIS; $P < 0{\cdot}05$). The present study shows the physiological effects of apple DF isolated from pomace extraction juices produced by enzymic liquefaction on intestinal fermentation. Results may be helpful for the development of such innovative juice products that are rich in DF of fruit origin.

Apple-juice colloids: Pomace extraction: Short-chain fatty acids: Microbiota

Dietary fibre (DF) plays an important role as indigestible food components in human nutrition due to their beneficial effects for health. Mainly consisting of NSP, oligosaccharides and resistant starch, they have water-binding properties thus increasing volume and viscosity of intestinal contents. DF are responsible for faecal bulking, enhancing gut motility and lowering transit time. Being indigestible in the small intestine, they finally reach the colon, where they are utilized as fermentation substrates by the gut microflora. In the colon, a symbiosis through fermentation exists between the host and intestinal bacteria. Released as main microbial fermentation products, the short-chain fatty acids (SCFA) acetate, propionate and butyrate are rapidly absorbed by the colonic epithelium, which stimulates water and Na^+ absorption (Mortensen & Clausen, 1996; Velázquez *et al.* 1997). Furthermore, especially butyrate is a preferred substrate for the colonocyte. It serves as an energy source and is known to contribute a trophic effect on colonic mucosa (Roediger, 1980; Velázquez *et al.* 1996; Salminen *et al.* 1998). With the knowledge of these beneficial effects of DF, a great deal of effort has been made to positively modulate the composition of intestinal microflora and of SCFA via pre- and probiotics (Steer *et al.* 2000); for example, the bifidobacteria with inulin and oligofructose to maintain a normal and healthy gut microflora and also immune regulation in the colon (Gibson *et al.* 1995; Gibson & Roberfroid, 1995; Gibson, 1999; Kruse *et al.* 1999).

In apples, the main DF constituents are NSP such as cellulose, hemicelluloses and pectin, which have been shown to be good fermentation substrates for intestinal bacteria in previous studies (Vince *et al.* 1990; Tietgemeyer *et al.* 1991; Guillon *et al.* 1995; Casterline *et al.* 1997; Lebet *et al.* 1998; Van Laere *et al.* 2000). Such cell-wall components DF character are partially released into juice during apple-juice production. By using liquefying enzymes, for example, pectinases and cellulases, physiologically valuable apple juices can be produced in a two-step mode

Abbreviations: AIS, alcohol-insoluble substance; DF, dietary fibre; FISH, fluorescent *in situ* hybridization; SCFA, short-chain fatty acid.

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(Will *et al.* 2000). The first step consists of a common pectolytic mash treatment yielding the premium juice (A-juice) after separation. Subsequently, the remaining pomace is enzymically extracted a second time with cellulases and/or pectinases. Besides higher juice yields, this resulting pomace extraction juice (B-juice) contains higher amounts of polyphenols and up to ten times more DF compared with the corresponding A-juice (Bauckhage *et al.* 2000; Sembries *et al.* 2000; Will *et al.* 2000). The latter is equivalent to commercially available cloudy juices. DF-rich colloids isolated from B-juices have been characterized previously (Mehrländer *et al.* 2002).

Applying this process efficiently enhances the extraction of valuable fruit ingredients from apples (for example, DF and polyphenols) from which very high concentrations otherwise remain unused in pomace. B-juices high in DF and polyphenols are promising as new fruit-juice products; they can therefore be considered a natural alternative for functional drinks. No external addition of DF components should be necessary due to the presence of a sufficient amount of DF released from fruits during enzymic treatment. Concerning the daily recommended DF intake, B-juices from apple pomace seem to be more ‘healthy’ than clarified apple juices containing practically no DF at all. Another advantage of applying this two-step mode is the fact that the underlying engineering process necessary to obtain these valuable fruit-juice products merely requires applying some already existing techniques of common juice technology.

Here, the present study tested the potential health-promoting effects of isolated DF-rich B-juice colloids for their potential use as a food ingredient in B-juices.

To our knowledge there are no data available in the literature about the physiological effects of such apple juice colloids isolated from pomace extraction juices on intestinal fermentation. Therefore, the effects of corresponding B-juice colloids on fermentation products and degrading organisms were investigated. In the present study, DF-rich colloids from B-juices were isolated and examined in rats with regard to food intake, body and faecal weights, SCFA profile and some intestinal microbiota. Additionally, an alcohol-insoluble substance (AIS) was prepared from apples and tested. Having an almost intact cell-wall structure, the AIS served as a counterpart to soluble juice colloids released during the enzymic pomace treatment.

Materials and methods

Juice colloids and alcohol-insoluble substance

Juice colloids were isolated from apple pomace extraction juices 1B and 4B, which were produced by enzymic liquefaction (Will *et al.* 2000). The extraction juice 1B was produced solely by water extraction of pomace without any additional enzymes, whereas juice 4B was obtained after a pectolytic and cellulolytic pomace treatment. For isolation of juice colloids, both pomace extraction juices were concentrated using preparative ultrafiltration first (Bucher/Abcor, Niederweningen ZH, Switzerland; cut-off 18 000 Da). Subsequently, to one part of retentate five parts of 96 % (v/v) ethanol were added to precipitate colloids. Filtrated colloids were washed in ethanol and dried at 60°C. The total DF content of isolated juice colloids 1B was 56·9 % (54·3 % soluble, 2·6 % insoluble DF); DF content of juice colloids obtained from extraction juice 4B was 80·1 % (78·3 % soluble, 1·8 % insoluble DF) as determined by the Association of Analytical Chemists method (Prosky *et al.* 1988). ‘Soluble’ DF-rich colloids mainly consisted of oligo- and polymeric arabinans, rhamnogalacturonans and arabinogalactans (Mehrländer *et al.* 2002). 1B colloids had the following monosaccharide composition (mol %): rhamnose, 3; arabinose, 20; galactose, 8; glucose, 11; xylose, 1; galacturonic acid, 57. 4B colloids had the following monosaccharide composition (mol %): rhamnose, 5; arabinose, 23; galactose, 6; glucose, 7; xylose, 1; galacturonic acid, 57. AIS was prepared from freshly harvested apples (variety ‘Boskoop’; Werder Frucht, Glindow, Germany). In portions, 45 kg washed apples with skins and cores were crushed into small pieces in two parts of 96 % (v/v) ethanol using a blender and an Ultra-Turrax T25 (Jahnke & Kunkel, IKA Labortechnik, Staufen, Germany) and boiled under reflux for 15 min. The liquid phase was removed by suction; thereafter, the residue was washed with 65 % (v/v) ethanol and extracted again. Then, it was sequentially dehydrated in 65, 80 and 96 % (v/v) ethanol followed by acetone. The vacuum-dried AIS was milled to a particle size of $\leq$0·5 mm. It contained 96·2 % total DF (22·9 % soluble, 73·3 % insoluble DF). The ‘soluble’ DF parts of AIS consisted of 20·7 % total pectin (4·3 % water- and 6·5 % EDTA-soluble) with a degree of esterification of 83 %.

Animals and diets

The experimental protocol was performed in accordance with the guidelines of the ethics committee of the Ministry of Agriculture, Nutrition and Forestry (State Brandenburg, Germany, permission no. L8-3560-0/3). Forty male Wistar rats (Shoe-Wistar; Tierzucht Schönwalde, Schönwalde, Germany) weighing 177 (SD 4) g were randomly divided into four groups of ten animals each and were kept in a temperature-controlled environment (22 $\pm$ 2°C) with a 12 h light–dark cycle. After adapting to the semi synthetic control diet (Table 1) for a period of 1 week, the three test groups were fed a diet supplemented with either DF-rich colloids isolated from extraction juice 1B or 4B, or with AIS from apples (Table 1) for 6 weeks. The control group rats were maintained on the control diet. Rats had free access to water and to their respective diets. Food consumption and body weight were monitored weekly.

Sampling procedures

At the end of the adaptation period and on weeks 3 and 6 of the experimental period, fresh faecal samples were collected directly from the anus and were immediately processed for microbial counts. For analysis of SCFA, samples were frozen and stored at -20°C until preparation for GC analysis. Fresh sample aliquots were taken for the determination of pH values and dry weights. Microbial counts in faeces were done at the end of the adaptation period as well as at the end of feeding the respective diets on

Table 1. Composition (g/kg) and dietary fibre (DF) content (g/100 g dry weight) of diets

	Diet			
Ingredients	Control	Colloids 1B	Colloids 4B	AIS
Casein*	200	200	200	200
Wheat starch†	630	580	580	580
Sunflower-seed oil‡	50	50	50	50
Microcrystalline cellulose§	50	50	50	50
Mineral mixture‖	50	50	50	50
Vitamin mixture‖	20	20	20	20
Colloids 1B	–	50	–	–
Colloids 4B	–	–	50	–
AIS	–	–	–	50
DF content¶				
Total	6·2	9·8	8·4	12·7
Soluble	0·2	4·0	1·7	2·4
Insoluble	6·0	5·8	6·7	10·3

AIS, alcohol-insoluble substance.
* Hermann Kröner GmbH Co. KG, Ibbenbüren, Germany.
† Bayerische Milchindustrie eG, Landshut, Germany.
‡ Plus Vertriebs GmbH, Mühlheim/Ruhr, Germany.
§ J. Rettenmaier & Söhne GmbH & Co., Ellwangen-Holzmühle, Germany.
‖ Altromin GmbH, Lage, Germany. Mineral mixture contained (mg/kg): Ca, 146 070·34; P, 97 355·04; Mg, 8784·27; Na, 39 229·41; K, 116 496·45; S, 10 535·81; Cl, 63 510·38; Fe, 2931·22; Mn, 1733·86; Zn, 387·54; Cu, 85·21; I, 7·50; Mo, 3·31; F, 70·08; Se, 3·84; Co, 2·06; Al, 0·07. Vitamin mixture contained (mg/kg): vitamin A, 225; vitamin D_3, 0·625; vitamin E, 7500; vitamin K_3, 500; vitamin B_1, 1000; vitamin B_2, 1000; vitamin B_6, 750; vitamin B_{12}, 1500; nicotinic acid, 2500; pantothenate, 2500; folic acid, 500; biotin, 10; choline chloride, 50 000; *p*-aminobenzoic acid, 5000; inositol, 5000; vitamin C, 1000; methionine, 173 250.
¶ Determined by the Association of Analytical Chemists method.

week 6. After 6 weeks of diet, rats were killed and luminal contents from caecum and colon were removed and analysed for SCFA. Aliquots of the caecal samples were immediately processed for microbial counts. Wet and dry weights and pH values of intestinal contents were also determined.

Short-chain fatty acid analysis

For analysis of SCFA, faecal samples were homogenized in distilled water (1:5, w/v) and centrifuged at 20 000 ***g*** and 4°C for 5 min. Then, 50 µl internal standard (10 mm-i-butyric acid), 280 µl $HClO_4$ (0·36 M) and 270 µl NaOH (1 M) were added to 100 µl supernatant fraction. Samples were mixed, frozen in liquid N_2 and lyophilized. Lyophilizates were acidified with 200 µl formic acid (5 M) and sample volume was adjusted to 1 ml by adding 800 µl distilled water. Subsequently, mixed samples were transferred into vials and analysed by GC. SCFA were determined in two parallel preparations on an HP 5890 Series II Plus GC (Hewlett-Packard, Waldbronn, Germany) equipped with an HP Autosampler, HP GC Autosampler Controller and an HP Injector 7673. Separation of SCFA was done on an HP-free fatty acid phase capillary column (cross-linked free fatty acid phase; 30 m × 0·53 mm × 0·1 µm; split 1:1; flame-ionization detection; injection volume 1 µl). He gas was used as the carrier. The initial oven temperature of 85°C was kept constant for 0·5 min, increased to 135°C at a rate of 7°C/min and in 0·1 min to 160°C at a rate of 70°C/min, kept for 4 min at 160°C and decreased to the initial temperature of 85°C in 1 min at a rate of 70°C/min. The temperature of the injector was set at 200°C and that of the detector at 260°C. SCFA concentrations are expressed in units of µmol SCFA/g dry weight of intestinal content. Total mol yield in SCFA (µmol) was calculated by multiplying SCFA concentration (µmol/g dry weight) with total weight (g dry weight) of caecal content.

Microbial studies

Approximately 0·2 g fresh collected faeces was immediately placed into pre-weighed tubes and diluted with pre-reduced buffered peptone water. In duplicates, 0·05 ml of each dilution was plated on non-selective and selective media. Columbia blood agar (BioMérieux, Nurtingen, Germany), Endo agar (BioMérieux) and Rogosa agar (Fluka, Taufkirchen, Germany) were incubated aerobically at 37°C for the determination of total aerobes (for 48 h), coliformic bacteria (for 24 h) and aerobic lactobacilli (for 48 h), respectively. Numbers of total anaerobes, *Bacteroidaceae* and *Bifidobacterium* sp. were determined after a 48 h anaerobic incubation of Columbia blood agar, Columbia blood agar supplemented with neomycin (0·1 g/l; Fluka) and sodium deoxycholate (0·2 g/l; Fluka) and Haenel–Müller-Beuthow media (composition (g/l): peptone, 10; yeast extract, 7; NaCl, 3; Na_2HPO_4, 2; agar, 10; cystine, 0·5; cysteine, 0·5; NaN_3, 0·1; bromkresol green, 0·0125; neutral red, 0·00025; saline B, 2·5 ml). Saline B consisted of (g/l): $MgSO_4.7H_2O$, 40; $FeSO_4.7H_2O$, 2; NaCl, 2; $MnSO_4.2H_2O$, 2·35. The faecal microbial counts are expressed as $\log_{10}$ colony-forming units/g dry weight.

In addition, total bacteria and selected groups of plant-cell-wall-degrading organisms were counted in caecal samples by fluorescent *in situ* hybridization (FISH) using the following 16S rRNA targeted oligonucleotide probes (5′ labelled with the indocarbocyanine dye; Interactiva, Ulm, Germany): (1) an equimolar mixture of five bacteria-directed probes (Eub 338, Eub 785, Eub 927, Eub 1055, Eub 1088) (Giovannoni *et al.* 1988; Amann *et al.* 1990; Lee *et al.* 1993), referred to as Eub mix (Kleessen *et al.* 2001), to detect all bacteria; (2) Bac 303 to detect *Bacteroides* (Manz *et al.* 1996) and Erec 482 to detect the *Eubacterium rectale* cluster (Franks *et al.* 1998). Approximately 0·3 g fresh caecal content was suspended in 500 µl sterile PBS at pH 7·4 (130 mm-NaCl, 10 mm-Na_2HPO_4–NaH_2PO_4) and fixated for at least 4 h by adding three vol. of fresh paraformaldehyde (40 g/l)–PBS (pH 7·2) at 4°C according to Amann *et al.* (1990). Samples were washed in PBS once, re-suspended in a 1:1 mixture of PBS and 96 % (v/v) ice-cold ethanol and stored at −20°C until FISH analysis. FISH was done by a modified method of Schwiertz *et al.* (2000). Before hybridization fixed samples were briefly vortexed and immediately centrifuged at 9 ***g*** and 4°C for 3 min. Then, 10 µl of each supernatant fraction was applied to a single well on a 3-aminopropyltriethoxysilane- (Sigma, Taufkirchen, Germany) coated slide (Maddox & Jenkins, 1987). Air-dried samples were dehydrated in 60, 80 and 96 % (v/v) ethanol (3 min each), dried and treated with 10 µl lysozyme buffer (100 mm-tris(hydroxymethyl)-aminomethane–HCl (pH 8·0), 50 mm-EDTA, 1 mg lysozyme (130 000 U/mg; Boehringer, Mannheim, Germany)) on ice for 8 min to improve the permeability of cell envelopes. After washing slides in water and dehydration in the ethanol series as described earlier, hybridizations were performed for 16 h at 46°C (50°C) in humid chambers after addition of a mixture of 1 µl probe (50 pmol/µl) and 10 µl hybridization buffer (0·9 M-NaCl, 0·1 g SDS/l and 10 mM-tris(hydroxymethyl)-aminomethane–HCl, pH 7·4). Slides were washed in hybridization buffer for 20 min at 48°C (52°C), subsequently treated with SlowFade® Antifade Kit (Molecular Probes, Leiden, The Netherlands) and then examined using a Carl Zeiss Axioplan epifluorescence microscope (Carl Zeiss, Jena, Germany) equipped with a 100 × 1·30 Plan Neofluar Ph3 oil immersion objective, HBO 100 W/3 Hg lamp, the filter block 15 and a 12-bit cooled CCD camera (SensiCam® 370 KL; PCO Computer optics, Kehlheim, Germany). Images were taken and fluorescent cells were counted by KS300 software (Carl Zeiss). Microbiota in caecal contents are expressed as $\log_{10}$ total organisms and were calculated by multiplying caecal microbiota concentration ($\log_{10}$ organisms/g dry weight) with total weight (g dry weight) of caecal content.

Statistical analysis

Results are expressed as mean values and standard deviations. Before statistical analysis, microbial cell counts were transformed to $\log_{10}$ numbers in order to improve homogeneity of variance. Data concerning SCFA, faecal pH values, dry and wet weights of intestinal contents as well as microbial counts, respectively, were analysed by an unpaired Student's *t* test to determine significant differences between control and experimental groups. *P* values of $<0·05$ were considered significant.

Results

Food intake and body-weight gain

Throughout the entire experimental period, rats were in good health. No significant differences in food intake between the control group (22·3 (SD 1·6) g/d) and the rats fed the juice colloid diets (20·3 (SD 4·6) g/d for both colloids) were observed. Rats fed with the AIS diet consumed 24·0 (SD 2·8) g food/d. At the end of the experimental feeding period, the mean body weight of all rats had risen to 180 (SD 19) g. Weight gain in the control group was highest (207 (SD 31) g) and lowest in the rat group fed with juice colloids 4B (156 (SD 19) g; $P<0·05$). Supplementation of diets with juice colloids 1B or AIS led to a gain in body mass of 170 (SD 26) and 189 (SD 17) g within 6 weeks, respectively. The weight of the rats of the former diet group differed significantly from that of the control group ($P<0·05$).

Total intestinal contents and pH values

Intake of apple DF resulted in an increase in wet and dry weight of caecal contents ($P<0·05$; Table 2). These results were also detected for distal colon contents in rats supplemented with juice colloids 4B. However, no significant differences in weight were found for proximal colon contents (data not shown). Furthermore, DF from apples lowered luminal pH values in caecum and colon ($P<0·05$), which was most prominent if the AIS-containing diet was given.

Short-chain fatty acids

Consumption of apple DF positively affected the total yield in SCFA in intestinal segments examined as indicated by a rise in total SCFA concentration (Table 3). Especially in the caecum, the main site of bacterial fermentation in rats, significant differences were detectable between control rats and rats fed with either juice colloids or AIS ($P<0·05$). Due to rapid absorption of SCFA by the colonic epithelium, luminal SCFA concentrations continuously decreased throughout the gut passage (caecum to distal colon) and reached their minimum in faeces. Rats fed with apple DF excreted faeces containing higher total SCFA concentrations than control ($P<0·05$). In all rat groups, acetate was the dominant SCFA. Furthermore, its concentration was higher in caecal contents if apple DF

Table 2. Effect of colloids isolated from apple pomace extraction juices produced by enzymic liquefaction and of alcohol-insoluble substance (AIS) from apples on pH values, total wet and dry weight (g) of intestinal contents in rats†

(Mean values and standard deviations for ten rats per group)

	Control		Colloids 1B		Colloids 4B		AIS	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
pH value								
Caecum	7·2	0·1	6·9*	0·2	7·0*	0·2	6·6*	0·2
Colon‡	6·8	0·1	6·6*	0·1	6·6*	0·1	6·3*	0·1
Wet weight								
Caecum	2·81	0·40	3·76*	0·46	3·60*	0·51	6·51*	0·99
Colon‡	0·75	0·40	0·94	0·35	1·15*	0·26	0·81	0·44
Dry weight								
Caecum	0·72	0·11	0·91*	0·10	0·88*	0·14	1·40*	0·34
Colon‡	0·41	0·16	0·45	0·10	0·59*	0·12	0·35	0·17

* Mean value was significantly different from that for the control group ($P<0·05$).
† For details of diets and procedures, see Table 1 and p. 608.
‡ Distal colon.

Table 3. Effect of colloids isolated from apple pomace extraction juices produced by enzymic liquefaction and of alcohol-insoluble substance (AIS) from apples on caecal, colonic and faecal concentrations (μmol/g dry weight) of short-chain fatty acids (SCFA) in rats†

(Mean values and standard deviations for ten rats per group)

	Diets							
	Control		Colloids 1B		Colloids 4B		AIS	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total SCFA‡								
Caecum	196·3	34·8	325·9*	41·1	396·0*	95·4	265·0*	63·4
Proximal colon	74·0	24·4	127·4	53·1	157·1*	96·1	80·5	50·0
Distal colon	45·5	18·4	91·4*	33·0	59·2	17·1	34·4	9·6
Faeces	30·8	10·3	46·1*	15·3	49·9*	16·7	46·8*	14·5
Acetate								
Caecum	140·5	25·1	243·6*	21·4	291·4*	63·1	189·5*	43·4
Proximal colon	50·5	18·2	98·4	41·1	111·6*	71·6	58·9	39·5
Distal colon	31·9	11·4	64·7*	20·8	45·9*	11·3	24·0	6·3
Faeces	26·6	8·6	41·2	12·3	44·2	12·6	35·8	8·2
Propionate								
Caecum	39·0	6·0	67·6*	14·8	88·5*	28·2	47·1	11·8
Proximal colon	15·6	4·2	23·7	9·8	37·9*	20·5	14·0	6·2
Distal colon	7·6	3·8	19·6*	9·9	9·0	4·3	6·1	2·0
Faeces	2·2	1·0	2·9	1·9	3·8	3·0	6·0	2·4
Butyrate								
Caecum	16·8	3·7	14·7	4·9	16·1	4·1	28·4*	8·3
Proximal colon	7·9	2·0	5·3	2·2	7·6	3·9	7·6	4·3
Distal colon	6·0	3·1	7·1	2·4	4·3	1·6	4·3	1·4
Faeces	2·0	0·7	2·0	1·1	1·9	1·2	5·0	3·9

* Mean value was significantly different from that for the control group ($P<0{\cdot}05$).
† For details of diets and procedures, see Table 1 and p. 608.
‡ Total SCFA represents the sum of acetate, propionate and butyrate.

were present in the diet ($P<0{\cdot}05$). Even the molar proportion of acetate was increased in the caecum of juice colloid-fed rats (data not shown). The caecal fermentation of juice colloids by gut bacteria resulted in distinctly higher concentrations of propionate as compared with the control group ($P<0{\cdot}05$). In addition, when calculating total mol yields of SCFA (μmol) for caecal contents, even more than twice as many mol of acetate and propionate could be found in all apple DF-fed rats as compared with the control group ($P<0{\cdot}05$; Fig. 1). However, only rats fed with the AIS diet had raised caecal butyrate levels also, due to microbial breakdown of the almost intact apple cell-wall material ($P<0{\cdot}05$; Table 3 and Fig. 1).

Microbial studies

In caecal contents, counts of total and some plant-cell-wall-degrading bacteria were done by FISH. When feeding AIS numbers of total bacteria were higher as compared with control (Table 4). However, members of the *E. rectale* cluster ($P<0{\cdot}05$ for AIS diet) as well as of the genus *Bacteroides* were increased in numbers if DF from apples were fed with the diets. In faeces, no significant differences were found in microbial plate counts with the single exception of the numbers of *Bacteroidaceae*, which nearly increased by 1 $\log_{10}$ in rats fed with DF from apples (either juice colloids or AIS; $P<0{\cdot}05$). Faecal concentrations of total anaerobes showed a slight tendency to increase with apple DF whereas those of lactobacilli tended to decrease with colloids 4B or AIS. The slight increase in total aerobes and lactobacilli occurred with colloids 1B in the diet. However, bifidobacteria remained under the limit of detection in all investigated rat groups.

Discussion

A supplementation of diet with juice colloids from apple pomace extraction juices produced by enzymic liquefaction

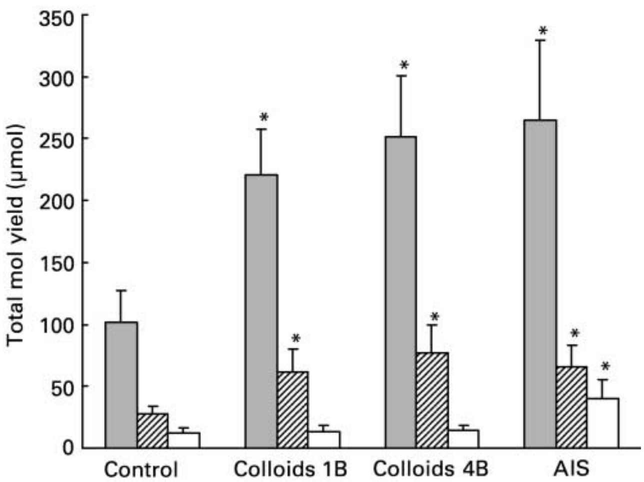

Fig. 1. Total mol yields of short-chain fatty acids (μmol) of caecal contents in rats fed control or 5 % apple dietary fibre diet (1B or 4B colloids from pomace extraction juices or alcohol-insoluble substance; AIS) for 6 weeks. Values are means for ten rats with standard deviations represented by vertical bars. *Mean values were significantly different from control ($P<0{\cdot}05$). (▒), Acetate; (▨), propionate; (□), butyrate. For details of diets and procedures, see Table 1 and p. 608.

Table 4. Effect of colloids isolated from apple pomace extraction juices produced by enzymic liquefaction and of alcohol-insoluble substance (AIS) from apples on microbiota in caecal contents (log_{10} total organisms) and on faecal microbiota concentrations (log_{10} colony-forming units/g dry weight) in rats†

(Mean values and standard deviations for five or six rats per group)

Diet...	Control		Colloids 1B		Colloids 4B		AIS	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Caecal microbiota (*n* 5)								
Total bacteria	10·94	0·25	10·88	0·26	10·84	0·16	11·21	0·23
Eubacterium rectale cluster	9·58	0·41	9·95	0·48	9·95	0·24	10·47*	0·59
Bacteroides	10·37	0·32	10·67	0·17	10·52	0·20	10·55	0·18
Faecal microbiota (*n* 6)								
Total aerobes	7·78	0·71	8·30	0·50	7·88	0·59	7·83	0·26
Total anaerobes	9·27	0·59	9·60	0·13	9·72	0·29	9·78	0·38
Coliforms	6·55	1·12	6·68	0·31	6·48	0·41	6·85	0·33
Bacteroidaceae	7·83	0·32	8·68*	0·53	8·65*	0·35	8·48*	0·46
Bifidobacteria	<3·30	–	<3·30	–	<3·30	–	<3·30	–
Lactobacilli	8·02	0·81	8·40	0·47	7·28	0·93	7·55	0·36

* Mean value was significantly different from that of control after 6 weeks of diet ($P<0·05$).
† For details of diets and procedures, see Table 1 and p. 608.

did not significantly affect food intake, but lowered the body-weight gain in rats. A weight gain-lowering effect by dietary soluble NSP has been previously reported by Seal & Mathers (2001) and Dongowski *et al.* (2002) although the food intake was unaffected by 5 or 10 % guar gum or sodium alginate and 6·5 % pectin, respectively. In contrast, feeding the AIS diet resulted in a 10 % greater food consumption than control for covering energy requirements. The AIS diet contained the highest DF content according to the Association of Analytical Chemists method, mainly consisting of insoluble fibres (Table 1). When comparing soluble fibre, for example, B-juice colloids, with insoluble fibre, for example, AIS, a lower intake and body-weight gain was observed in juice colloid-fed rats. This finding is consistent with Frias & Sgarbieri (1998), who fed guar gum or cellulose as soluble and insoluble fibre, respectively. Arjmandi *et al.* (1992) also reported that body-weight gains were significantly lower in pectin-fed rats than those fed cellulose.

DF derived from apples positively influenced the luminal concentrations of SCFA as demonstrated by their increased levels in intestinal contents. As a consequence, a decrease in pH values occurred. Similar results were reported by Berggren *et al.* (1993) for pectin. In the literature, a good fermentability is known for several DF sources from apples such as pectin (Englyst *et al.* 1987; Vince *et al.* 1990; Titgemeyer *et al.* 1991; Mortensen & Nordgaard-Andersen, 1993; Barry *et al.* 1995), cell-wall components (Stevens *et al.* 1988; Guillon *et al.* 1995; Casterline *et al.* 1997) and pomace (Lebet *et al.* 1998). With apple DF diets, total amounts of acetate and propionate were two times higher than in control, which indicates the microbial fermentation of pectin (Englyst *et al.* 1987; Titgemeyer *et al.* 1991; Barry *et al.* 1995; Casterline *et al.* 1997) and arabinogalactan (Englyst *et al.* 1987) (Fig. 1). Also in other *in vivo* studies acetate was observed as the dominant SCFA released by intestinal fermentation of pectin (Berggren *et al.* 1993; Dongowski *et al.* 2002). Microbial degradation of the pectin monosaccharide unit galacturonic acid is correlated with the production of acetate (Salvador *et al.* 1993). Bourquin *et al.* (1993) observed, in fermentation experiments with different cell-wall preparations from vegetables *in vitro*, that galacturonic acid was the most quickly fermented unit (up to 97 %) followed by arabinose (up to 96 %) and galactose (up to 90 %). From these results they concluded that the breakdown of cell-wall components by gut bacteria proceeds in a certain hierarchy depending on the fermentability of each monosaccharide. Similar results were reported by Lebet *et al.* (1998) for apple pomace, as well as by Guillon *et al.* (1995) and Englyst *et al.* (1987) for apple cell-wall material and pectin from apples, respectively. In their *in vitro* experiments, degradation of galacturonic acid and arabinose was enhanced in residues of DF components from apples examined, whereas xylose was purely degraded. Our *in vivo*-tested juice colloids mainly consisted of oligo- and polymeric arabinans, rhamnogalacturonans and arabinogalacatans (Mehrländer *et al.* 2002) containing the monomers galacturonic acid, arabinose and galactose, which would also explain their good fermentability by gut microbiota in rats.

During passage through the intestinal tract, SCFA were absorbed by colonic mucosa as indicated by decreasing luminal SCFA concentrations (Table 3). In contrast to juice colloids, AIS resulted in an increase in butyrate level in caecal contents ($P<0·05$), probably as a result of fermentation of a DF component only present in AIS. This component was obviously limited or only partially degradable because no further differences in butyrate concentrations were detected for the lower intestinal parts.

For a better comparison of the fermentation results the calculation of total mol yields is more favourable than that of concentrations. Therefore, the total mol yields of SCFA were calculated for the whole caecal contents, the caecum being the main location of fermentation activity. In rats fed with juice colloids, no differences in total mol yields in butyrate of caecal contents were found (Fig. 1). However, the AIS diet resulted in a four-fold rise in total mol of caecal butyrate as compared with control ($P<0·05$). An increase in luminal butyrate levels is a prominent goal in nutritional colon cancer prevention (Jacobasch *et al.* 1999; Jacobasch & Dongowski, 2000) due to its important role in homeostasis of colon epithelium (Roediger, 1980; Velázquez *et al.* 1996, 1997; Singh *et al.* 1997). Especially dietary fructo-oligosaccharides, oligofructose as well as resistant starch are known butyrogenic substrates for the intestinal microflora (Campbell *et al.* 1997; Schwiertz *et al.* 2002). Together with increasing butyrate in the caecum of AIS-fed rats the total numbers of the *E. rectale* cluster also increased by 1 $\log_{10}$ unit. In rats fed juice-colloid diets, only a two-fold rise in this cluster was observed. Despite inducing higher intestinal butyrate levels resistant starch had no effect on members of the *E. rectale* cluster *in vivo* as reported by Schwiertz *et al.* (2002). With its almost intact cell-wall structure, AIS mainly consisted of insoluble DF components. Besides cell-wall polysaccharides such as xyloglucans, arabinans, arabinogalactans and rhamnogalacturonans AIS also contains cellulose in contrast to juice colloids. In primary cell walls, cellulose is partially fermentable by intestinal bacteria (Gray *et al.* 1993), if pectin is previously completely degraded (Guillon *et al.* 1995). The rise in the *E. rectale* cluster (cluster XIV according to Collins *et al.* 1994) and total mol of caecal butyrate could be explained by the partial fermentation of cellulose present in AIS. Some species of the *E. rectale* group are known cellulose-degrading organisms releasing acetate; for example, *E. cellulosolvens*, *Clostridium lentocellum*, and *C. celerescens* (Hippe *et al.* 1992). The latter organism also produces butyrate as a fermentation endproduct. Furthermore, there are some further butyrogenic species in this cluster that generate butyrate from acetate via the butyryl-coenzyme A–acetyl coenzyme A-transferase pathway (Barcenilla *et al.* 2000). Although the genus *Bacteroides* provides some members of cellulose degraders (Hill, 1995), no remarkable increase in cell numbers was detected for *Bacteroides* in caecal contents. However, it is not exactly known which of these species in the *E. rectale* cluster really belongs to the normal gut microflora in rats.

In faeces samples, bacterial cell numbers were determined by classic plate count procedures. When feeding either colloids from pomace extraction juices or AIS, members of the *Bacteroidaceae* were present in almost 1 $\log_{10}$ higher counts than in faeces of control rats. Besides being cellulose degraders, they belong to the arabinogalactan- and pectin-degrading organisms (Hill, 1995; Dongowski *et al.* 2000; Van Laere *et al.* 2000).

Another known arabinogalactan- and arabinan-degrading genus is bifidobacteria (Hill, 1995; Van Laere *et al.* 2000). Bifidobacteria have also been reported to be selectively growth stimulated by pectic oligosaccharides *in vitro* (Olano-Martin *et al.* 2002). In contrast to human intestinal microflora, bifidobacteria are only present in the large bowel of rats in low counts. In our experiments this genus remained under the detection limit in faeces and was therefore not determined in caecal contents of our Wistar strain. Noack *et al.* (1998) reported similar results for a 10 % dietary pectin supplementation, whereas feeding 10 % guar gum clearly stimulated bifidobacteria growth in rats. The present study provides data on the effects of juice colloids isolated from apple pomace extraction juices produced by enzymic liquefaction on intestinal fermentation products and microbiota in rats. Having the advantage of animal studies that allow access to intestinal samples of all gut segments, a clear rise in intestinal SCFA yield due to microbial fermentation of apple DF was found. Furthermore, results indicate a slight tendency to modulate gut microbiota by extraction juice colloids. However, our findings should also be verified by a study using human subjects in the future. In addition, plant-cell-wall-degrading members of the *E. rectale* cluster need to be identified in rat gut and their possible health-promoting properties examined.

From our point of view, juice colloids from pomace extraction juices tested *in vivo* are useful DF components from apples and may help to diminish the lack of daily recommended DF intake of at least 30 g. Furthermore, pomace extraction juices providing such DF of fruit basis are a promising healthy and natural alternative to functional drinks.

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