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Research Article

Model experiments mimicking the human intestinal transit and metabolism of D-galacturonic acid and amidated pectin

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In order to study the human intestinal transit and metabolism of D-galacturonic acid and amidated pectin a number of model experiments were carried out. Both substrates were incubated under aerobic conditions at 37°C using saliva (2 min) and simulated gastric juice (4 h). Under anaerobic conditions the substrates were incubated at 37°C using human ileostomy and colostomy fluids, each obtained from three different donors, for 10 and for 24 h, respectively. D-Galacturonic acid, SCFA (acetic acid, propionic acid, and butyric acid), as well as methanol were analyzed photometrically after carbazole reaction, GC-flame ionization detection (GC-FID), and headspace solid-phase microextraction GC/MS (HS-SPME-GC/MS), respectively. D-Galacturonic acid and amidated pectin were found to be stable during incubations with saliva and simulated gastric juice, whereas both substrates underwent degradation in the course of human ileostomy and colostomy fluid incubations. D-Galacturonic acid was practically completely decomposed within 10 h and SCFA, with acetic acid as the major representative, were formed up to 98% of the incubated substrate in colostomy effluent. The amidated pectin was only degraded in part, revealing stable amounts of 22–35% and 3–17% in ileostomy (after 10 h) and colostomy fluid (after 24 h), respectively. SCFA were generated up to 59% of the applied amidated pectin. In parallel, 19–60% and 52–67% of the available methyl ester groups were cleaved in the course of incubations with ileostomy and colostomy fluids, respectively. The results demonstrate for the first time that D-galacturonic acid and amidated pectin are stable in human saliva and simulated gastric juice. The degradation of both compounds during incubation with ileostomy effluent is highlighted, providing evidence for a considerable metabolic potential of the small intestine.

Keywords: Amidated pectin / Colostomy fluid / D-Galacturonic acid / Gastric juice / Ileostomy fluid

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1 Introduction

Pectin is a heteropolysaccharide found in the middle lamella and the primary cell wall of higher plants. The main backbone of the molecule is composed of a polymer of D-galacturonic acid, called homogalacturonan (homopolymer of [1 → 4]α-D-galactopyranosyluronic acid with part of the carboxyl groups methylesterified) and rhamnogalacturonan I (RG-I) (heteropolymer of repeating [1 → 2]α-L-rhamnosyl-[1 → 4]α-D-galactosyluronic acid disaccharide units [1]. Apart from RG-I, the more complex rhamnogalacturonan II (RG-II) has been identified. The pectin molecule is branched at the rhamnogalacturonan part by side chains such as arabinans, galactans, or arabinogalactans, which are linked by [1 → 4] linkages to rhamnose. In the main side chains, the arabinose units are α[1 → 5] linked and the galactose units are joined by β[1 → 4] linkages. Apart from these neutral sugars the side chains of pectins can also contain D-xylopyranose, D-glucopyranose, and L-fucopyranose, whereas in RG-II D-apiose, 2-*O*-methyl-D-xylose, and 2-*O*-methyl-L-fucose are present [2]. In RG-I the galacturonic acid residues are often acetylated at the C_2 or C_3 posi-

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Abbreviations: DE, degree of methylesterification; **DP,** degree of polymerization; **HM,** high methylesterified; **HS-SPME-GC/MS,** headspace solid-phase microextraction GC/MS; **LM,** low methylesterified

tion, but acetylation has also found in the homogalacturonan region [1]. The cell biology of pectin and the impact of molecular genetic approaches have been reviewed in terms of the functional analysis of pectic saccharides [3].

Pectin – usually obtained in large quantities from apple pomace and citrus peels – has a broad range of applications as a gelling agent, thickener, texturizer, emulsifier, and stabilizer in the food industry. The degree of polymerization (DP) and degree of methylesterification (DE) determine the conditions for use. The DP varies from a few hundred to 1000 saccharide units corresponding to molecular weights from about 50 to 150 kDa. Depending on the number of methylesterified galacturonic acid residues, pectins are classified as low (LM) (25–50%) and high (HM) (50–80%) methylesterified pectins. In general, LM-pectin is obtained from HM-pectin by a treatment at mild acidic or alkaline conditions. The DE determines the gelling properties, and also the textural properties are influenced by the DE, but also by the sugar composition of the side chains and cross-linking of pectins. About 80% of the world production of HM-pectin are used in the manufacturing of jams and jellies.

Amidated pectin is obtained from HM-pectin when ammonia is used in the alkaline deesterification process. Thus, the LM-pectins contain some amide groups at the C_6 position of the galacturonic acid unit. The useful properties of amidated pectin may vary with the proportion of ester and amide units and with the DP. Pectin has been evaluated and cleared toxicologically by JECFA and a group ADI "not specified" was established for pectins and amidated pectins.

In medicine, pectin is traditionally used because of its antidiarrhea effect. In addition, a number of health promoting effects have been described; among them the serum cholesterol lowering effect was highlighted [4]. As physiologically active compound in the group of dietary fibers [5, 6], the intestinal degradation of pectin has already been studied extensively, as shown, *e.g.*, in the work of Saito *et al*. [7], with quite large differences in the results. Information about the amidated pectin, however, is rather scarce [8]. Most surprisingly, even though D-galacturonic acid is known to be the major intestinal metabolite of pectin, there is no information available about the behavior of this sugar acid in the course of the intestinal metabolism.

In this paper, we report the results of the human intestinal transit and metabolism of amidated pectin and D-galacturonic acid obtained by *in vitro* and *ex vivo* studies, *i.e.*, using human saliva, simulated gastric juice, as well as ileostomy and colostomy fluids, respectively. In previous investigations the use of ileostomy effluent has been shown as reliable tool to determine the availability of physiologically active compounds [9, 10].

2 Materials and methods

2.1 Subjects

Saliva samples from three healthy subjects (23–35 years old) were collected in the morning with the subject abstaining from tooth brushing since the previous evening.

Ileostomy effluents were provided by three healthy subjects (34–39 years old; donors 1–3) with a terminal ileostomy undergone colectomy (all suffering from Crohn's disease without an involvement of the ileum) 5–6 years prior to the study. Due to the medical history of each no ileal resection was performed by surgery.

Colostomy effluents were provided by three healthy subjects (30–60 years old; donors 4–6), all with left-sided colostomies after suffering from colon cancer or prestages undergone colectomy 12–30 years prior to the study. After removal of the affected part of the colon no further symptoms for colon cancer were noticed.

All the subjects had their weights taken and were medically examined. All of them were medically normal. A flyer was handed out to the probands to omit the consumption of pectin and D-galacturonic acid containing food such as vegetables, fruits, jams and jellies, juices, and milk products 48 h ahead of the study. None of the patients was treated with antibiotics for the last 4 wk.

2.2 Chemicals

All chemicals and solvents were of analytical grade. Solvents were redistilled before use. Methanol-D_3 was purchased from Deutero (Kastellaun, Germany). Butyric acid, carbazole, D-galacturonic acid, methanol, sulfuric acid, and sodium tetraborate were from Fluka (Deisenhofen, Germany). Acetic acid and ammonium sulfate were from Grüssing (Filsum, Germany) and 2-methyl-1-pentanol, propionic acid, and perchloric acid were obtained from Sigma–Aldrich (Steinheim, Germany). Hydrochloric acid was purchased from VWR International (Fontenay sous Bois, France). Ethanol absolute was obtained from Riedel-de Haën (Seelze, Germany). Amidated pectin (AF 020; declared DE 30% and degree of amidation [DA] 20%, respectively) was generously provided by Herbstreith & Fox KG (Neuenbürg, Germany).

2.3 Preparation of saliva

For the incubations, collected saliva (directly transferred from the oral cavity into tubes) was diluted 1:1 with distilled water and shaken to reduce viscosity according to ref. [11]. The diluted saliva was centrifuged at 5000 × *g* and the supernatant was used as the incubation medium.

2.4 Preparation of simulated gastric juice

The conditions in the stomach during the incubations were simulated with artificial gastric juice according to ref. [12].

2.5 Preparation of inoculum (ileostomy/colostomy fluids)

After removal, the ileostomy/colostomy bag was immediately placed in an anaerobic jar containing AnaeroGen™ from Oxoid (Hampshire, UK) to create an anaerobic atmosphere. It was transported straight into the laboratory where the jar was transferred immediately into an anaerobic chamber (self-constructed) flushed with a N_2/CO_2 gas mixture (80:20 v/v). The ileostomy/colostomy fluid was diluted with the same volume of anaerobic carbonate – phosphate – buffer (pH 6.3 for ileostomy fluid, pH 7.4 for colostomy fluid) according to ref. [13]. The ileostomy/colostomy fluid was mixed and coarse particles were removed by filtration using glass wool. The filtrate was used as inoculum.

2.6 Incubation conditions

2.6.1 Saliva

Diluted saliva (2.5 mL) was added to pregassed (N_2) incubation vessels, each containing 5 mg D-galacturonic acid. For incubation with amidated pectin, 1.25 mL diluted saliva was added to 1.25 mL of an amidated pectin stock solution (4 g/L). The incubation vessels were sealed tightly and after shaking stored at 37°C for 0, 30, 60, and 120 s. To stop enzymatic reactions the vessels were placed in liquid nitrogen and then lyophilized.

For methanol analysis 5 mL diluted saliva was added to 5 mL of an amidated pectin stock solution (4 g/L) and after shaking incubated at 37°C for 0, 30, 60, and 120 s. To stop enzymatic reactions the vessels were placed in liquid nitrogen. For blank assays adequate volumes of distilled water instead of D-galacturonic acid or amidated pectin were applied.

2.6.2 Simulated gastric juice

Simulated gastric juice (2.5 mL) was added to pregassed (N_2) incubation vessels, each containing 5 mg D-galacturonic acid. For incubation with amidated pectin, 1.25 mL simulated gastric juice was added to 1.25 mL of an amidated pectin stock solution (4 g/L). The incubation vessels were sealed tightly and after shaking stored at 37°C for 0, 1, 2, 3, and 4 h. To stop enzymatic reactions the vessels were placed in liquid nitrogen and then lyophilized.

For methanol determination 5 mL simulated gastric juice was added to 5 mL of an amidated pectin stock solution (4 g/L) and after shaking incubated at 37°C for 0, 1, 2, 3, and 4 h. To stop enzymatic reactions the vessels were placed in liquid nitrogen. For blank assays adequate volumes of distilled water instead of D-galacturonic acid or amidated pectin were applied.

2.6.3 Ileostomy/colostomy fluids

Inoculum (2.5 mL) was added to pregassed (N_2) incubation vessels, each containing 5 mg D-galacturonic acid. For incubation with amidated pectin, 1.25 mL inoculum was added to 1.25 mL of an amidated pectin stock solution (4 g/L). The incubation vessels were sealed tightly and after shaking stored at 37°C for 0, 0.5, 1, 2, 4, 6, 8, and 10 h (ileostomy fluid) and 0, 1, 2, 4, 6, 8, 10, and 24 h (colostomy fluid). To stop enzymatic reactions the vessels were placed in liquid nitrogen and then, in the case of D-galacturonic acid and amidated pectin determination, lyophilized. For SCFA determination see Section 2.7.3.

In addition, model stability studies of D-galacturonic acid and amidated pectin were conducted using anaerobic carbonate – phosphate buffer (pH 6.3 and 7.4) without inoculum as described above.

For methanol measurements 5 mL inoculum was added to 5 mL of an amidated pectin stock solution (4 g/L) and after shaking incubated at 37°C for 0, 0.5, 1, 2, 4, 6, 8, and 10 h (ileostomy fluid) and 0, 1, 2, 4, 6, 8, 10, and 24 h (colostomy fluid). To stop enzymatic reactions the vessels were placed in liquid nitrogen. For blank assays adequate volumes of distilled water instead of D-galacturonic acid or amidated pectin were applied.

2.7 Sample preparations

2.7.1 D-Galacturonic acid and amidated pectin

The freeze-dried samples were extracted twice using 1.25 mL distilled water. After centrifugation ($5000 \times g$ for 10 min) the supernatants were pooled and filtered (polyvinylidene difluoride, 0.45 μm). Blanks (without substrates) were treated identically. Aliquots of the extracts (1 mL for saliva; 500 μL for simulated gastric juice and ileostomy/colostomy inocula, respectively) were diluted (1:100 for saliva; 1:50 for simulated gastric juice and ileostomy/colostomy inocula, respectively) and measured photometrically (*cf*. Section 2.8).

2.7.2 Methanol

The frozen samples were thawed and measured by headspace solid-phase microextraction GC/MS (HS-SPME-GC/MS; *cf*. Section 2.9).

2.7.3 SCFA

For determination of SCFA, the incubated samples were centrifuged ($5000 \times g$ for 10 min), 50 μL of 2-methyl-1-pentanol (internal standard), 280 μL of 0.36 mol/L perchloric acid solution, and 270 μL of 1 mol/L potassium hydroxide solution were added rapidly to 100 μL of the supernatant. The freeze-dried samples were homogenized in 950 μL of 0.5 mol/L HCl. After centrifugation the supernatant was measured by GC-FID (*cf*. Section 2.10). Linearity was given from 1 to 200 mg/L SCFA, coefficients of correlation of the calibration curves were at least 0.99. The

assay was accurate and reproducible. The lower LOQ was 1 mg/L for all SCFA.

2.8 Photometric determination of D-galacturonic acid and amidated pectin

D-Galacturonic acid and amidated pectin concentrations were measured by the modified uronic acid carbazole reaction [14, 15], as applied by Plätzer *et al*. [16]. Thus, 1.25 mL of a sodium tetraborate solution (0.025 mol/L sodium tetraborate × 10 H_2O in sulfuric acid) was placed in tubes and cooled to 4°C. The prepared samples (210 µL) (*cf*. Section 2.7.1) were carefully layered above the acid and the closed tubes were then shaken at constant cooling for 5 min. Subsequently, the tubes were heated for 10 min in a boiling water bath and cooled to room temperature. After that 40 µL carbazole solution (0.125% carbazole in absolute ethanol v/v) was added and the tubes were shaken again. Then they were heated for 15 min in a boiling water bath, cooled down to room temperature and the absorption of the samples was measured at 524 nm in a Shimadzu UVmini-1240 spectrophotometer (Kyoto, Japan) with Hellma precision cell Quartz Suprasil® cuvettes (light path: 10 mm; Müllheim, Germany), against blanks (distilled water instead of added sample). This method of determination only comprises the free D-galacturonic acid (not esterified or amidated) residues of the used amidated pectin; this amount was determined to be 47.5 ± 0.7%. Consequently, for the applied amidated pectin concentrations (4 and 2 g/L) corrected values of 1.9 g/L and 0.95 g/L, respectively, had to be considered. Linearity was given from 1 to 100 mg/L D-galacturonic acid, coefficients of correlation of the calibration curves were at least 0.99.

2.9 HS-SPME-GC/MS

SPME fibers (Supelco, Bellefonte, USA; 70 µm Carbowax-DVB) were conditioned prior to the measurements as recommended by the manufacturer. The assay was, except little modifications, performed according to Savary and Nuñez [17]. For this 6 g $(NH_4)_2SO_4$ and 1.25 mL methanol-D_3 (internal standard) were mixed with 10 mL of the thawed sample in a headspace vial. Vials were capped and heated at 70°C for 30 min in an aluminum block heater. After that, the needle of the SPME device was inserted through the septum and the fiber was exposed to the headspace vapor for 30 min. Then the fiber was retracted and transferred to the GC injection port. For sample desorption the fiber remained exposed in the injection port for 1 min and was then transferred to another injection port for equilibration.

An HP Agilent 6890 Series gas chromatograph with split injection (1:20) was directly coupled to an HP Agilent 5973 Network mass spectrometer (Agilent Technologies) using a DB-Wax fused-silica capillary column (J&W, Agilent, Waldbronn, Germany) (30 m × 0.25 mm id; d_f = 0.25 µm) and helium (1.0 mL/min) as carrier gas. The temperature program was the following: 30°C for 10 min, 25°C/min heating up to 240°C. The injector and interface temperatures were 220°C. Mass-selective detection (70 eV, electron impact) was performed in the scan mode (10 – 50 amu).

2.10 GC-flame ionization detection (GC-FID)

One µL (split 1:20) of the prepared sample (*cf*. Section 2.7.3) was analyzed on a 30 m × 0.25 mm (id) J&W DB-Wax column using the following temperature program: isothermal at 40°C for 3 min, then raised at 4°C/min up to 240°C. The GC system (Hewlett-Packard, Waldbronn, Germany) consisted of an HP gas chromatograph 5890 Series II, HP 7673 autosampler and FID-detector. Data analysis was performed with HP Chemstation software.

2.11 Statistical analysis

Mean values and SD were calculated for all data (n = 6 independent experiments; each experiment was carried out in duplicate). Data sets were analyzed by one-way ANOVA with *post hoc* Bonferroni's multiple comparison test. Statistical significance was defined as a significance level of $p \leq 0.05$. Due to the very limited sample number a pretest was performed to test the normal distribution of the residuals. Therefore, the residuals of each data group were calculated and the ratio of range to SD was analyzed according to David *et al*. [18]. On one data set a reciprocal transformation was performed for normal distribution of the residuals and subsequent ANOVA analysis. Due to the limited number of data p values should be interpreted very cautiously.

3 Results

D-Galacturonic acid and amidated pectin were incubated under *aerobic* conditions using saliva and simulated gastric juice. Under *anaerobic* conditions the substrates were incubated using ileostomy fluids (donors 1 – 3) and colostomy fluids (donors 4 – 6). In addition, stability studies of both substrates were carried out using anaerobic carbonate – phosphate buffer (pH 6.3 and 7.4) without inoculum to check their chemical stability. The target compounds to be determined were D-galacturonic acid, SCFA and methanol (in case of incubations with amidated pectin).

D-Galacturonic acid was found to be stable during incubations with saliva, simulated gastric juice as well as in the model stability studies (data not shown), whereas complete degradation was observed after incubation of 6 h using the ileostomy fluids from donors 1 and 2. However, after the same time of incubation with ileostomy fluid from donor 3, 0.3 g/L D-galacturonic acid remained and was found to be almost stable (0.2 g/L) until 10 h (Fig. 1). SCFA increased continuously during the ileostoma incubation of D-galactur-

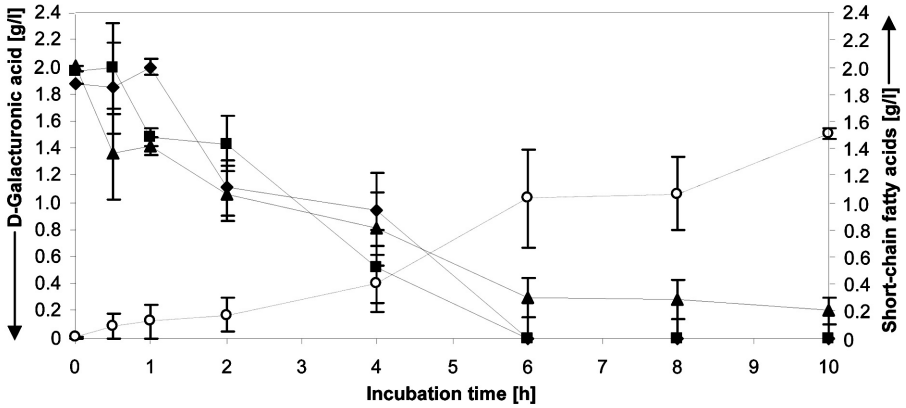

Figure 1. Time course of the incubation of D-galacturonic acid (2 g/L) with human ileostomy fluids obtained from three donors. D-Galacturonic acid and SCFAs were determined photometrically (524 nm) after carbazole reaction and by GC-FID, respectively. D-Galacturonic acid: donor 1 (■), donor 2 (♦), donor 3 (▲); SCFA: (○). D-Galacturonic acid values are the means each of donors 1 – 3 ± SD; for better clarity of presentation SCFA values are given as means ± SD from the donors.

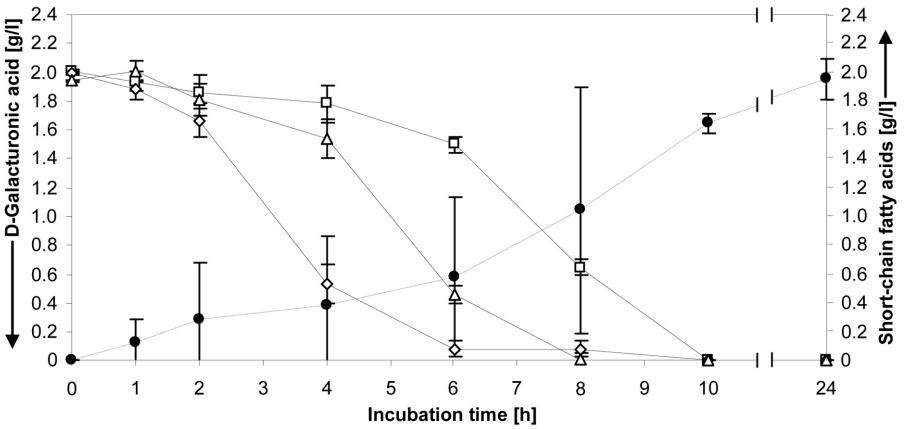

Figure 2. Time course of the incubation of D-galacturonic acid (2 g/L) with human colostomy fluids obtained from three donors. D-Galacturonic acid and SCFAs were determined photometrically (524 nm) after carbazole reaction and by GC-FID, respectively. D-Galacturonic acid: donor 4 (□), donor 5 (◇), donor 6 (△); SCFA: (●). D-Galacturonic acid values are the means each of donors 4 – 6 ± SD; for better clarity of presentation SCFA values are given as means ± SD from the donors.

Table 1. Formation of SCFA in human ileostomy and colostomy fluids during incubation of D-galacturonic acid and amidated pectin, respectively

Incubation medium	Incubation time (controls) (h)	Controls[a)] (mmol/L)	Substrate	Incubation time (h)	Acetate (mmol/g)	Propionate (mmol/g)	Butyrate (mmol/g)	Total SCFA[b)] (mmol/g)
Ileostomy fluid	0	7.14	D-Galacturonic acid	10	12 ± 0.24**	0.28 ± 0.13	0.18 ± 0.15	12.4 ± 0.51
	10	7.03	Amidated pectin	10	8.71 ± 2.87	0.05 ± 0.03	0.05 ± 0.04	8.81 ± 2.94
Colostomy fluid	0	65.50	D-Galacturonic acid	24	16 ± 0.8*	0.12 ± 0.12	0.06 ± 0.04	16.2 ± 0.96
	24	72.00	Amidated pectin	24	10.1 ± 1.5**	0.02 ± 0.02	0.01 ± 0.002	10.1 ± 1.5

Values represent the means ± SD of $n = 6$ independent experiments (two tests *per* sample of an individual human subject each; samples collected once).

a) Sum of acetate, propionate, and butyrate (mmol/L inoculum); incubations without addition of substrate.

b) Sum of acetate, propionate, and butyrate (mmol/g substrate); values determined as difference from the incubations with substrates and controls.

* Significantly higher than **($p < 0.001$) as determined by one-way ANOVA.

onic acid up to a mean concentration of 1.5 ± 0.04 g/L (Fig. 1).

The incubation of D-galacturonic acid with the colostomy fluids revealed initially stronger individual differences concerning its degradation and SCFA formation. Thus, after 6 h of incubation D-galacturonic acid was found from 1.5 g/L (donor 4), 0.46 g/L (donor 6) to 0.08 g/L (donor 5) but disappeared completely within 10 h. In parallel, SCFA concentration increased, showing the expected corresponding high deviations, up to a maximum of 1.95 ± 0.14 g/L (Fig. 2). Except for one donor, SCFA analysis revealed acetic acid as the major constituent being generated during the ileostoma as well as colostoma incubations of D-galacturonic acid (Fig. 7). In addition, Table 1 gives detailed information about the amounts of acetic acid, propionic acid, butyric acid, and total SCFA.

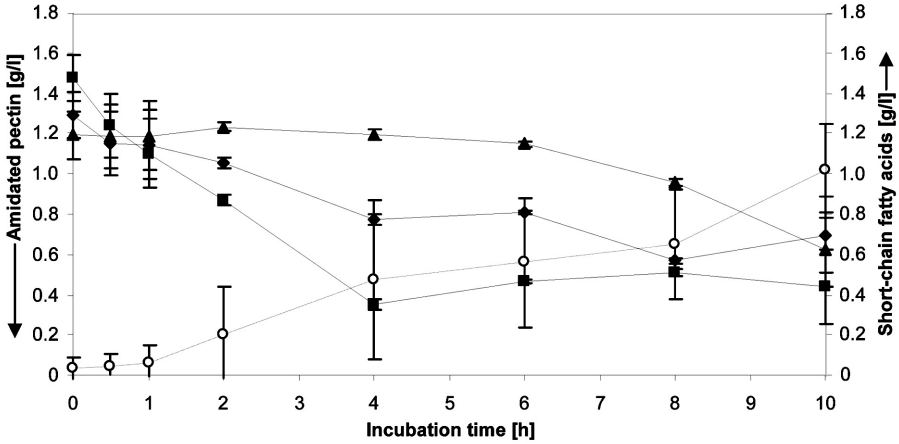

Figure 3. Time course of the incubation of amidated pectin (2 g/L) with human ileostomy fluids obtained from three donors. Amidated pectin and SCFA were determined photometrically (524 nm) after carbazole reaction and by GC-FID, respectively. Amidated pectin: donor 1 (■), donor 2 (♦), donor 3 (▲); SCFA: (○). Amidated pectin values are the means each of donors 1 – 3 ± SD; for better clarity of presentation SCFA values are given as means ± SD from the donors.

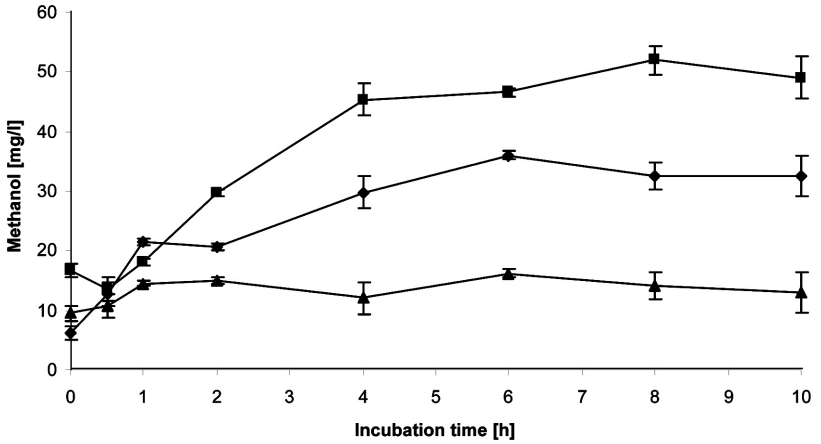

Figure 4. Methanol liberation during the incubation of amidated pectin (2 g/L) with human ileostomy fluids obtained from three donors. Methanol was determined *via* HS-SPME-GC/MS. The methanol values are the means ± SD from donor 1 (■), donor 2 (♦), donor 3 (▲), respectively.

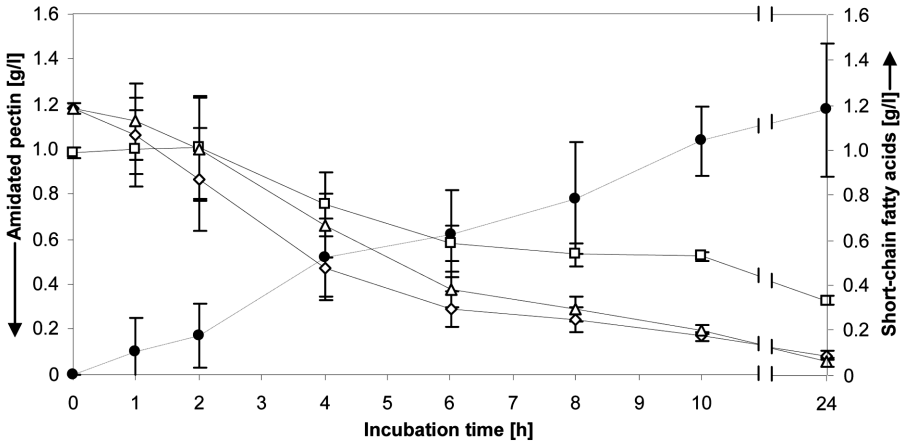

Figure 5. Time course of the incubation of amidated pectin (2 g/L) with human colostomy fluids obtained from three donors. Amidated pectin and SCFA were determined photometrically (524 nm) after carbazole reaction and by GC-FID, respectively. Amidated pectin: donor 4 (□), donor 5 (◇), donor 6 (△); SCFA: (●). Amidated pectin values are the means each of donors 4 – 6 ± SD; for better clarity of presentation SCFA values are given as means ± SD from the donors.

Amidated pectin was found to be stable during incubations with saliva, simulated gastric juice, and in all model stability studies (data not shown). The incubations with the ileostomy fluids from donors 1 to 3 led to very different degradation rates. Thus, after 6 h of incubation the initial concentrations of amidated pectin (donor 1: 1.47 g/L, donor 2: 1.3 g/L, donor 3: 1.2 g/L) decreased to 0.47, 0.81, and 1.15 g/L, respectively, resulting in a mean SCFA concentration of 0.56 ± 0.32 g/L (after 6 h) and a maximum concentration of 1 ± 0.23 g/L. After 10 h of incubation still 0.44 – 0.7 g/L amidated pectin remained unaffected (Fig. 3). In parallel, methanol liberation was observed up to maximum concentrations of 52 mg/L (donor 1), 36 mg/L (donor 2), and 16 mg/L (donor 3) (Fig. 4).

In contrast to the results observed in our studies with ileostomy fluid, the incubation of amidated pectin with colostomy fluid showed lower individual differences concerning degradation rates and SCFA composition. Thus,

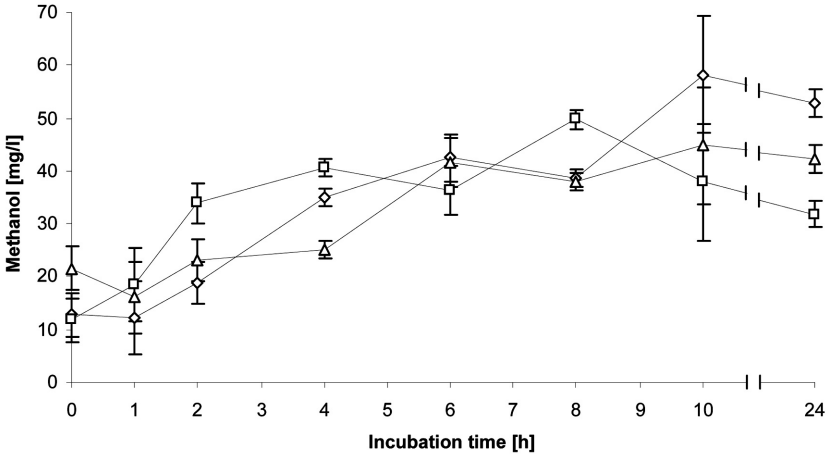

Figure 6. Methanol liberation during the incubation of amidated pectin (2 g/L) with human colostomy fluids obtained from three donors. Methanol was determined *via* HS-SPME-GC/MS. The methanol values are the means ± SD from donor 4 (□), donor 5 (◇), donor 6 (△), respectively.

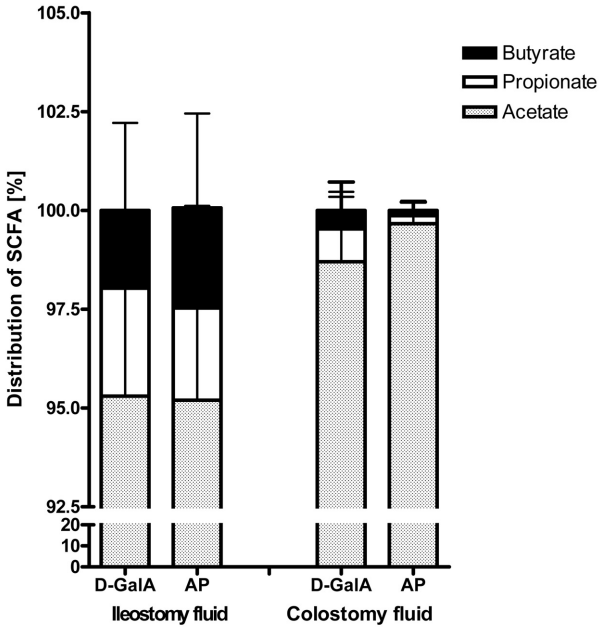

Figure 7. Distribution of SCFA [%] after incubation of D-galacturonic acid (D-GalA) and amidated pectin (AP) with ileostomy (10 h) and colostomy fluids (24 h). The SCFA values are the means ± SD.

after 6 h of incubation amidated pectin concentrations ranged from 0.58 g/L (donor 4), 0.38 g/L (donor 6) to 0.29 g/L (donor 5) and decreased to minimum concentrations of 0.33–0.06 g/L after 24 h. In parallel, SCFA concentration increased, showing the expected corresponding deviations up to a maximum of 1.18 ± 0.3 g/L (Fig. 5). Analogously, methanol liberation was observed up to maximum concentrations of 49.7 mg/L (donor 4), 58.1 mg/L (donor 5), and 44.8 mg/L (donor 6) (Fig. 6).

Acetic acid consistently was the major SCFA being generated during the ileostoma as well as colostoma incubations (Fig. 7). The percentage distribution of the SCFA after incubation of D-galacturonic acid with ileostomy (10 h) and colostomy fluids (24 h) was 91.2–98% acetate, 1–4.3% propionate, 0.4–4.5% butyrate as well as 96.8–99.7% acetate, 0.1–2.2% propionate, 0.1–1% butyrate, respectively (Fig. 7). Aside, those determined after incubation of amidated pectin with ileostomy (10 h) and colostomy fluids (24 h) were 89.6–98.8% acetate, 0.8–5.2% propionate, 0.6–5.2% butyrate and 99–100% acetate, 0–0.6% propionate, 0–0.4% butyrate, respectively (Fig. 7).

By comparison of each pair of variates (*cf.* Section 2.11) only the acetate values showed certain significance (Table 1). The acetate concentrations at the end of the incubations of D-galacturonic acid with ileostomy fluids (12 ± 0.24 mmol/g substrate) were significantly lower ($p < 0.001$) compared with those of the colostomy incubations (16 ± 0.8 mmol/g substrate) (Table 1). However, after D-galacturonic acid incubations with colostomy fluid, significantly higher ($p < 0.001$) acetate values (16 ± 0.8 mmol/g substrate) were formed than after colostomy incubations of amidated pectin (10.1 ± 1.5 mmol/g substrate) (Table 1). There were no significant differences in the concentrations of propionate and butyrate at the end of any incubation.

4 Discussion

Although the intestinal degradation of high- and low-esterified pectin as well as pectic acid has already been studied extensively [7, 19, 20], information about amidated pectin and the monomer D-galacturonic acid is rather scarce [8, 21, 22]. In addition, information about the metabolism of these compounds in the oral cavity and the stomach is still lacking.

Hydrolytic activity of the oral cavity is known for a long time and was linked, *inter alia*, with the activation of flavonoid glycosides [11]. In the course of our experiments carried out with saliva, the substrates under study, D-galacturonic acid and amidated pectin, were found to be stable. Following the digestion pathway partial degradation of pectic substances seems to be possible under the physico-chemical conditions of the stomach [19]. Nonetheless, as recently

shown [23], when pectin beads were used for colonic delivery of the drug ketoprofen, little or no drug was released in the gastric medium, attesting the stability of pectin under such conditions. Our findings that both D-galacturonic acid and amidated pectin were stable during incubation with gastric juice fit well with this observation.

In our study, we mostly observed complete decomposition of D-galacturonic acid but only partial degradation of amidated pectin by ileostomy as well as colostomy fluids. Neither the complete decomposition of the monomer nor the partial depolymerization of the polymer by the small intestinal microflora has been reported up to date. Although pectin is known to be degraded by the large intestinal microflora [24], it was thought to be completely stable under small intestinal conditions [19]. Our results were confirmed by the formation of SCFA and by the liberation of methanol. In general, amidated pectin showed higher stability after incubation with ileostomy (Fig. 3) than with colostomy fluid (Fig. 5). Therefore, the stable amount of the physiologically active dietary fiber would reach the colon and fulfill the described health effects [5, 6].

SCFA and methanol as known products of pectin metabolism [25, 26] were found in degradation related amounts; the formation of oligogalacturonic acids as intermediate products [20] was not considered in our study. Theoretically, during incubation of the amidated pectin, free galacturonic acid residues may be additionally formed resulting in an increased substrate concentration. However, this effect was not observed.

SCFA, primarily acetate, propionate, and butyrate as chief end products (together with the gases CO_2, CH_4, and H_2) of polysaccharide metabolism [27], are regarded to play physiological roles in the colon. In general, SCFA are, after their rapid and efficient absorption in the colon (with only 5 – 10% SCFA being excreted in feces [28]), nutrients for the colonic epithelium, modulators of colonic and intracellular pH and cell volume, regulators of proliferation, differentiation and gene expression [29], and utilized for lipid synthesis [30].

In agreement with the findings of recent animal studies [22], acetate was found as principal SCFA in our experiments. Compared with the results of our model studies, however, higher percentages of propionate and butyrate were determined [22]. Acetate is known to be readily absorbed and transported to the liver and hence is less metabolized in the colon [29]. In contrast to propionate, acetate has been shown to increase cholesterol synthesis [27]. Therefore, substrates that can decrease the acetate/propionate ratio (*e.g.*, propionylated starch derivatives [31]) may reduce serum lipids and possibly cardiovascular disease [27]. Since butyrate was found to be a minor fermentation product only in our studies, its detailed function is not further discussed.

The maximum methanol concentrations determined during our incubations ranged from 16 to 58.1 mg/L; they are in agreement with previously reported data obtained from experiments with pectin and apples [26, 32]. According to earlier observations [32] the methanol concentrations are far below toxic levels. In addition, early studies involving human subjects on large doses of pectin (15 – 50 g/day) showed no toxic side effects [33, 34].

Summarizing, our results demonstrate for the first time that D-galacturonic acid and amidated pectin are stable in saliva and gastric juice. Whereas the monomer showed the expected decomposition after incubation with ileostomy and colostomy effluents, the amidated pectin was only degraded in part, revealing stable amounts of 22 – 35% and 3 – 17% in ileostomy (after 10 h) and colostomy fluid (after 24 h), respectively.

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5 References

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