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Formation of Phenolic Microbial Metabolites and Short-Chain Fatty Acids from Rye, Wheat, and Oat Bran and Their Fractions in the Metabolical in Vitro Colon Model

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ABSTRACT: Rye bran and aleurone, wheat bran and aleurone, and oat bran and cell wall concentrate were compared in their in vitro gut fermentation patterns of individual phenolic acids and short-chain fatty acids, preceded by enzymatic in vitro digestion mimicking small intestinal events. The formation of phenolic metabolites was the most pronounced from the wheat aleurone fraction. Phenylpropionic acids, presumably derived from ferulic acid (FA), were the major phenyl metabolites formed from all bran preparations. The processed rye, wheat, and oat bran fractions contained more water-extractable dietary fiber (DF) and had smaller particle sizes and were thus more easily fermentable than the corresponding brans. Rye aleurone and bran had the highest fermentation rate and extent probably due to high fructan and water-extractable arabinoxylan content. Oat samples also had a high content of water-extractable DF, β-glucan, but their fermentation rate was lower. Enzymatic digestion prior to in vitro colon fermentation changed the structure of oat cell walls as visualized by microscopy and increased the particle size, which is suggested to have retarded the fermentability of oat samples. Wheat bran was the most slowly fermentable among the studied samples, presumably due to the high proportion of water-unextractable DF. The in vitro digestion reduced the fructan content of wheat samples, thus also decreasing their fermentability. Among the studied short-chain fatty acids, acetate dominated the profiles. The highest and lowest production of propionate was from the oat and wheat samples, respectively. Interestingly, wheat aleurone generated similar amounts of butyrate as the rye fractions even without rapid gas production.

KEYWORDS: *bran, aleurone, rye, oat, wheat, dietary fiber, gut, colon, fermentation, in vitro*

INTRODUCTION

Epidemiological studies have repeatedly shown that diets rich in whole grain foods reduce the risk of type 2 diabetes mellitus[1] and cardiovascular disease.[2] The fiber complex of cereal grain has been suggested as one of the main constituents behind the protective effects.[3] Interaction between the gut microbiota and the insoluble dietary fiber (DF) has been proposed as a link between DF and reduced diabetes risk.[4] The interactions between dietary factors, gut microbiota, and host metabolism are important for maintaining homeostasis and health,[5] and research into the role of different characteristics of DF and phytochemicals in gut microbiota-mediated signaling is in its early phases.

The cereal DF complex is composed of a combination of DF polymers, such as arabinoxylan (AX), β-glucan, fructan, cellulose, and lignin, and small molecular weight bioactive compounds. The structure, content, and interactions of the DF components may change during the processing of grains for food applications.[6] The DF in cereal grains is concentrated in the bran fraction, that is, pericarp and aleurone layers of grain. The main part of DF of bran is insoluble in water, which causes both technological and nutritional challenges in food production.

The physiological effects of DF are dependent on the physicochemical properties, which are mainly influenced by particle size, cell wall architecture, solubility, degree of polymerization and substitution, distribution of side chains, and degree of cross-linking of the polymers. Water-insoluble DF is generally more resistant to colonic fermentation than soluble DF.[7] The suggested hypothesis that water-insoluble DF is the major contributor of the protective effects of whole grain type cereal foods emphasizes the importance of DF structure and the conversions of both carbohydrates and polyphenols in the large intestine.[4,8,9] There is also an increasing amount of evidence that the soluble cereal DF constituents can contribute to the observed health effects.[10] Van Craeyveld et al.[11] showed that the effect of water-soluble arabinoxylo-oligosaccharides on short-chain fatty acid (SCFA) production in rat feces depended on the average degree of polymerization—the preparations with low degree of polymerization increased colonic acetate and butyrate production, whereas the preparation with a higher degree of polymerization decreased the branched SCFA concentration.

In addition to the metabolism of carbohydrates, large intestinal fermentation of the nondigested plant material results

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in liberation and further microbial metabolism of the DF-associated phenolic compounds.[8,9,12] The delivery and microbial conversion of phenolic compounds have significance in the circulating metabolite pool present in human body,[12] which may in turn influence signal pathways and partly explain the beneficial health effects of plant foods in our body. DF delivers the phenolic compounds to the colon for release and conversion, and after the release of phenolic acids from DF, they are converted in the colon to phenylpropionic, phenylacetic acid, and benzoic acid (BA) metabolites.[8,9] The water solubility of the DF has a major effect also on the bioavailability of DF-associated phenolic compounds. In wheat bran, ferulic acid (FA) is the most abundant phenolic compound, most of which is covalently bound to the cell wall structures, and its bioaccessibility in physiological conditions is thus low.[13] Our previous studies[14] and also the work of Napolitano et al.[15] show that the bioavailability of FA acid can be significantly increased by partial hydrolysis of cereal bran and DF with fermentation and enzyme treatments. The reduction of particle size or dry fractionation of wheat bran into aleurone fraction also have been reported to increase the bioavailability of FA acid.[16]

The aim of the current work was to elucidate the grain-specific factors as well as the effect of fractionation of rye, wheat, and oat bran on the release and conversion of phenolic compounds and degradation of DF complex detected as SCFA formation and gas pressure evolution. The in vitro gut fermentation patterns of individual phenolic acids and SCFAs from the brans and their dry-processed fractions were reflected in the chemical and structural characteristics of the cereal samples.

MATERIALS AND METHODS

Bran Samples. Rye bran (code R4500), partly destarched with a bran finisher MLU 302 (Buhler, Uzwil, Switzerland), was supplied by Fazer Group (Lahti, Finland). Oat bran was a commercial product (Elovena Plus) supplied by Ravintoraisio Oy (Raisio, Finland). Regular coarse wheat bran was obtained from wheat grains (cultivar Tiger, white wheat, harvested in 2006 in Germany), by using a conventional roller milling process, carried out on a Test-mill in the Department of Safety & Quality of Cereals, in the Federal Research Centre for Nutrition and Food (MRI), Germany.

Dry Processing of Rye, Wheat, and Oat Bran. To produce the aleurone fraction of rye and wheat bran, micronization and electrostatic separation of rye and wheat bran particles were performed as described by Hemery et al.[17,18] Shortly, rye and wheat brans were first finely milled using an impact mill (Hosokawa-Alpine, type 100 UPZ, Augsburg, Germany), operated at ambient temperature at a speed of 18000 rpm, with a selection grid of 0.3 mm (the material was milled until it passed through the grid). Three successive grinding steps were carried out as described by Hemery et al.[17] Then, two (rye) and three (wheat) successive steps of electrostatic separation based on tribo-charging and subsequent separation in electric field were carried out using a pilot electrostatic separator (TEP System, Tribo Flow Separations, Lexington, United States).[18]

To separate starch, degrade cell wall structures, and concentrate subaleurone β-glucan of oat bran, the oat bran raw material was fractionated using consecutive milling and air classification techniques. Oat bran was first ground in Hosokawa Alpine 100 UPZ-Ib Fine impact mill with grinding track (Hosokawa Alpine AG, Augsburg, Germany) at a rotor speed of 15000 rpm. The ground material was then air classified using Minisplit Classifier (British Rema Manufacturing Co. Ltd., Chesterfield, United Kingdom). During the classification, the rotor speed was 3000 rpm, and the air flow was 220 $m^3\ h^{-1}$. The coarse fraction from the first air classification step was further milled at a rotor speed of 17700 rpm using the same mill with pin disk grinders. This ground coarse fraction was then again air classified with the same rotor speed and air flow rate (3000 rpm, 220 $m^3\ h^{-1}$). The coarse fraction from the second air classification step was milled using the same mill with a grinding track at anticlockwise rotation and rotor speed of 17700 rpm. This ground second coarse fraction was air classified the third time using the same parameters as previously to obtain the third fine and coarse fraction. To concentrate the β-glucan-rich cell walls from this third fine fraction, the sample was air-sieved using Alpine model A200 LS Air Jet Sieve (Alpine AG, Augsburg, Germany) with a sieve size of 40 μm. The coarse fraction was collected from the air sieving and used in the further work.

In Vitro Digestion and Colon Model. Prior to in vitro colon fermentation, the starch and protein contents of the cereal samples were reduced by in vitro enzymatic digestion mimicking the digestion in the upper intestine according to Aura et al.,[19] and the digestion products were removed by dialysis.[20] After the dialysis, the retentates were freeze-dried. The microbial degradation of cereal matrix and formation of SCFAs and gases from carbohydrates and release and conversion of phenolic acids were then studied in an in vitro colon model. In vitro fecal fermentation experiments were performed with the predigested brans and their fractions principally according to Barry et al.[21]

For measurement of SCFA and phenolic acid metabolites, 200 mg (on dry weight basis) of rye, wheat, or oat brans or their fractions was weighed in the fermentation bottles (50 mL) and hydrated with 4 mL of medium one day before inoculation. Human feces were collected from six healthy volunteers, who had not received antibiotics for at least 6 months and had given a written consent. The collection of fecal samples was performed with an approval of and according to the guidelines given by the Ethical Committee of Technical Research Centre of Finland. Freshly passed feces were immediately taken in an anerobic chamber or closed in a container with an oxygen-consuming pillow (Anaerocult Mini; Merck, Darmstadt, Germany) and a strip testing the anaerobiosis (Anaerotest; Merck, Darmstadt, Germany). The fecal suspension was prepared under strictly anaerobic condititions. Equal amounts of fecal samples were pooled and diluted to a 12.5% (w/v) suspension, 16 mL of which was dosed to the fermentation bottles to obtain a 10% (w/v) final fecal concentration described previously by Aura et al.[22] The fermentation experiments were performed in triplicate, and a time course of 0, 2, 4, 6, 8, and 24 h was followed using the same inoculum for all of the substrates. Incubation was performed at 37 °C in tightly closed bottles and in magnetic stirring (250 rpm). The fecal background was also incubated without the addition of the supplements.

Analysis of Fermentation Metabolites. SCFAs were analyzed by gas chromatography (GC) after diethylether extraction according to Aura et al.[22] SCFA formation was expressed as a sum of acetic, propionic, and butyric acids. The relative proportions of the individual SCFA were calculated from the averages of three replicates at a time point of 24 h. Phenolic acids were analyzed using a Leco Pegasus 4D GCxGC-TOFMS instrument, equipped with an Agilent GC 6890N from Agilent Technologies (United States) and a Combi PAL autosampler from CTC Analytics AG (Switzerland). The modulator, secondary oven, and time-of-flight mass spectrometer were from Leco Inc. (United States). The GC was operated in split mode with a 1:20 ratio. Helium with a constant pressure of 39.6 psig was used as the carrier gas. The first dimension GC column was a relatively nonpolar RTX-5 column, 10 m × 0.18 mm × 0.20 μm (Restek Corp., United States), coupled to a polar BPX-50 column, 1.50 m × 0.10 mm × 0.10 μm (SGE, Australia). The temperature program was as follows: initial temperature, 50 °C; 1 min → 310 °C; and 7 °C/min, 1 min. The secondary oven was set to +20 °C above the oven temperature. Inlet and transfer line temperatures were set to 260 °C. The second dimension separation time was set to 6 s. The mass range used was 45−800 amu, and the data collection speed was 100 spectra/s. The phenolic acids were quantitated with authentic standards. The following compounds were used as standards: BA, 3-hydroxybenzoic acid (3-OHBA), 3-(4′-hydroxyphenyl)-propionic acid (4-OHPPr), and 3-(3′,4′-dihydroxyphenyl)propionic acid (3,4-diOHPPr) were products from Aldrich (Steinheim, Germany). 4-Hydroxybenzoic acid (4-OHBA), 2-(3′-hydroxyphenyl)acetic acid (3-OHPAc), and 2-

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(3′,4′-dihydroxyphenyl)acetic acid (3,4-diOHPAc) were purchased from Sigma (St. Louis, MO). 3-Phenylpropionic acid (3-PPr) and 3,4-dihydroxybenzoic acid (3,4-diOHBA) were from Fluka (Buchs, Switzerland), and 3-(3′-hydroxyphenyl)propionic acid (3-OHPPr) was purchased from Alfa Aesar (Karlsruhe, Germany). 4-Methylcatechol (Aldrich), vanillic acid (3-methoxy-4-hydroxybenzoic acid; Fluka), 4-coumaric acid (p-CA) (Sigma), gallic acid (Extrasynthése, Genay, France), and FA (Sigma-Aldrich) and sinapic acid (SA) (Fluka) were used.. The internal standard was 2-hydroxycinnamic acid (mainly *trans*; Aldrich Inc., H2,280-9; 97%; St. Louis, MO). *N*-Methyl-*N*-trimethylsilyl-trifluoracetamide was from Pierce (Rockford, IL), and methoxyamine 2% hydrochloride in pyridine (Pierce) was used as the silylation reagent.

For the measurement of gas formation during fermentation, 100 mg (on dry weight basis) of cereal samples was weighed in headspace bottles (100 mL), hydrated with 2 mL of the medium one day before inoculation, and inoculated with 8 mL of fecal suspension as described above. Bottles were sealed with rubber stoppers and transported to the tempered room on ice. The system was placed in a room at 37 °C, where the bottles were in constant rotation of 150 rpm. The stoppers were punctured with an infusion needle connected to a pressure sensor (Honeywell Division Micro Switch 185 PC 30 DT) and linked to a computer (Dell System 325SX and 386 SX microprocessor) with measurement cards (Kethley MetraByte). The device recorded the gas pressure (bar) in 20 min intervals. The measurement was continued until 26 h from the start of the incubation. The data collection program was based on Test Point software.

The responses in the fermentation experiment were measured in triplicate, and two-way analysis of variance with repeated measures using a Bonferroni adjustment was used to test significancies ($p < 0.05$) between samples. The statistics were performed with a program and using MatLab Version R2008b. When the response differed significantly ($p < 0.05$) from the fecal control (no added substrate), it was indicated with a small letter. Significantly different response levels between cereal ingredients within a time point were indicated with different letters (a, b, c, ...).

Chemical Characterization of the Bran Samples. The DF content of brans and their fractions was analyzed by AOAC method 991.43,[23] with β-glucan using the Megazyme mixed-linkage β-glucan kit (Megazyme, Ireland)[24] and fructan using the Megazyme fructan assay kit (AOAC Method 999.03).[25] The AX content was analyzed by a gas chromatographic method as described by Blakeney et al.[26] with corresponding standard compounds and internal standard (myo-inositol). The protein content was analyzed using the Kjeldahl method,[27] fat was analyzed by the AOAC method 922.06,[28] starch was analyzed by the AOAC Method 996.11,[29] phenolic acids were analyzed by high-performance liquid chromatography as described by Mattila et al.,[30] and ash was analyzed gravimetrically as an inorganic residue after burning samples at 550 °C to remove water and organic material. The chemical analyses were performed in duplicate.

Structural and Physicochemical Analyses. Analysis of the particle size distribution of brans and their fractions was performed using LS Particle Size Analyzer (Beckman Coulter) using the liquid mode of the equipment (with ultra H_2O as liquid). Solubility and dissolving of samples were analyzed by the LS Particle Size Analyzer (Beckman Coulter) following the changes in the particle size distribution as function of time. The water absorption capacity was analyzed using the Bauman device.[31] The sample (25 mg) was weighted on a filter paper (grade 5, cut off over ⌀ 2.5 μM particles) after which the absorption of deionized water (mL) was followed for 60 min at ambient temperature.

Microscopy. Brans and their fractions were first embedded in 2% agar and then fixed in 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0). The samples were then washed, dehydrated in a series of ethanol solutions of increasing concentration, and embedded in hydroxyethyl methylacrylate for infiltration (Leica Historesin embedding kit, Heidelberg, Germany). Polymerized samples were sectioned (2 μm sections) in a rotary microtome HM 355 (Microm Laborgeräte GmbH, Walldorf, Germany) using a steel knife, and the sections were transferred onto glass slides. Protein was stained with aqueous 0.1% (w/v) Acid Fuchsin (BDH Chemicals Ltd., Poole, Dorset, United Kingdom) in 1.0% acetic acid, and β-glucan was stained with aqueous 0.01% (w/v) Calcofluor White (fluorescent brightener 28, Aldrich, Germany). In exciting light (epifluorescence, 400−410 nm; fluorescence, >455 nm), intact cell walls stained with Calcofluor appeared blue, and proteins stained with Acid Fuchsin appeared red. Starch was unstained and appeared black. The samples were examined with an Olympus BX-50 microscope (Olympus Corp., Tokyo, Japan). Micrographs were obtained using a PCO SensiCam CCD camera (PCO AG, Kelheim, Germany) and the CellP imaging software (Olympus).

RESULTS

In Vitro Fermentation Pattern. The behavior of rye bran, rye aleurone, wheat bran, wheat aleurone, oat bran, and oat cell wall concentrate were compared in the batch in vitro colon model, which uses a pooled microbial population as a source of conversion activity from the microbial enzymes for the degradation of fiber complex and conversion of microbial metabolites. The evolution of gas pressure showed significant ($p < 0.05$) differences from the fecal control from 4 to 6 h time points for all of the cereal samples (Figure 2). The rye bran, its aleurone, and the oat cell wall concentrate showed earlier significant gas pressure evolution than the rest of the fractions. At 6 h, the wheat and oat bran and the wheat aleurone fraction showed significantly ($p < 0.05$) lower gas pressure than the rye aleurone, indicating lower gas formation rate. In general, rye samples showed higher gas evolution rates and extents than the wheat samples, the oat samples being at the intermediary level. Dry fractionation enhanced gas evolution in the colon model regardless of cereal grain.

When the time course of total SCFA was analyzed, components of rye bran and especially its aleurone fraction were fermented at the highest rate and to the largest extent in 24 h (Table 3). The slowest fermentation was exhibited with wheat bran and its aleurone and oat bran. When the relative proportions of the three individual SCFA were investigated (Table 4), the relative proportion of propionic acid was most pronounced with oat bran (6−24 h) and its fraction, followed by rye aleurone and rye bran, whereas wheat aleurone, followed by rye bran and its aleurone, showed the highest relative proportions of butyric acid (8 and 24 h). Again, the dry processing enhanced SCFA responses regardless of the grain.

The formation of phenolic microbial metabolites was monitored during the in vitro fermentation, and the cereals and their fractions were compared in relation to their total and individual phenolic metabolite formations displayed in Table 5. The total phenolic metabolites were calculated as a sum of phenylpropionic acid, phenylacetic acid, and BA derivatives, including plant lignans, enterolactone, and enterodiol, as minor metabolites. Significant total phenolic metabolite formation was exhibited only for wheat bran and its aleurone at 6, 8, and 24 h time points and for oat cell wall concentrate at 6 h time point. Dry fractionation enhanced the formation of total phenolic microbial metabolites as the sum in wheat samples but not in rye.

When individual phenolic metabolites were observed, 3-hydroxyphenylpropionic acid was formed significantly already after 2 h of incubation from all of the cereal samples, and dry fractionation enhanced its formation from both rye and wheat grains. However, the metabolite disappeared in 24 h from wheat aleurone incubation, indicating further metabolism. Wheat bran and wheat aleurone showed the highest extents of the formation of 3-PPr. A high background of 3-PPr in the

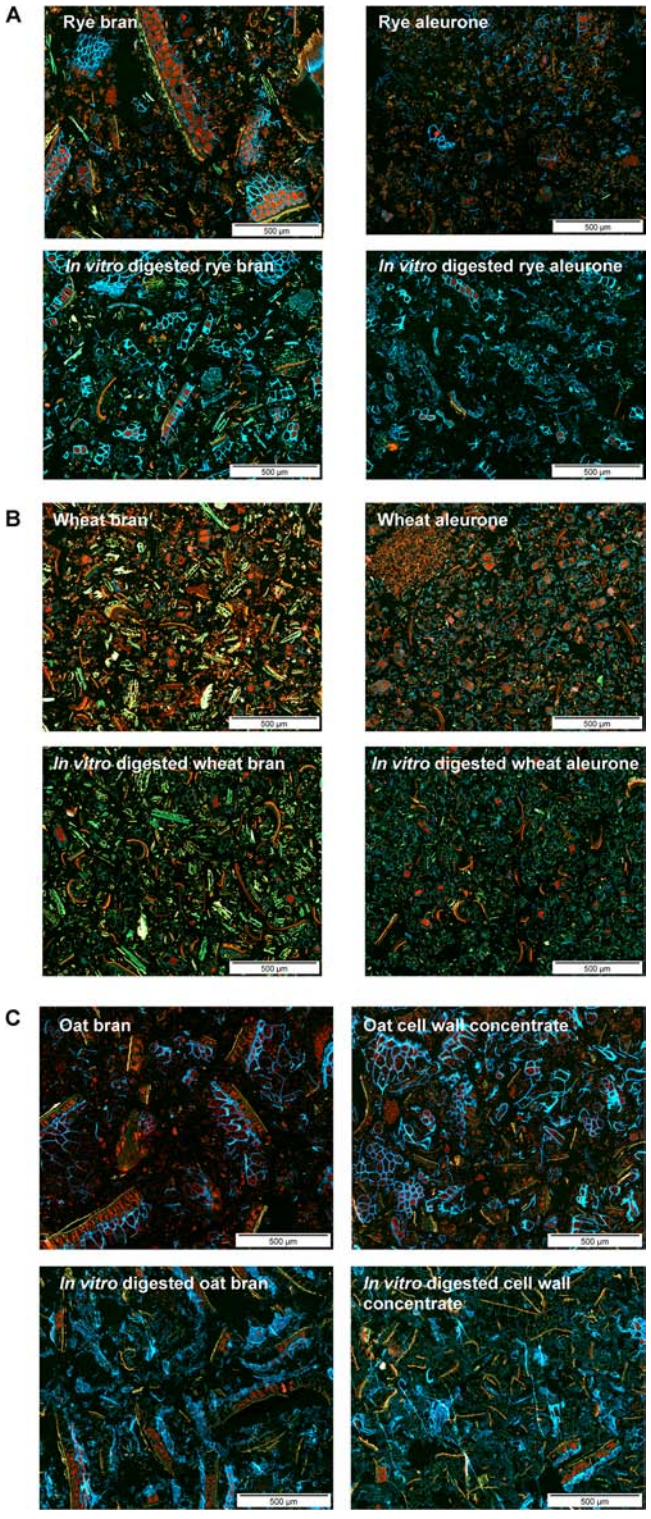

Figure 1. Microstructure of rye (A), wheat (B), and oat (C) bran samples and their dry-processed fractions before (raw material) and after enzymatic in vitro digestion process (in vitro digested). β-Glucan-rich endosperm and aleurone cell walls are stained in blue, the pigment strand (between pericarp and aleurone layer) is stained in orange, the pericarp layer is stained in light green and yellowish, and proteins are stained in red and reddish brown.

controls suggests that other precursors are present in the faecal inoculum, most likely originating from the diet of the fecal donors.

The following most abundant, intermediary, but significant metabolite was BA, the formation of which showed neither grain nor process specific enhancement. The other minor metabolites, differing significantly from fecal control, were 3-OHBA and 3,4-diOHPAc, which did not show remarkable cereal- or process-specific differences due to their low concentrations, and mammalian lignan enterodiol, whose formation in contrast was slightly suppressed by the dry fractionation from rye and wheat samples (8 h time point; Table 5). The other mammalian lignin, enterolactone, was formed in all of the samples, including the control; however, the highest formations were observed from the controls (results not shown).

The fermentation rate of carbohydrates was reflected by the pH values of the colon model samples. The pH values of the suspensions with cereal substrates decreased more rapidly than those of the control samples, showing significant differences already after 2 h of incubation. The most rapid pH decrease was in samples containing rye aleurone (range 6.97−6.17), followed by rye bran (range 6.95−6.25), which was in accordance with their higher SCFA production. The slowest decrease of pH was in wheat (7.03−6.34) and oat brans (7.01−6.36), whereas their fractions showed intermediary pH decrease rates, again reflecting their SCFA productions. However, wheat aleurone showed the lowest final pH (range 6.98−6.04), possibly reflecting released and converted phenolic acids, whereas the oat cell wall concentrate (range 6.98−6.30) showed the same final level as wheat and oat brans. Controls were in the range of 6.99−6.67.

Chemical Composition. Chemical characterization of the different bran preparations was performed before and after the enzymatic in vitro digestion (Table 1), and in general, the chemical composition of the bran samples was in accordance with the previous data.[20,32] The DF content was clearly highest in wheat bran (61.2%) and aleurone (51.8%) as compared to that in the oat (14.9−28.9%) and rye (37.4−21.0%) bran preparations. As compared to the original bran samples, the relative content of water-extractable DF was higher in all processed fractions, and the content was highest in the oat cell wall concentrate (11.6%). The fructan content was highest in rye (5.9% in bran and 4.8% in aleurone) and lowest in the oat samples (0.4−0.8%). Clearly, the highest contents of β-glucan were analyzed in oat samples (7.6% in bran and 15.1% in fraction), and the wheat bran had the lowest content of β-glucan (2.6%). The highest content of water-unextractable AX was in wheat (30.9% in bran and 42.4% in aleurone) and lowest in oat bran (5.5%) and rye aleurone (7.3%), whereas the rye samples had the highest content of water-extractable AX (1.9% in bran and 1.8% in aleurone). Wheat samples had the highest content of phenolic acids, due to the high content of FA (5310 μg/g in bran and 7867 μg/g in aleurone). However, the SA content of rye bran (429 μg/g) was similar to that of wheat bran (418 μg/g). Oat bran clearly had the lowest content of the analyzed phenolic acids (Table 1).

Enzymatic in vitro digestion was performed to reduce the amount of starch and protein and thus to concentrate the DF content of the samples prior to the in vitro colon model (Table 1). The starch content decreased by the enzymatic digestion; however, especially in rye aleurone (18.6%) and also in oat bran (9.9%), the residual starch content was still relatively high. The distinctive DF characteristics as observed for the initial bran samples also remained after the digestion step; for example, the wheat samples were most concentrated in insoluble DF (68.6−

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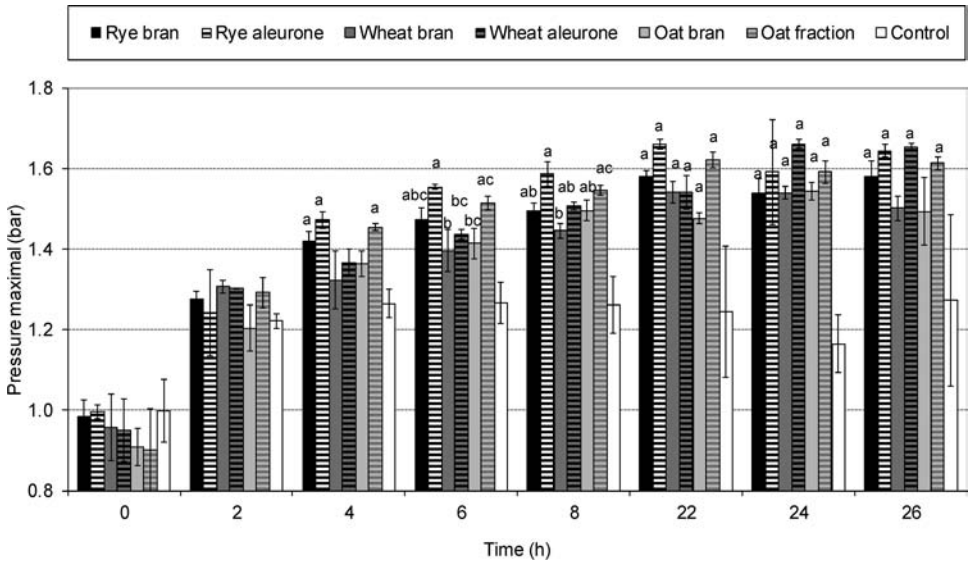

Figure 2. Time course of gas pressure (measured as maximal pressure, bars) developed by human fecal microbiota from rye, wheat, and oat bran samples and their dry-processed fractions. Letters indicate a significant difference ($p < 0.05$) from fecal control (FBL) and different letters (a, b, c) indicate significant differences between brans and fractions within a time point.

64.8%), oat samples in β-glucan (12.6−19.7%), and rye samples in fructan (4.8%). The fructan content of wheat samples decreased from 3.2 to 0.7% in bran and from 4.5 to 1.3% in aleurone, whereas in rye and oat bran, the change in the fructan content by the digestion step was small. The content of ferulic and *p*-coumaric acids increased by the digestion process, whereas the sinapic acid content clearly decreased in rye and wheat samples and remained stable in oat samples (Table 1).

Particle Size and Water Absorption Capability. The aleurone fractions had smaller average particle size (49 μm in rye and 46 μm in wheat) than the corresponding bran samples (100 μm in rye and 58 μm in wheat). The processed oat cell wall concentrate (127 μm) on the contrary had a larger average particle size than the original oat bran (78 μm) (Table 2). The particle size of all samples increased in the in vitro enzymatic digestion of starch and proteins (Table 2). The oat cell wall concentrate clearly had the highest water absorption capability, followed by the rye and oat bran, whereas the wheat bran samples absorbed the least water (Table 2). The water absorption capability of the bran materials was changed by the in vitro digestion. After the digestion, the capability of the samples to absorb water increased especially with the wheat samples (Table 2), and in fact, the wheat samples absorbed water the most efficiently among the samples after the digestion process. DF has a high water absorption capacity,[33] and thus, it was likely that the water binding of samples increases according to the concentration of the DF in the samples after the in vitro digestion step. However, the influence of water absorption rate on fermentation rate in the colon model was eliminated by early hydration of the samples.

Microstructure. The microstructure of the bran samples and fractions was analyzed before and after in vitro digestion. In contrast to native brans, the rye and wheat aleurones contained none or minor amounts of pericarp layer (Figure 1A,B). In accordance with the particle size analysis (Table 2), the average size of the particles in the rye and wheat aleurone fractions was clearly smaller than that of bran samples. As compared to the oat bran consisting of pericarp, aleurone, and subaleurone, the oat cell wall concentrate contained mostly large particles of subaleurone layer (Figure 1C). Even though in vitro digestion of the samples resulted in a decrease in reddish/brown color in the micrographs, indicating hydrolysis of proteins, the presence of proteins was still detectable in some of the aleurone cells. In the rye and wheat samples, the in vitro digestion did not remarkably influence the cell wall structures, although in the rye bran sample, some disintegration of the pericarp from aleurone layer was detected (Figure 1A,B). In the oat samples, the impact of the in vitro digestion was more evident: the subaleurone cells had collapsed. The cell walls were still linked to each other, but the structure appeared as smeared because of unformed particles (Figure 1C).

DISCUSSION

The aim of the present work was to elucidate factors affecting microbial metabolite formation (phenolic acid metabolites and SCFA) in the in vitro colon model from wheat, rye, and oat brans and their corresponding fractions by focusing on their physicochemical parameters and microstructure. Previously, Karppinen et al.[20] showed that brans of wheat, oat, and rye have grain-specific differences in the fermentation in colon simulation, whereas Harris et al.[34] and Amrein et al.[35] reported that when comparing wheat bran and aleurone, the aleurone fraction shows a higher degree of degradation in colonic environment. However, these studies concentrated on the catabolism of DF detected only as formation of SCFAs. To our knowledge, the present work is the first report to compare brans from different grains with respect to the concurrent conversions in an in vitro colon model of phenolic compounds and carbohydrates from the DF complex. The batch in vitro colon model applied in the present study uses a pooled microbial population as a source of conversion activity from the microbial enzymes for degradation of fiber complex and conversion of microbial metabolites. The microbial composition is constant during the incubation and alive only for one life cycle for the conversion. During the incubation, degradation of DF complex and release and conversion of phenolic compounds can occur at a rate that is determined by factors related to the substrates only. For these reasons, the design of

Table 1. Chemical Composition (% of DM) of Rye, Wheat, and Oat Brans and Bran Fractions before and after the Enzymatic in Vitro Digestion Process

	rye bran	digested rye bran	rye aleurone	digested rye aleurone	wheat bran	digested wheat bran	wheat aleurone	digested wheat aleurone	oat bran	digested oat bran	oat cell wall concentrate	digested oat cell wall concentrate
						composition (% DM)						
starch	34.0 ± 0.4	6.1 ± 0.1	56.2 ± 0.9	19.3 ± 0.0	6.2 ± 0.1	0.7 ± 0.4	11.3 ± 0.4	1.5 ± 0.1	49.9 ± 0.4	10.2 ± 0.3	25.9 ± 0.1	5.1 ± 0.6
protein	17.6 ± 0.1	17.7 ± 0.0	17.6 ± 0.2	17.0 ± 0.0	16.6 ± 0.0	17.3 ± 0.0	21.3 ± 0.1	21.2 ± 0.1	18.1 ± 0.0	17.8 ± 0.2	22.0 ± 0.2	22.4 ± 0.3
fat	3.7 ± 0.0	6.6 ± 0.2	2.3 ± 0.1	5.8 ± 0.2	4.4 ± 0.1	6.2 ± 0.4	4.4 ± 0.1	7.1 ± 0.1	8.0 ± 0.0	13.5 ± 0.1	10.4 ± 0.2	12.1 ± 0.4
ash	3.9 ± 0.0	3.7 ± 0.0	2.3 ± 0.0	4.2 ± 0.0	7.3 ± 0.0	6.1 ± 0.0	6.9 ± 0.0	5.3 ± 0.3	3.0 ± 0.0	6.0 ± 0.0	4.4 ± 0.0	6.2 ± 0.0
total DF[a]	37.4 ± 0.3	57.4 ± 0.4	21.0 ± 0.3	41.7 ± 1.9	61.2 ± 0.7	68.6 ± 0.5	51.8 ± 0.4	64.8 ± 1.3	14.9 ± 1.2	33.8 ± 0.9	28.9 ± 1.5	36.3 ± 0.3
soluble DF	5.3 ± 0.1	9.7 ± 0.4	6.1 ± 0.2	11.8 ± 0.7	4.3 ± 0.3	5.7 ± 0.1	6.4 ± 0.2	9.5 ± 1.9	4.5 ± 0.8	14.4 ± 0.4	11.6 ± 1.4	14.1 ± 0.2
total AX	15.5 ± 0.9	33.7 ± 0.1	9.1 ± 0.1	21.4 ± 0.0	31.8 ± 0.0	44.3 ± 0.2	30.1 ± 0.6	48.8 ± 0.7	5.8 ± 0.1	9.1 ± 0.2	10.7 ± 0.1	14.0 ± 0.2
soluble AX	1.9 ± 0.1	5.3 ± 0.0	1.8 ± 0.1	6.2 ± 1.0	0.9 ± 0.0	1.9 ± 0.2	1.5 ± 0.0	3.0 ± 0.1	0.3 ± 0.0	1.3 ± 0.2	0.6 ± 0.0	1.4 ± 0.3
β-glucan	3.7 ± 0.1	5.5 ± 0.0	3.0 ± 0.1	5.4 ± 0.0	2.6 ± 0.0	2.8 ± 0.0	5.8 ± 0.0	7.3 ± 0.0	7.6 ± 0.9	12.6 ± 0.0	15.1 ± 0.1	19.7 ± 0.2
fructan	5.9 ± 0.1	4.8 ± 0.0	4.8 ± 0.1	4.8 ± 0.1	3.4 ± 0.1	0.7 ± 0.0	5.0 ± 0.2	1.4 ± 0.0	0.4 ± 0.0	0.6 ± 0.0	0.8 ± 0.0	0.8 ± 0.0
						phenolic acids (μg/g)						
total FA	2426 ± 33	3705 ± 104	870 ± 24	1370 ± 18	5310 ± 95	5786 ± 107	7867 ± 72	9098 ± 197	552 ± 68	809 ± 29	1021 ± 26	1507 ± 412
free FA	10 ± 0	8 ± 1	5 ± 0	3 ± 3	13 ± 1	2 ± 1	11 ± 0	3 ± 0	4 ± 0	ND	6 ± 0	ND
total *p*-CA	82 ± 1	120 ± 4	19 ± 0	30 ± 1	161 ± 4	175 ± 0	252 ± 1	327 ± 5	16 ± 1	19 ± 0	21 ± 1	23 ± 6
free *p*-CA	ND[b]	ND	ND	ND	ND	ND	1 ± 0	ND	ND	ND	1 ± 0	ND
total SA	429 ± 1	248 ± 12	213 ± 2	97 ± 7	418 ± 14	204 ± 1	276 ± 8	191 ± 1	123 ± 20	139 ± 5	207 ± 3	201 ± 53
free SA	7 ± 0	2 ± 0	4 ± 0	ND	3 ± 0	ND	ND	ND	4 ± 0	ND	4 ± 0	ND

[a]Includes fructans. [b]Not detected.

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Table 2. Mean Particle Size (μm) and Water Absorption (μL) as a Function of Time (after 1, 10, and 60 min) of Rye, Wheat, and Oat Bran Samples and Bran Fractions before and after the Enzymatic in Vitro Digestion Process

sample	rye bran	digested rye bran	rye aleurone	digested rye aleurone	wheat bran	digested wheat bran	wheat aleurone	digested wheat aleurone	oat bran	digested oat bran	oat cell wall concentrate	digested oat cell wall concentrate
mean particle size (μm)	100 ± 15	128 ± 8	49 ± 3	107 ± 10	58 ± 2	65 ± 1	46 ± 1	59 ± 3	78 ± 9	450 ± 43	127 ± 9	225 ± 1
water absorption (μL)												
after 1 min	42 ± 8	61 ± 0	22 ± 3	30 ± 2	11 ± 0	82 ± 0	11 ± 0	102 ± 4	45 ± 1	39 ± 2	74 ± 3	87 ± 1
after 10 min	54 ± 9	88 ± 2	31 ± 4	56 ± 2	23 ± 1	92 ± 2	22 ± 2	112 ± 2	52 ± 3	64 ± 1	84 ± 2	109 ± 1
after 60 min	57 ± 4	97 ± 1	42 ± 3	90 ± 0	40 ± 2	99 ± 4	37 ± 6	122 ± 2	58 ± 4	78 ± 3	93 ± 1	122 ± 2

Table 3. Production of SCFAs (mM) from Rye, Wheat, and Oat Bran Samples and Their Dry-Processed Fractions during the in Vitro Fermentation (0–24 h)[a]

time (h)	rye bran	rye aleurone	wheat bran	wheat aleurone	oat bran	oat cell wall concentrate	fecal control
			total SCFAs (mM)				
0	13	13	12	13	11	12	12
2	41 a	47 b	31 cd	36 d	31 c	35 cd	22
4	54 a	72 b	42 c	49 d	48 d	57 ae	25
6	66 a	81 b	48 c	59 d	57 d	59 d	26
8	76 a	89 b	53 c	68 ad	59 cd	68 ad	29
24	92 a	104 b	69 c	89 a	73 c	80 d	36
			acetic acid (mM)				
0	8	8	8	8	7	8	8
2	27 a	33 b	19 c	23 d	20 c	23 cd	14
4	35 a	49 b	26 c	30 d	29 cd	35 a	17
6	41 a	51 b	30 c	36 d	33 d	36 d	17
8	47 a	57 b	31 c	40 d	35 cd	40 d	19
24	57 a	66 b	39 c	48 d	42 c	48 d	23
			propionic acid (mM)				
0	2	2	2	2	2	2	2
2	7 ad	8 b	6 c	7 cd	8 ab	8 b	4
4	9 a	13 b	8 c	9 ac	14 b	13 b	4
6	13 a	16 b	10 c	12 a	16 b	16 b	5
8	14 ac	15 ad	10 b	12 bc	17 d	15 ad	5
24	19 ab	20 ab	15 b	21 a	21 a	18 ab	7
			butyric acid (mM)				
0	2	2	2	2	2	2	2
2	7 a	6 abd	6 bc	7 ab	4	5	4
4	10 a	10 a	8 b	10 a	6	9 ab	4
6	12 ac	14 a	8 b	11 c	7 b	8 b	4
8	15 a	16 a	12 b	16 a	8 c	12 b	5
24	16 a	17 a	14 b	20 c	10 d	14 b	6

[a]Total SCFAs comprise the sum of acetic acid, propionic acid, and butyric acid. The letters after the numerical value indicate a significant difference ($p < 0.05$) from fecal control, and different letters indicate significant differences between brans and fractions within a time point.

the colon model is particularly suitable for comparisons related to the effect of processing of solid cereal ingredients on the prediction of colon fermentation parameters. Thus, conversion patterns reflect the characteristics of the rye, wheat, and oat brans and their fractions prepared with dry fractionation.

Table 4. Relative Proportions of the Individual SCFA, Acetic, Propionic, and Butyric Acids, during the in Vitro Fermentation (0–24 h), Indicated as Percentages (%) from the Total SCFA

time (h)	rye bran	rye aleurone	wheat bran	wheat aleurone	oat bran	oat cell wall concentrate	fecal control
			acetic acid (%)				
0	65	65	66	66	65	65	65
2	66	70	62	64	62	64	64
4	65	68	63	61	60	61	65
6	62	63	62	61	58	60	65
8	62	64	58	59	58	59	65
24	62	64	57	54	58	60	65
			propionic acid (%)				
0	16	16	16	16	16	16	16
2	17	17	19	18	24	23	18
4	17	18	19	18	28	23	17
6	20	20	21	21	29	26	18
8	18	17	20	18	28	23	18
24	20	20	23	24	29	22	19
			butyric acid (%)				
0	18	19	18	18	19	19	19
2	17	13	18	18	13	14	18
4	18	14	18	20	12	16	17
6	18	17	17	19	13	14	17
8	20	19	22	23	14	18	17
24	18	17	20	22	13	18	16

Impact of Fractionation of Brans on the Fermentation Pattern. In general, the fractionation of the brans to the rye and wheat aleurone and to the oat cell wall concentrate increased the formation of the carbohydrate and phenolic metabolites in the colon model with one exception. In the case of rye, the dilution of phenolic compounds by starch due to the fractionation process resulted in the lower formation of phenolic metabolites from the rye aleurone than from the rye bran. Conversion of total phenolic metabolites was most pronounced in wheat aleurone followed by wheat bran. In addition to the clearly highest content of phenolic acids, the smallest particle size among all of the samples after the enzymatic digestion prior to colon model can have contributed to the easier release and conversion of phenolic acids from the wheat aleurone fraction. Reduction of particle size or dry fractionation of wheat bran into aleurone fraction has also been reported earlier to increase the bioavailability of FA.[16] Hemery et al.[16] demonstrated how the particle size of the dry fractionated wheat bran was able to increase the bioaccessibility

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Table 5. Formation of Total and Individual Phenolic Microbial Metabolites (μM) during the in Vitro Fermentation (0–24 h)[a]

time (h)	rye bran	rye aleurone	wheat bran	wheat aleurone	oat bran	oat cell wall concentrate	fecal control
				total phenolic metabolites (μM)			
0	201.5	168.6	174.4	188.1	191.4	172.5	166.8
2	221.9	216.6	220.2	220.5	207.0	238.6	223.8
4	226.8	230.2	249.8	289.5	220.2	246.7	226.7
6	228.6	209.8	324.9 a	325.4 a	225.5	299.3 a	236.5
8	252.5	225.6	358.9 a	411.8 a	260.9	245.0	249.2
24	296.4	271.6	376.3 a	502.3 b	267.7	273.5	292.6
				3-OHPPr (μM)			
0	50.3	49.5	52.8	50.8	59.8	47.8	47.2
2	40.7 a	58.8 b	52.4 ab	56.0 b	55.9 b	57.2 b	22.7
4	42.4 a	60.7 ab	51.0 ab	72.8 b	59.2 ab	53.2 ab	8.6
6	37.1 a	48.3 b	55.4 c	64.2 d	50.6 bc	52.0 bc	5.9
8	52.0 ad	52.3 ad	65.5 ab	120.7 c	61.2 ab	38.3 d	5.9
24	22.4	43.0 a	14.1	42.6 a	39.9 a	22.9	8.5
				3-PPr (μM)			
0	101	81	80	83	96	91	90
2	133	109	125	118	106	128	169
4	131	119	151	159	111	145	187
6	142	120	217	198	128	191	197
8	150	130	246	239	150	163	206
24	211	173	314a	413b	174	184	246
				BA			
0	6.6 a	6.3 a	5.5	5.5	6.5 a	6.8 a	5.4
2	11.0 ac	13.2 c	9.5	9.4	10.4 a	12.5 ac	7.1
4	12.2 a	13.4 a	10.8 a	11.7 a	12.0 a	13.4 a	6.9
6	12.2 a	11.8 a	11.6 a	11.1 a	11.4 a	16.1 b	7.1
8	11.4	12.3 a	9.8	10.2	13.6 a	12.9 a	8.1
24	14.4 a	16.3 a	12.7	13.3 a	15.6 a	16.3 a	9.1
				3-OHBA (μM)			
0	1.1 a	1.0	0.9	0.9	1.0	1.0	0.9
2	2.7 a	2.1 ac	1.7 bc	1.7 bc	2.2 ac	3.3 d	0.8
4	2.7 ac	2.4 ab	1.7 b	1.7 b	2.5 ac	3.3 c	0.8
6	1.8 ab	1.7 b	1.6 b	1.7 b	2.1 a	2.7 d	0.7
8	1.3 a	1.1	1.4 a	1.4 a	1.4 a	1.6 a	0.7
24	0.9 a	0.7	1.3 bc	0.9 ac	1.6 b	1.0 ac	0.3
				3,4-diOHPAc (μM)			
0	2.8	2.5	2.6	2.6	3.0	2.7	2.7
2	3.3 a	3.9 a	3.2 a	3.1	3.4 a	3.9 a	2.5
4	3.7 a	4.0 a	3.6 a	3.8 a	3.7 a	4.2 a	1.9
6	3.4 a	3.6 a	3.6 a	3.6 a	3.8 a	4.5 b	1.4
8	4.3 ab	4.2 ab	3.5 ab	4.0 a	4.7 b	4.4 ab	1.1
24	3.8 a	4.1 a	3.7 a	3.7 a	4.7 b	4.0 a	0.8
				3-OHPAc (μM)			
0	6.8	6.3	6.2	6.1	7.1	6.4	6.4
2	7.9	8.5	7.4	7.3	7.0	8.1	7.9
4	8.0	7.9	7.3	7.5	7.2	8.1	8.8
6	7.7 a	7.5 a	7.9 a	7.7 a	7.3 a	9.1	10.1
8	8.0 a	7.5 a	7.1 a	7.2 a	8.4 a	7.2 a	11.4
24	7.5 a	8.2 ab	9.2 b	7.7 a	8.0 a	7.7 a	12.1
				enterodiol (μM)			
0	0.37 a	0.34	0.26	0.33	0.40 a	0.40 a	0.24
2	0.63	0.49	0.58	0.49	0.50	0.53	0.56
4	0.66	0.60	1.38 a	0.73	0.56	0.74	0.69
6	1.05	0.72	2.19 a	1.14	0.69	0.74	0.55
8	1.57 a	1.02 b	2.16 c	1.69 a	0.80 b	0.98 b	0.20
24	1.65 a	1.03 bc	0.74 c	1.41 ab	0.88 bc	1.23 abc	0.09

[a]A letter after the numerical value indicates significant differences ($p < 0.05$) between the sample and the fecal control, and different letters indicate significant ($p < 0.05$) differences between cereal samples within a time point.

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of phenolic acids from breads rich in dry-fractionated wheat bran in the gastrointestinal model TIM-1 mimicking the upper intestine. In the present study, the major phenolic colonic metabolites from all of the bran preparations were phenylpropionic acids, 3-OHPPr, and 3-PPr, which corresponds to the in vitro and in vivo studies of Mateo Anson et al.,[14,36] in which the bioaccessibility of FA, *p*-CA, and SA and conversion of the acids were enhanced by enzymatic and fermentation processes of wheat bran.

Fractionation increased the fermentability of the bran samples but did not affect the relative proportion of the SCFA, suggesting that the SCFA ratios are grain-specific and more dependent on the carbohydrate composition than on the physical characteristics of the DF. Fractionation of the brans increased the proportion of water-extractable DF and decreased the mean particle size in the case of rye and wheat. As indicated by the microscopic analysis and similarly to the work of Hemery et al.,[18] the higher proportion of water-extractable DF, especially in rye and wheat fractions, was due to the removal of water-unextractable pericarp. The reduced particle size and disintegration of the cell wall structures result in an increased surface area of the bran particles and are thus also relevant factors in explaining the differences in the fermentation rates of the different bran preparations in the current study. Microbes have a larger area to adhere and degrade the fiber matrix, which causes the release of monosaccharides and phenolic acids, which, finally, are converted to SCFA, gases, and microbial phenolic acid metabolites, respectively. According to Guillon and Champ,[6] small particle size and increased surface area increase the fermentation rate of DF, and also when different sized wheat bran particles were compared, small particles had higher SCFA production in vitro.[37]

Grain-Specific Factors Affecting the Fermentation Pattern. The fermentation rate and extent of the rye, wheat, and oat bran were dependent more on water-extractable DF content rather than the total DF content of the digested cereal samples. Rye samples caused the most rapidly evolving gas and SCFA production, which were most probably related to the high content of fructan and water-extractable AX.[38,39] Water-extractable AX and fructo oligosaccharides are known to be rapidly fermentable by gut microbiota.[11,20,40] Oat bran and its fraction possessed the highest proportion of water-extractable DF, mainly β-glucan, but the high viscosity caused by the high β-glucan content of oat may have retarded the fermentation rate of oat samples. Indeed, the oat samples were found to form large amorphous/viscous like structures after the in vitro digestion, as detected by microscopy and also by the particle size analysis. Although the reason for this disintegration of the aleurone and subaleurone cell wall structures of oat samples is not clear, the phenomenon could be related to the high concentration of β-glucan in oat cell walls. The higher water extractability of β-glucan, as compared to for example AX, might have caused the disintegration of the aleurone cells during the digestion process. There are also structural differences in the β-glucans of the different grains, which affect the water solubility of the polymers.[41,42] The polymer structure (the molar ratio of cellotriose to cellotetraose units, DP3:DP4) has been argued to increase the solubility of oat as compared to wheat and rye.[42] These specific characteristic of oat could partly explain the characteristics detected after the in vitro digestion and could also relate to lower fermentation rate of the oat samples than expected based on the chemical composition. However, the influence of the oat bran particle size and the changes in the bran structure by the in vitro digestion on the fermentation pattern should be proved by future studies.

The particle size of wheat bran preparations after the in vitro digestion step was clearly smallest when compared to oat and rye, but the wheat samples were fermented at the lowest rate and extent, indicating a small impact of particle size on the fermentability. Hence, a high proportion of water-unextractable DF, which was clearly highest in wheat, is proposed as a more important factor for the fermentation rate when different bran preparations were compared. The removal of fructans during the dialysis after the in vitro digestion presumably also decreased the fermentability of the wheat samples. The size of the wheat fructans is smaller than those of rye,[43] and thus, wheat fructans may have been removed by the dialysis step of the in vitro digestion. On the other hand, rye and oat samples, but not wheat, were found to form a gel when they were hydrated before the colon model (data not shown), suggesting high viscosity also in the in vitro digestion process, which might have hindered the removal of rye and oat fructans during the dialysis step. High molecular weight water-soluble AXs and β-glucans are known to form high viscosity or gels in water solution,[44,45] and the rye and oat samples had a relatively high content of water-extractable AX and β-glucan. As compared to wheat, water-extractable AX of rye has also been shown to have a higher degree of polymerization, thus producing more viscous solutions in water.[46]

Formation of Individual Microbial Metabolites, Phenolic Acids, and SCFA. The formation patterns of specific phenolic metabolites were affected by the origin of the DF matrix. Wheat bran preparations were most concentrated in FA, thus explaining the highest formation of the hydroxylated phenylpropionic acids. The phenylpropionic acid metabolites have already previously been proposed by Mateo Anson et al.[36] to be connected to FA. FA is the major phenolic acid of grains, and it is mostly linked to the *O*-5 position of the arabinofuranose substituents in the AX and may also be cross-linked as diferulates by ester and ether bonds to AX and lignin. The in vitro digestion removed most of the free FA from the brans and equalized the free FA content among the samples. Thus, the bound FA acid must have been released and metabolized during the fermentation, since the wheat bran samples were clearly highest in the phenolic acid metabolites.

BA derivatives were the second highest metabolite group that increased during the in vitro incubation of the bran samples in the in vitro colon model. Oat and rye bran samples showed the highest formation of the BA derivatives. BAs can be derived from various compounds present in grains. BAs can be formed directly from vanillic acid (3-hydroxy-4-methoxybenzoic acid) or 3,4-dimethoxybenzoic acid by demethylation to form 3-OHBA or BA, respectively. The metabolism of 3-OHBA, the concentration of which was decreased during the incubation, can be continued by dehydroxylation to form BA. BAs can also be derived from the phenylpropionic acids or -acetic acids by β- or α-oxidation as has been shown for caffeic acids,[47] or they can be formed from 3,5-dihydroxybenzoic acid released from alkylresorcinols, as reported earlier.[48,49] Alkylresorcinol content is known to be high in rye but low in oats;[50] thus, the alkylresorcinols cannot explain the elevated BA metabolite formation in the case of oat.

In addition to phenylpropionic acids and BAs, 3,4-diOHPAc was formed in significantly higher concentrations than in fecal control, even though 3-OHPAc remained significantly below the control. The phenylacetic acids are known mainly as

flavonol metabolites[12] but reflect also the metabolism of oxygen-linked diferulates as reported by Braune et al.[51] Braune et al.[51] showed that, for example, the 8-*O*-4-derivative of diferulate degraded to transiently appearing monomeric FA acid and further to 3-(4-hydroxy-3-methoxy)phenylpyruvic acid, 3-(3,4-dihydroxyphenyl)propionic acid, and via caffeic acid to 3,4-diOHPAc.

Enterodiol, formed from lignans, was derived to a low degree in this experiment, and enterolactone concentrations in the presence of cereal samples were below fecal controls in the current study. The low inoculum concentration used was not favorable for enterolactone formation, because enterolactone-converting microbiota is a minor population,[52] and the population might not have been strong enough for the slow conversion, as earlier reported by Aura et al.[22] This is also supported by Clavel et al.,[52] who showed that enterodiol is converted by 2000-fold larger population of microbiota than enterolactone-producing population. However, the highest maximum at 8 h and disappearance of enterodiol at 24 h time point in the wheat bran incubation indicate further metabolism. Extractable cell walls or water-soluble, phenolic fraction has been shown to suppress enterolactone formation,[22,53] whereas rigid nonextractable rye bran residue (after the removal of extractable cell walls)[53] was able to exhibit the formation of mammalian lignans, indicating that slow degradation of DF matrix and a slow decrease of pH favors their formation. It was also apparent in the study of Aura et al.[53] that rye itself was not an inhibitory factor, but the design of the in vitro batch model enables product-induced inhibition, when acidic compounds are not removed, as occurs in the intestine in vivo.

The formation of the individual SCFA is connected to carbohydrate content in the cereal samples. When specific SCFA formation was compared, the relative proportion of acetic acid varied between 54 and 65% in samples and fecal control, and acetate showed the same trend as the total SCFA formation. Thus, the focus was on differences in the propionic acid and butyric acid production between the in vitro-digested DF residues. Propionic acid formation reached its maximum earlier than butyric acid, and its formation was most pronounced with oat bran and oat cell wall concentrate. High and early propionic acid production in oat samples can be explained by the high content of β-glucan in oat samples, which is in accordance with Karppinen et al.[20] who compared oat bran results with those from rye and wheat bran. Kedia et al.[54] showed higher in vitro propionic acid production from oat bran than from fructo-oligosaccharides, glucose, or oat flour. Kim and White[55] showed significant propionic acid formation capacity of β-glucan from oat independent of the molecular weight of β-glucan. In the present wok, significant differences in the propionate production were not found between the oat bran and its cell wall concentrate with disintegrated cell walls; instead, the production of butyric acid was higher from oat bran than from the oat cell wall concentrate.

Rye and wheat brans and their aleurone fractions showed the high relative proportions of butyric acid. The high butyric acid formation in rye could be attributed to high water-extractable AX and fructan content, which are the main DF components of rye grain.[56] The fermentation of AX is most often related to the increased butyrate production in colon.[38,57,20] However, Van den Abbeele et al.[58] recently showed in humanized rats that propionate was increased most by fermentation of water-extractable AX and butyrate by fermentation of oligofructose. Haskå et al.[59] also related the high formation of propionic acid from wheat sample to the high water-extractable AX content, and they also indicated that the wheat fructans produced more propionic acid and less butyric acid than oligofructose. Furthermore, resistant starch has been reported to enhance both the relative proportions of butyric and propionic acids and that the type of starch affects both the fermentation rate and the relative proportions of the SCFA.[60] Starch might have contributed to the more pronounced butyric acid formation from rye aleurone than from rye bran and similarly to the more pronounced butyric acid production from oat cell wall concentrate than from oat bran, since the processed bran fractions contained a comparably high amount of remaining starch even after the enzymatic digestion and the removal of the hydrolyzed (digested) components.

CONCLUSIONS

Dry fractionation of rye, wheat, and oat brans enhanced the release and conversion of phenolic acids and degradation of the DF complex. The higher fermentation rate was related to the physical characteristics (higher portion of water-extractable DF, more degraded cell wall structures, and smaller particle size of bran samples) and, thus, to the better accessibility of microbes to fermentable DF and associated phenolic compounds. The results also indicated that the SCFA ratios are grain specific and more dependent on the carbohydrate composition than on the physical characteristics of the fiber complex. The hydroxylated phenylpropionic acid metabolites were found to be the major microbial metabolites and are related to the high content of FA acid in the cereal samples. Minor analyzed metabolites were BA derivatives. Because the analysis of the present work covered only a fragment of possible phenolic metabolites from these grains, more detailed analysis of grain phenolic compounds and their metabolite profiling using novel metabolomics platform will be needed to complete the picture of phenolic and other metabolites from rye, wheat, and oat.

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ABBREVIATIONS USED

AX, arabinoxylan; BA, benzoic acids; DF, dietary fiber; FA, ferulic acid; GC, gas chromatography; *p*-CA, 4-coumaric acid;

SA, sinapic acid; SCFA, short-chain fatty acids; 3,4-diOHPAc, 2-(3,4-dihydroxyphenyl)acetic acid; 3-OHPPr, 3-(3-hydroxyphenyl)propionic acid; 3-OHPAc, 2-(3-hydroxyphenyl)acetic acid; 3-PPr, 3-Phenylpropionic acid; 3-OHBA, 3-Hydroxybenzoic acid

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