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The composition of dietary fibre-rich extrudates from oat affects bile acid binding and fermentation in vitro

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Abstract

A series of extrudates was prepared from oat meal, oat bran and Novelose 330®, differing in concentrations of individual dietary fibre (DF) components, such as β-glucan and resistant starch, as well as total DF. After simulated digestion, the digested DF-rich oat-based extrudates were used to evaluate their physiological effects in vitro. A strong interaction occurred between the digested extrudates and bile acids (BA). The binding of BA increased with increasing proportions of oat bran, total DF, insoluble DF and β-glucan in the extrudates. Dihydroxy-BA was more strongly bound to the extrudates than trihydroxy-BA. Interactions at pH 5.0 were greater than at pH 6.5. During fermentation of digested extrudates with human faecal samples, concentrations of short-chain fatty acid (SCFA) formed and the molar proportion of butyrate increased continuously. Higher SCFA concentrations were found when extrudates contained more oat bran, soluble and insoluble DF and β-glucan. Extrudates, on the basis of oat, have several beneficial nutritional and protective effects in vitro. Therefore, physiological effects occurring in the small and large intestine are also related to the DF composition of the cereal products.

Keywords: Oat extrudate; Dietary fibre; β-Glucan; Resistant starch; Bile acid binding; Fermentation

1. Introduction

Cereal products, particularly from whole grains, are the most important source of dietary fibre (DF) in the western diet. A high intake of DF, which is an essential component in nutrition, is positively related to several physiological and metabolic effects. However, the daily intake of DF is below the recommended levels in most industrial countries (Spiller, 2001).

Oat (*Avena sativa* L.) products are well accepted in human nutrition. Oat is an excellent source of different DF types, such as mixed-linked $(1\rightarrow3),(1\rightarrow4)$-β-D-glucan (β-glucan), arabinoxylans and cellulose, and it contains relatively high levels of protein, lipids (unsaturated fatty acids), vitamins, antioxidants, phenolic compounds and minerals (Emmons & Peterson, 1999; Hampshire, 1998; Panfili, Fratianni, & Irano, 2003; Peterson, 2001; Zadernowski, Nowak-Polakowska, & Rashed, 1999). In German oat cultivars, average values of 4.7% β-glucan were found, whereas, in oat bran, the β-glucan content can be increased to more than 8% (Ganssmann, 1998).

Extrusion cooking is widely used in the food industry for the production of breakfast cereals and snacks (Kokini, Ho, & Karwe, 1992; Østergård, Björk, & Vainionpää, 1989). It may result in changes of starch components, in solubility of DF or in functional properties of the cereal products (Ralet, Thibault, & Della Valle, 1990; Vasanthan, Gaosong, Yeung, & Li, 2002). It is possible to generate resistant starch (RS) by extrusion of starch-rich materials (Unlu & Faller, 1998) such as pea meal (Berghofer & Horn, 1994) or wholegrain meal from barley (Huth, Dongowski, Gebhardt, & Flamme, 2000). RS is defined as the starch or starch degradation products, which are not absorbed in the

Abbreviations: AIS, alcohol-insoluble substance; BA, bile acid; DF, dietary fibre; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; RS, resistant starch; SCFA, short-chain fatty acid.

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small intestine of healthy individuals, and is classified as a DF (American Association of Cereal Chemists, 2001). RS is a major substrate for bacterial fermentation and a good source for butyrate production in the large intestine (Asp, van Amelsvoort, & Hautvast, 1996; Brouns, Kettlitz, & Arrigoni, 2002).

The consumption of β-glucan- and RS-rich diets results in several beneficial physiological effects. Thus, β-glucan lowers the postprandial blood glucose and insulin responses in normal individuals (Braaten et al., 1991). Serum cholesterol and lipoprotein concentrations can be reduced in humans and animals by β-glucans from oat and barley or by cereal products (Braaten et al., 1994; Brown, Rosner, Willett, & Sacks, 1999; Delaney et al., 2003b; Kahlon, 2003; Kalra & Jood, 2001). A reason for the cholesterol-lowering effects of β-glucan-rich diets may be the greater excretion of bile acids (BA) (Zhang et al., 1992). Furthermore, β-glucans are fermented by the intestinal microflora, resulting in the formation of short-chain fatty acids (SCFA), which are protective to the colon mucosa (Daniel, Wisker, Rave, & Feldheim, 1997; McBurney, 1991).

Food processing can influence the digestibility of starch and metabolic effects of cereal products (Bornet, 1993). There is scant information on the influence of the composition of extruded products on their physiological effects. Chang, Martinez-Flores, Martinez-Bustos, and Sgarbieri (2002) investigated the hypocholesterolemic properties of extrudates prepared from mixtures of cassava starch and Novelose or oat fibre in hamsters. However, proportions of the components in the extrudates were not given. In in vitro experiments, physiological effects of food or food products may be simulated for subsequent animal and human studies.

The objective of this study was to evaluate physiological effects of DF-rich oat-based extrudates of the whole-grain type in vitro. The extrudates tested differed in their contents of individual DF types such as β-glucan and RS, as well as of total DF. Our hypothesis was that the composition of the oat-based extrudates is important for direct or indirect physiological effects in the intestinal tract. Therefore, effects of the oat-based extrudates on the interactions with BA, occurring in vivo in the small intestine, and on their fermentation with formation of SCFA, occurring in the large intestine, were investigated in model systems.

2. Materials and methods

2.1. Cereal products

Oat meal (oat groats) was prepared from commercial oat kernels by milling (61.4% starch; 11.6% crude protein; 6.2% total fat; 2.6% soluble DF; 8.4% insoluble DF; 3.5% β-glucan). Oat bran was obtained from Peter Kölln Köllnflockenwerke (Elmshorn, Germany). The bran preparation used contained 8.9% β-glucan, 0.5% soluble and 17.8% insoluble DF but no RS. Novelose 330® is a commercial RS product from National Starch and Chemical (Hamburg, Germany). It contained 45.5% RS type III (non-granular, retrograded starch).

2.2. Preparation and composition of extrudates

A series of extrudates was prepared from oat meal, oat bran and/or Novelose 330 in a single screw laboratory extruder DN 20 from Brabender (Duisburg, Germany) (Table 1). The following conditions were used during extrusion: moisture content 25%, mass temperature 130 °C, screw speed 200 rpm and die width 4 mm (circular in shape).

2.3. Analytical methods

RS was measured by a modified Englyst-method (Englyst, Klingman, & Cummings, 1992). First, the digestible starch was hydrolysed by incubation with pancreatin (Merck, Darmstadt, Germany) and amyloglucosidase (Sigma, St. Louis, MO, USA) in acetate buffer (pH 5.2) for 2 h at 37 °C, simulating starch hydrolysis in the small intestine. After addition of the fourfold amount of 96% EtOH and centrifugation (10 min at 4 °C and 2800*g*), the hydrolysed starch products

Table 1
Composition of original and digested extrudates (in %)

Extrudate	Composition of extrudates			Original extrudates				Digested extrudates				
	Oat meal	Oat bran	Novelose 330	β-Glucan	Resistant starch	Soluble DF	Insoluble DF	Yield	β-Glucan	Resistant starch	β-Glucan[a]	Resistant starch[a]
A	100	0	0	4.29	0.43	1.54	6.89	52.00	5.53	0.20	2.88	0.10
B	80	0	20	4.55	8.30	1.63	11.58	62.30	4.69	11.29	2.92	7.03
C	62.6	17.4	20	5.36	9.01	3.03	13.21	67.80	7.83	10.91	5.30	7.40
D	40	40	20	5.67	9.02	4.01	15.29	69.80	6.08	11.46	4.24	8.00
E	0	80	20	7.09	8.69	2.12	18.72	82.50	7.25	8.32	5.98	6.86

DF, dietary fibre.
[a] Related to original extrudates. Values are means ($n = 3$–6).

were extracted twice (with 80% EtOH). The freeze-dried RS-containing residue was dissolved in 1 M NaOH. The diluted solution was hydrolysed with amyloglucosidase at pH 4.6, and the released glucose was determined enzymatically using the hexokinase and glucose-6-phosphate dehydrogenase kit (Boehringer, Mannheim, Germany).

After its extraction with 1 M NaOH, the total starch content was analysed enzymatically using amyloglucosidase and the Boehringer glucose kit.

For determination of β-glucan, sample material was suspended in phosphate buffer (pH 6.5) and mixed for 5 min at 100 °C. The diluted suspension was hydrolysed with lichenase (Megazyme International, Bray, Ireland) for 60 min at 45 °C (McCleary & Mugford, 1997). After dilution and centrifugation (10 min at 1000*g*), a part of the supernatant was incubated with β-glucosidase (Megazyme) in acetate buffer at pH 4.5 and 40 °C for 15 min. The glucose released was determined with the hexokinase/glucose-6-phosphate dehydrogenase kit from Boehringer.

Total, insoluble and soluble DF were analysed by the enzymatic-gravimetric AOAC method (Prosky et al., 1988).

Water binding was determined using the capillary suction method, in the variant of Heinevetter and Kroll (1982) at 20 °C for 15 min. The water uptake of the samples was expressed as g H_2O/g substance.

2.4. In vitro digestion of the extrudates

Before in vitro experiments, extrudates were digested enzymatically to remove digestible starch, simulating physiological conditions by a treatment with a mixture of pancreatin (Merck) and amyloglucosidase (Sigma), at pH 5.2 and 37 °C for 2 h. Then, a fourfold amount of 96% EtOH was added and the mixture was centrifuged (10 min at 4 °C and 2800*g*). The residue was extracted twice (with 80% EtOH), heated under reflux in 96% EtOH for 15 min (enzyme inactivation), centrifuged and then dried.

2.5. Binding of bile acids by digested extrudates

The binding experiments were performed using the conjugated BA glycocholic acid (GCA) and glycochenodeoxycholic acid (GCDCA) as sodium salts from Sigma.

Digested extrudates (100 mg) were suspended in 10 ml of Sörensen buffer (pH 5.0 or 6.5) containing 0.5 mM BA and treated for 2 h at 37 °C under shaking. After centrifugation (10 min at 4 °C and 3000*g*), 0.5 ml of the supernatant (containing the unbound BA) was purified by solid-phase extraction on Bakerbond spe C_{18}-columns in the BAKER spe-12G system (J.T. Baker, Gross Gerau, Germany).

The BA were estimated on a non-polar stationary phase (Nucleosil 100 Å ; C_{18}; 5 μm; 250 × 4.6 mm) at 40 °C by HPLC (Gynkotek, Germering, Germany), after pre-column derivatization with 4-bromomethyl-7-methoxycoumarin, using 18-Crown-6 as a catalyst (Wang, Stacey, & Earl, 1990) and fluorescence detection (excitation λ 320 nm; emission λ 385 nm). Linear gradients, consisting of acetonitrile (30–100%), methanol (40–0%) and water (30–0%) were applied (Dongowski, Huth, & Gebhardt, 2003).

2.6. Fermentation and determination of SCFA

For simulating fermentation in the large intestine, 15 mg of the digested extrudates were incubated anaerobically (under N_2) with 1 ml of a suspension from fresh human faeces (20 g/60 ml, 0.1 M phosphate buffer; pH 6.5) for up to 8 or 24 h at 37 °C with constant shaking. At different times, samples were taken, under sterile conditions, and analysed for their contents of SCFA. Faecal materials were used from three healthy persons receiving no antibiotics or laxatives for 6 months before the experiment. The samples were immediately frozen at −80 °C.

For estimation of SCFA using gas-chromatography (Brighenti, 1997), 350 mg of the thawed samples were mixed with 1500 μl of water and were homogenized. After centrifugation (5 min at 4 °C and 15,000*g*), 50 μl of *iso*-butyrate (internal standard), 280 μl of 0.36 M perchloric acid solution and 270 μl of 1 M sodium hydroxide solution were added to 100 μl of the supernatant. The mixture was freeze-dried. The dried samples were homogenized in a mixture of 200 μl of 5 M formic acid and 800 μl of water. After centrifugation, one microlitre of the supernatant was assayed on a HP–FFAP capillary column (30 m × 0.53 mm; 1 μm), using a temperature programme. The chromatography system (Hewlett–Packard, Waldbronn, Germany) consisted of a HP gas-chromatograph 5890 Series II, HP 7673 GC/SFC Injector, HP GC Auto Sampler Controller, Detector FID and Software – HP Chemstation. Helium was used as the carrier. All samples were analysed in duplicate and quantified by external and internal calibration with standards (SCFA standard mix from Sigma –Aldrich, Deisenhofen, Germany).

Because small SCFA amounts are present in faeces samples, all determined SCFA values were corrected by the 0 h values. SCFA concentrations were calculated on the basis of 15 mg fermented substrate (e.g. digested extrudate) and on the basis of one gramme original extrudate.

2.7. Statistical analysis

Results are expressed as mean values ± SD. The statistical significance was determined using one-way

analysis of variance (ANOVA). $P < 0.05$ was taken to indicate a statistically significant difference.

3. Results

3.1. Characterization of the extrudates

Concentrations of β-glucan, RS, soluble and insoluble DF in the extrudates are shown in Table 1. In extrudate A, only a small amount of RS was found. The addition of 20% Novelose gave an increase in RS up to approximately 9%. This concentration is related to the estimated RS content in the used Novelose preparation of 45%. Extruded oat meal contained approximately 1.5% soluble and 7% insoluble DF. An addition of Novelose and/or oat bran gave higher concentrations of the insoluble DF fraction. Further, the contents of β-glucan increased from extrudate A to E. In contrast to extrusion of barley meal under optimised conditions (Huth et al., 2000), RS was practically not generated during extrusion of oat meal. It was assumed that the relatively high fat content of oat was the main reason for this effect.

In vitro digestion of the extrudates gave losses in mass between 17.5% and 48.0% (especially by removal of digestible starch). Therefore, concentrations of β-glucan and RS were higher in digested extrudates. However, if these values were related to original extrudates, up to 20% of RS and β-glucan were lost during digestion and preparation of the digested extrudates (Table 1).

3.2. Water binding

Water binding of extrudates and digested extrudates was measured using the Baumann method. Water uptake by the samples was finished within 3 min. This is in agreement with findings of Robertson et al. (2000). Oat meal-rich extrudates A and B had water uptakes of approximately 4.6 g H_2O/g (Fig. 1). Extrudates containing higher proportions of oat bran had a lower water uptake.

Water binding was lower in digested extrudates (2.2–2.7 g H_2O/g). This effect points to the distinct participation of the starch component in the water uptake of the original extrudates. If the water binding of the digested extrudates was related to original extrudates (using the yield of digested extrudates; see Table 1), the lowest water absorption was calculated for sample A (1.38 ± 0.18 g H_2O/g) and the highest for the sample E (2.16 ± 0.09 g H_2O/g). Besides the digestible part of starch, especially the complex cell wall seems to be involved in the water binding. Under the conditions used, polysaccharides, such as β-glucans or arabinoxylans, are only to a small degree soluble.

3.3. Binding of bile acids

Two of the three major BA present in human bile were used for the binding studies. GCA has hydroxyl groups at C-atoms 3, 7 and 12 of the steroid nucleus, whereas, GCDCA has hydroxyl groups at C-atoms 3 and 7. The carboxyl groups of the BA are amidated with the amino acid glycine. The experiments were done at

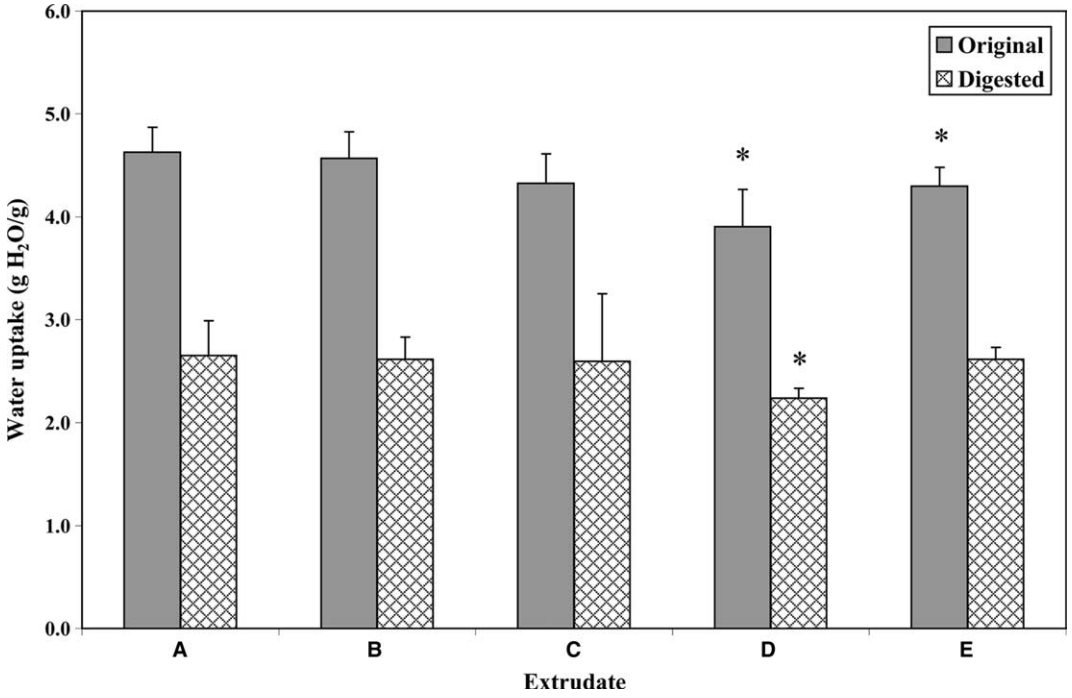

Fig. 1. Water uptake of original and digested extrudates at 20 °C. Values are means ± SD ($n = 6$); *$P < 0.05$ (compared to extrudate A).

pH values of 5.0 and 6.5, which are typical of the conditions in the upper and lower part of the small intestine, respectively.

Under the conditions used, a strong interaction was found between the digested extrudates and the BA. The concentrations of BA in the supernatants were significantly lower after incubation and centrifugation. The proportion of bound BA is given in Table 2. In the presence of all digested extrudates, the dihydroxy-BA was more strongly bound than the trihydroxy-BA. These interactions were greater at pH 5.0 than at pH 6.5.

The effect of DF composition of the extrudates on the binding of BA can be shown if the measured degrees of interactions are calculated on the basis of the original extrudates (Fig. 2). Under the conditions used, more than 40% of the BA interacted with the extrudates. This proportion was greater with a higher proportion of the RS preparation and, especially, with a higher proportion of oat bran in the original extrudates. Extrudate E, consisting of 80% oat bran, was most effective in BA binding. Interactions with BA were significantly greater if extrudates B–E were used rather than extrudate A ($P < 0.001$). In each case, interactions were more strong at pH 5.0 than at pH 6.5 ($P < 0.001$) and stronger with GCDCA than with GCA ($P \leqslant 0.02$). The order of intensity of interactions with all original extrudates was:

GCDCA at pH 5.0 > GCA at pH 5.0 > GCDCA at pH 6.5 > GCA at pH 6.5.

3.4. Fermentation of the extrudates and formation of SCFA

In the fermentation experiments, digested extrudates and selected 'standard' substances (e.g Novelose 330, glucose) were used as substrates. Acetate, propionate and butyrate are typical metabolites of carbohydrate (DF) fermentation, whereas both valerates are end products of protein fermentation. Besides a high total

Table 2
Interactions between glycoconjugated bile acids and digested oat-based extrudates

Bile acid	pH	Binding of bile acids (%) to digested extrudates				
		A	B	C	D	E
GCA	5.0	82.6 ± 1.25	83.2 ± 1.31	81.7 ± 0.35	85.5 ± 1.16*	90.3 ± 0.67*
GCA	6.5	75.2 ± 2.26	73.4 ± 1.17	74.6 ± 0.36	78.4 ± 0.52*	82.7 ± 1.15*
GCDCA	5.0	92.0 ± 0.47	90.1 ± 1.09*	86.9 ± 0.46*	89.9 ± 1.07*	92.8 ± 1.16
GCDCA	6.5	82.1 ± 0.91	77.7 ± 1.74*	77.7 ± 0.31*	84.1 ± 0.82*	87.7 ± 0.86*

GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid. Values are means ± SD ($n = 6$).
* $P \leqslant 0.005$ (compared to extrudate A).

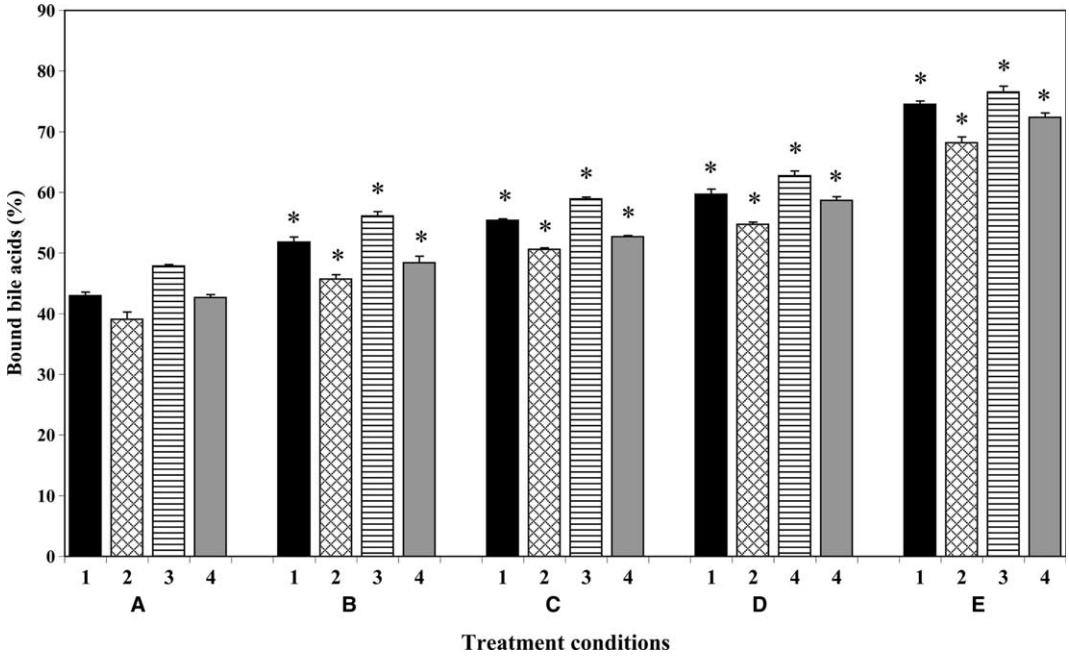

Fig. 2. Influence of pH value on the interactions between glycocholic acid (GCA) or glycochenodeoxycholic acid (GCDCA) and the digested extrudates A–E. Values are related to original extrudates. (1 = GCA at pH 5.0; 2 = GCA at pH 6.5; 3 = GCDCA at pH 5.0; 4 = GCDCA at pH 6.5). Values are means ± SD ($n = 6$); *$P < 0.001$ (compared to extrudate A).

amount of SCFA, high concentrations of butyrate are physiologically important.

During fermentation, concentrations of SCFA formed increased continuously up to at least 6 h. Later, substrate shortage may result in a diminished SCFA production. Table 3 shows these effects, related to 15 mg of selected digested extrudates. In this form of presentation, digested extrudate A seems to be the best substrate for SCFA production. Additionally, the molar proportions between the SCFA types differ in the course of fermentation. Fig. 3 shows this effect during fermentation of the digested extrudate C with the faecal sample from subject 2. Whereas, the proportion of acetate decreased, the proportion of propionate did not change. After 6 and 8 h of fermentation, the proportion of butyrate was significantly increased ($P < 0.05$).

In the following, concentrations of SCFA were calculated on the basis of original extrudates (see Table 1).

Table 3
Formation of SCFA during fermentation of selected digested extrudates with the faecal sample from subject 1

Extrudate	Time (h)	Acetate	Propionate	Butyrate	*iso*-Valerate	*n*-Valerate	Sum
		(μmol SCFA/15 mg digested substrate)					
A	1	11.8 ± 1.87	1.08 ± 0.13	4.13 ± 0.66	0.18 ± 0.06	0.31 ± 0.03	17.5 ± 2.69
	2	18.2 ± 2.40*	2.19 ± 0.21*	6.94 ± 0.73*	0.31 ± 0.03*	0.61 ± 0.06*	28.2 ± 3.42*
	4	22.2 ± 2.23*	3.07 ± 0.23*	8.93 ± 0.67*	0.44 ± 0.06*	0.91 ± 0.06*	35.5 ± 3.18*
	6	27.5 ± 0.94*	4.21 ± 0.15*	10.8 ± 1.06*	0.56 ± 0.09*	0.82 ± 0.64	43.9 ± 2.49*
	8	23.3 ± 1.59*	4.29 ± 0.18*	11.1 ± 0.68*	0.63 ± 0.02*	1.32 ± 0.03*	40.6 ± 2.34*
D	1	7.70 ± 0.52	0.70 ± 0.11	3.11 ± 0.17	0.09 ± 0.03	0.20 ± 0.02	11.8 ± 0.81
	2	10.8 ± 0.70*	1.18 ± 0.10*	4.58 ± 0.16*	0.15 ± 0.02*	0.33 ± 0.03*	17.0 ± 0.93*
	4	15.1 ± 1.41*	1.85 ± 0.14*	7.29 ± 0.59*	0.25 ± 0.03*	0.55 ± 0.05*	25.1 ± 2.11*
	6	16.1 ± 1.90*	2.11 ± 0.28*	8.19 ± 0.93*	0.29 ± 0.04*	0.65 ± 0.09*	27.3 ± 3.20*
	8	21.7 ± 0.54*	2.83 ± 0.09*	10.8 ± 0.40*	0.34 ± 0.02*	0.83 ± 0.03*	36.5 ± 1.02*
E	1	9.86 ± 0.49	0.69 ± 0.05	3.92 ± 0.18	0.11 ± 0.01	0.20 ± 0.02	14.8 ± 0.47
	2	14.7 ± 2.77*	1.42 ± 0.26*	6.53 ± 0.87*	0.19 ± 0.04*	0.42 ± 0.08*	23.3 ± 4.00*
	4	16.5 ± 0.94*	1.85 ± 0.03*	8.16 ± 0.07*	0.20 ± 0.01*	0.53 ± 0.01*	27.3 ± 0.99*
	6	15.9 ± 0.75*	1.91 ± 0.21*	8.60 ± 0.30*	0.21 ± 0.05*	0.60 ± 0.04*	27.2 ± 1.22*
	8	19.8 ± 1.50*	2.44 ± 0.20*	11.1 ± 0.31*	0.27 ± 0.05*	0.75 ± 0.06*	34.3 ± 2.01*

Values are means ± SD ($n = 4$).
* $P \leqslant 0.05$ (compared to SCFA concentrations after 1 h).

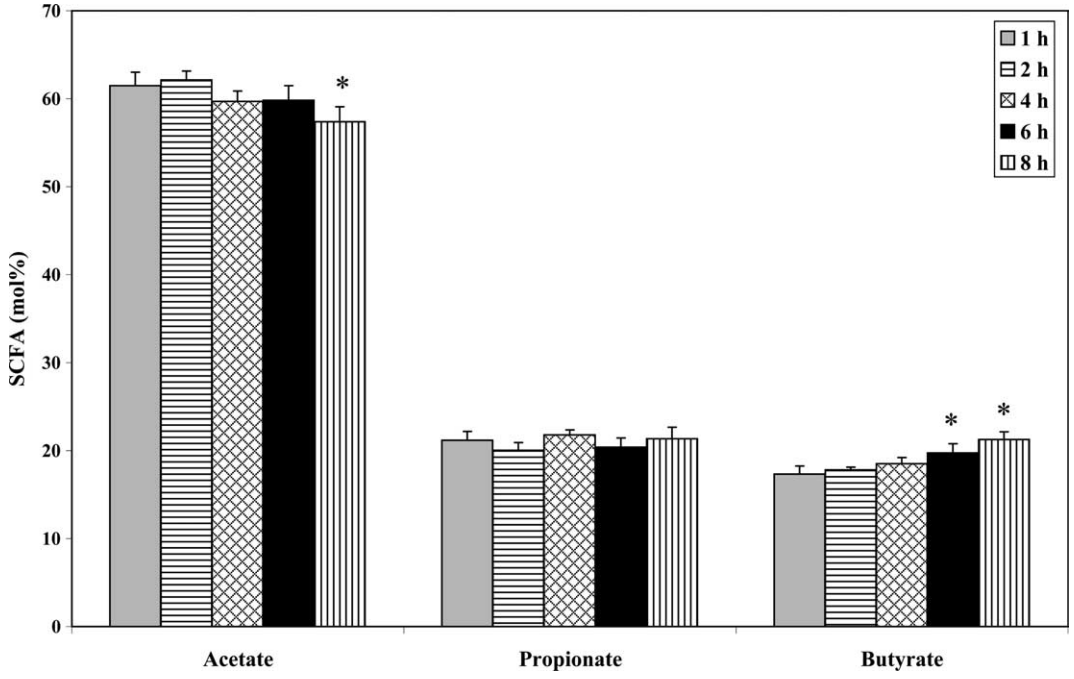

Fig. 3. Changes in the proportions of major SCFA during fermentation of digested extrudate C with the faecal sample from subject 2. Values are means ± SD ($n = 4$) *$P < 0.05$ (compared to 1 h).

Table 4
Influence of individual faeces flora from three subjects on the formation of SCFA after 8 h during fermentation of digested extrudates

Subject	Extrudate	Acetate	Propionate	Butyrate	*iso*-Valerate	*n*-Valerate	Sum
		(μmol SCFA/g original extrudate)					
1	A	807 ± 55.2	149 ± 6.4	386 ± 23.5	21.7 ± 0.6	45.8 ± 1.1	1408 ± 81.3
	B	691 ± 45.1*	178 ± 7.6*	462 ± 28.1*	26.0 ± 0.7*	54.8 ± 1.3*	1413 ± 37.8*
	C	854 ± 29.1*	124 ± 1.7*	452 ± 2.7*	16.7 ± 1.0*	37.7 ± 0.4*	1485 ± 29.5*
	D	1010 ± 25.0*	132 ± 4.0*	501 ± 18.6*	15.8 ± 1.1*	38.6 ± 1.4*	1697 ± 47.3*
	E	1089 ± 82.2*	134 ± 11.0	609 ± 18.6*	14.6 ± 2.5*	41.3 ± 3.3*	1888 ± 111*
2	A	710 ± 113	291 ± 32.5	244 ± 23.8	71.9 ± 2.3	83.9 ± 9.4	1401 ± 180
	B	794 ± 80.8	299 ± 16.5	280 ± 15.6	64.0 ± 2.1	95.9 ± 5.8	1532 ± 120
	C	978 ± 198	359 ± 56.2	359 ± 56.2*	86.1 ± 14.4	129 ± 28.8*	1910 ± 431
	D	923 ± 108	369 ± 38.7*	348 ± 22.4*	94.2 ± 11.3*	141 ± 12.6*	1876 ± 191*
	E	794 ± 82.8	322 ± 25.0	304 ± 21.4*	67.9 ± 9.4	108 ± 7.5*	1595 ± 132
3	A	571 ± 39.5	292 ± 23.0	274 ± 9.0	29.3 ± 3.0	47.7 ± 5.6	1214 ± 45.8
	B	617 ± 123	324 ± 16.0	370 ± 11.1*	33.0 ± 2.7	56.7 ± 1.1*	1401 ± 111
	C	838 ± 133*	391 ± 44.5*	412 ± 58.6*	35.5 ± 2.0*	67.3 ± 5.4*	1743 ± 241*
	D	1059 ± 60.8*	458 ± 11.7*	482 ± 25.1*	37.0 ± 1.5*	77.5 ± 2.7*	2113 ± 96.1*
	E	1021 ± 49.9*	443 ± 20.4*	500 ± 27.1*	34.1 ± 0.8*	74.8 ± 3.5*	2072 ± 90.9*

Values are means ± SD ($n = 4$).
* $P \leqslant 0.05$ (compared to extrudate A).

Amount and composition of the SCFA formed was dependent on the individual bacterial flora and on the DF composition of the (digested) extrudates. Table 4 shows the concentrations of SCFA formed after an incubation time of 8 h using faecal samples from the three healthy subjects. Highest acetate and total SCFA concentrations were found when the bran-rich extrudates C–E were used as substrates. Butyrate production was greatest with the faecal sample from subject 1, whereas faecal samples from subjects three and two were able to form significantly more propionate than those from subject one ($P < 0.05$). Highest *iso*- and *n*-valerate amounts were found when faecal sample from subject two was used.

The average molar proportions of the produced SCFA, acetate, propionate and butyrate, from all used extrudates after 8 h are summarised in Fig. 4 for the faecal samples from the three subjects. Thus, independent of the substrate used, the proportions of acetate and butyrate were highest and those of propionate were lowest when the faecal sample from subject one was used ($P < 0.05$). Finally, the average production of SCFA was evaluated for the faecal samples of all subjects (fermentation time: 8 h) (Fig. 5). Production of acetate, butyrate and total SCFA increased from substrate A to substrate D. Differences between substrates D and E were low. Generally, significantly more acetate, butyrate, and total SCFA were formed when extrudates C–E were used as substrates ($P < 0.05$). Amounts of propionate, *n*-valerate and *iso*-valerate were not different in this way of presentation. Therefore, fermentation of the digested extrudates gave higher concentrations of acetate, butyrate and total SCFA if the original extrudates contained less oat meal and more oat bran, soluble and insoluble DF, as well as β-glucan. Further, presence of RS-rich Novelose seems to play an important role in the appearance of high butyrate levels.

This effect was confirmed during in vitro fermentation of Novelose 330 alone: Molar proportion of butyrate increased continuously from 28.2% (1 h) to 37.5% whereas, that of propionate was not different during the fermentation time of 24 h (approximately 10.5 mol%) (faecal sample from subject three). Similar results were found during fermentation of glucose.

4. Discussion

Using extrusion technology, directly edible cereal products can be prepared. Combined extrusion of cereal products and Novelose gave products with improved textural properties and higher DF contents, including increased RS concentrations (Anon, 1996). The most important DF component in oat and barley is β-glucan. Administration of high β-glucan concentrations (up to 7%) showed no toxic effects in rats (Delaney et al., 2003a). Further, cereal bran (such as barley bran) significantly accelerates transit time and increases daily faecal weight in humans that are physiologically desired effects (Lupton, Morin, & Robinson, 1993).

The water uptake of our original extrudates was between 4 and 5 g H_2O/g sample. Bourquin, Titgemeyer, and Fahey (1996) found similar water-holding capacities in several oat fibres. This shows that the oat products bind distinct amounts of water. Despite decreased water binding in the digested extrudates, it must be mentioned that the cell wall architecture of the oat bran or meal is not completely fermented in vivo. Therefore, they are

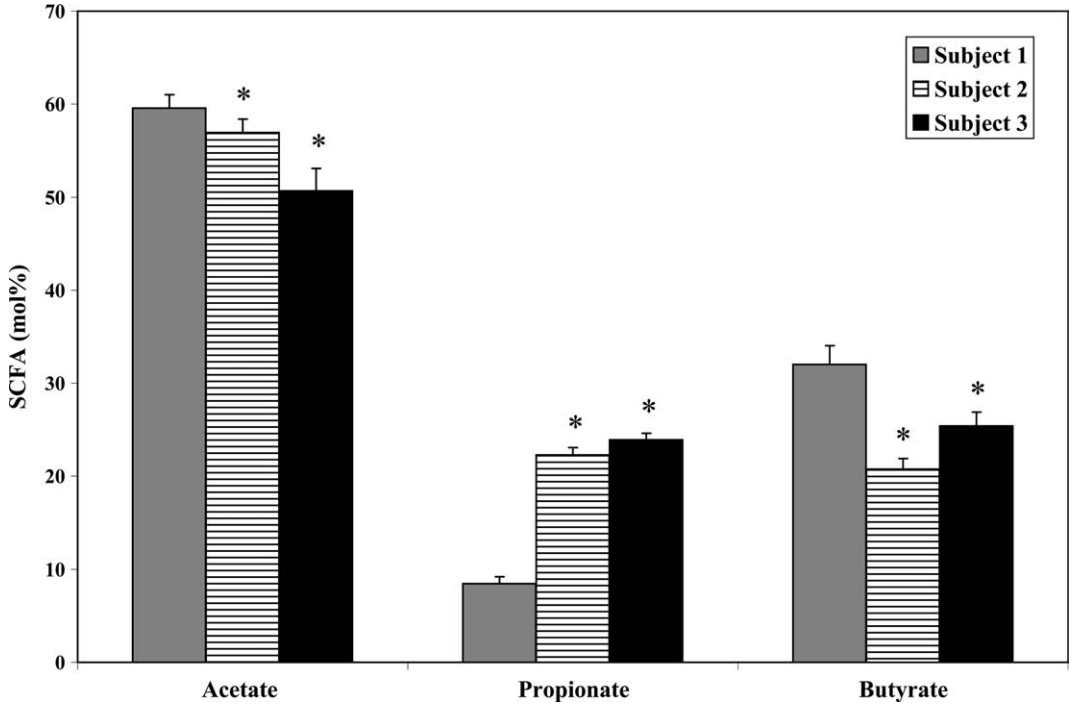

Fig. 4. Effect of individual faecal samples (3 subjects) on the average molar proportion of major SCFA during fermentation of digested extrudates A–E after 8 h. Values are related to one gramme of original extrudates. Values are means ± SD ($n = 5$); *$P < 0.05$ (compared to subject 1).

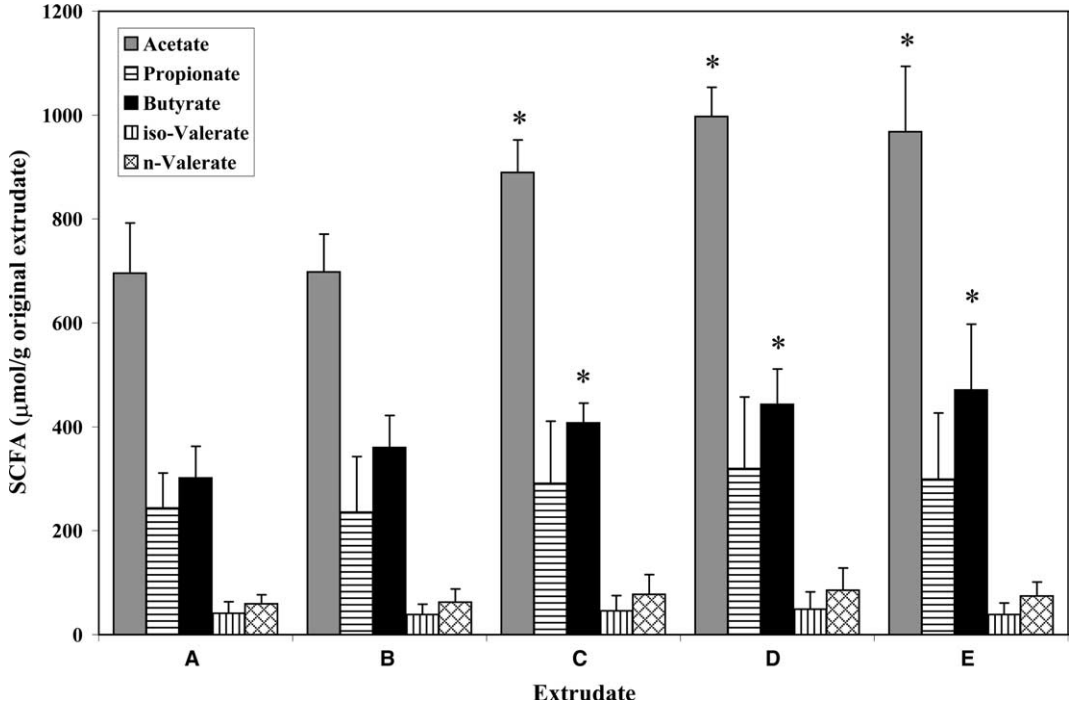

Fig. 5. Average concentrations of SCFA after an 8 h fermentation of digested extrudates A-E with the faecal samples from three subjects. Values are related to one gramme of original extrudates. Values are means ± SD ($n = 6$) *$P < 0.05$ (compared to extrudate A).

able to bind or hold water, also, in colonic contents, resulting in reduced transit times and in dilution effects.

BA are necessary for lipid digestion as part of the mixed micelles in the small intestine. Normally, BA are practically completely re-absorbed and transported to the liver via the enterohepatic circulation by active and passive mechanisms (Hofmann, 1994). However, several DF types such as isolated pectins, are able to interact

under the conditions of the small intestine with BA (Agte & Joshi, 1991; Dongowski, 1995; Hoagland & Pfeffer, 1987) resulting in a lower re-absorption rate of BA, in their further transport towards the large intestine (Zhang et al., 1992), in an increased microbial conversion (e.g. de-conjugation, de-hydroxylation) and in a higher excretion of BA (Dongowski et al., 2003; Lia et al., 1997; Marlett et al., 1994; Zhang et al., 1993). Higher excretion of BA requires an increased hepatic synthesis of BA from cholesterol, as shown during consumption of β-glucan from oat bran (Andersson, Ellegård, & Andersson, 2002). This is a major hypocholesterolemic pathway, occurring especially in hypercholesterolemic individuals or animals (Braaten et al., 1994; Garcia-Diez, Garcia-Mediavilla, Bayon, & Gonzalez-Gallego, 1996; Judd & Truswell, 1981). Recently, Chang et al. (2002) reported on hypocholesterolemic properties of extrudates prepared from cassava starch with Novelose 330 or an oat fibre in hamsters. The mechanisms for the interactions between DF and BA in vitro or in vivo are as yet unknown. Bowles, Morgan, Furneau, and Coles (1996) found no chemical binding between isolated β-glucan and BA. The intensity of interactions between isolated pectins and BA was affected by the molecular fine-structure of both the polysaccharides and the steroids, as well as by the pH of the media (Dongowski, 1995, 1997). Although BA are amphipathic detergent-like molecules, the hydrophobic effects seem to play a major role for the binding to DF. These interactions are also influenced by the surface and particle size and the botanical grown cell wall structure of the DF preparations, by the viscosity in the media or gut and/or by the temperature (Dongowski & Ehwald, 1999; Huang & Dural, 1995; Mongeau & Brassard, 1982). In the case of cell wall preparations from carrots, an influence of carrot variety on the binding of BA was observed (Kotcharian, Kunzek, & Dongowski, 2004). The BA binding by different cereal brans was related to the content of insoluble DF (Kahlon & Woodruff, 2003). As in this study, in vitro interactions with barley extrudates were higher with dihydroxy-BA than with trihydroxy-BA (Huth et al., 2000).

It is interesting that the interactions between the extrudates used and the glyco-conjugated BA are dependent on the composition of the oat product, on the fine-structure of the BA and on the pH of the media. The binding of BA was increased with increasing proportions of oat bran, total DF and insoluble DF but also with increasing contents of β-glucan in the extrudates. This effect points to an involvement of both the botanical grown cell wall structure and the viscosity effects of the soluble macromolecular DF (such as β-glucan) in the interactions with BA. Binding of BA seems to be a very complex process. It is important that not all bound BA are excreted. A part of the DF components is fermented by the microflora. During this process, bound BA were released and enzymatically converted (e.g. formation of secondary BA). Despite changed physicochemical properties, a part of the BA may be re-absorbed even from the large intestine (Hofmann, 1994).

DF is fermented partly or completely by the intestinal microflora, depending on their structural and physicochemical parameters (Bourquin et al., 1996; Wood, Arrigoni, Miller, & Amadò, 2002). Thus, β-glucan, RS or pectin can be fermented to a degree between 70% and 100%, whereas, cellulose is usually incompletely fermented or not fermented. Both types of DF, fermentable and un-fermentable, are important for healthy nutrition. The unfermented DF can increase colonic luminal contents and lower the transit time through the large intestine, and can bind water and "dilute" carcinogens and toxic substances. Fermentable DF is the essential source for formation of SCFA in the large intestine. In vitro fermentation simulates this degradation of DF by the microflora. During the complex fermentation, the polysaccharides must first be split in to their monosaccharide units by bacterial enzymes. Then the monosaccharides formed are further enzymatically decomposed via the Emden–Meyerhof–Parnas and pentose phosphate pathways (Macfarlane & Gibson, 1996). The disappearance of DF components during their fermentation can be followed by analysis of the neutral sugars (Wood et al., 2002). Major end-products of DF fermentation are the SCFA, acetate, propionate and butyrate. Further, some SCFA, such as *n*- and *iso*-valeric acid, are formed by de-amination of amino acids as a result of protein fermentation (De Schrijver, 1996). We used a simulated digestion before fermentation. Fresh whole faecal samples from three healthy subjects were applied to also show the influence of the individual flora on the formation of SCFA. Individual and total SCFA formed are strongly dependent on both the composition of the substrates and the properties of the microflora applied. As in our experiment, Bourquin et al. (1996) found variations in the ability to ferment DF between the faecal samples from different subjects. DF composition of used extrudate strongly influenced the SCFA production and composition. Propionate has been proposed as an inhibitor of hepatic cholesterol synthesis (Anderson, Deakins, & Bridges, 1990). Butyrate, the physiologically most important SCFA, is the major energy source for the colonic epithelium. It regulates cell growth and differentiation as well as plays a role in protection against colon cancer (Jacobasch & Dongowski, 2000; Roediger, 1982; Smith, Yokoyama, & German, 1998; Topping & Clifton, 2001). Elevated concentrations of butyrate with increasing the fermentation time were also reported in other in vitro studies (Förster, Dongowski, & Kunzek, 2002; Kotcharian et al., 2004).

In conclusion, extrudates based on oat have several beneficial nutritional and protective effects in vitro.

Because of their content of macromolecular DF, such as β-glucan, they may influence the viscosity within the gut. The extrudates are able to interact with BA under the pH conditions of the small intestine, resulting in vivo in a higher BA transport into the lower part of the intestinal tract (colon) and in a greater BA excretion. This effect is connected with the decrease of serum cholesterol reported in several studies (Delaney et al., 2003b; Rispin et al., 1992). The lower pH values present in the colon as a result of the SCFA formation during fermentation of the digested extrudates may lead to a lower formation rate of secondary BA, which may be involved in large-bowel carcinogenesis (Roy et al., 1999). High concentrations of SCFA in colon are a precondition for a healthy colon mucosa (Jacobasch & Dongowski, 2000; Topping & Clifton, 2001).

The effects occurring in vivo in the small and large intestine are related to the DF composition of the cereal products. It seems to be possible to influence the beneficial physiological effects of oat products by their DF composition. The results found in vitro will be proved in feeding experiments with animal models and in nutritional studies with human subjects.

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