JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 7529–7536 7529
DOI:10.1021/jf900788c

In Vitro Fermentation of Oat Flours from Typical and High β-Glucan Oat Lines

HYUN JUNG KIM AND PAMELA J. WHITE*

Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa 50011

Two publicly available oat (*Avena sativa*) lines, "Jim" and "Paul" (5.17 and 5.31% β-glucan, respectively), and one experimental oat line "N979" (7.70% β-glucan), were used to study the effect of β-glucan levels in oat flours during simulated in vitro digestion and fermentation with human fecal flora obtained from different individuals. The oat flours were digested by using human digestion enzymes and fermented by batch fermentation under anaerobic conditions for 24 h. The fermentation progress was monitored by measuring pH, total gas, and short-chain fatty acid (SCFA) production. Significant effects of β-glucan on the formation of gas and total SCFA were observed compared to the blank without substrate ($P < 0.05$); however, there were no differences in pH changes, total gas, and total SCFA production among oat lines ($P > 0.05$). Acetate, propionate, and butyrate were the main SCFA produced from digested oat flours during fermentation. More propionate and less acetate were produced from digested oat flours compared to lactulose. Different human fecal floras obtained from three healthy individuals had similar patterns in the change of pH and the production of gas during fermentation. Total SCFA after 24 h of fermentation were not different, but the formation rates of total SCFA differed between individuals. In vitro fermentation of digested oat flours with β-glucan could provide favorable environmental conditions for the colon and these findings, thus, will help in developing oat-based food products with desirable health benefits.

KEYWORDS: β-Glucan; oat; in vitro fermentation; short chain fatty acids

INTRODUCTION

Oats (*Avena sativa*) are well recognized as a whole-grain cereal, highly recommended as an important part of the daily diet. The health benefits of oat-based products are attributed to the mixed linkage (1→3)(1→4)-β-D-glucan (referred as β-glucan), a linear unbranched polysaccharide composed of 70% (1→4)-linked and 30% (1→3)-linked β-D-glucopyranosyl units (*1*). β-Glucan is a water-soluble dietary fiber derived from the cell-wall of oat endosperm and subaleurone layers (*2*). The consumption of β-glucan induces satiety, decreases glucose uptake, and insulin response, lowers cholesterol in the blood, and controls weight through prolonged satiety (*3*−*5*). In addition, β-glucans escape digestion in the small intestine and are fermented by the colonic microflora in the large intestine. Fermentation of β-glucan results in the formation of short-chain fatty acids (SCFA), primarily acetate, propionate, and butyrate (*6*, *7*). The low pH in the gut caused by the production of SCFA can prevent the growth of harmful bacteria and aid in the absorption of minerals such as calcium and magnesium (*8*).

Most of the SCFA are absorbed from the colonic lumen and metabolized by various body tissues (*8*). The individual SCFA have specific roles related to certain health benefits. Acetate largely escapes colonic and hepatic metabolism and provides energy for peripheral tissues (*8*). Propionate is converted to glucose in the liver and may modulate hepatic carbohydrate and lipid metabolism that could enhance the hypocholesterolemic effect (*9*). Butyrate seems to be almost exclusively utilized by the colonic epithelial cells (*10*) and has been found to protect against cellular differentiation and possibly inhibit tumor growth (*11*). Therefore, it is important to evaluate the amounts and patterns of SCFA formed from different substrates.

Different types of indigestible polysaccharides form different amounts and patterns of SCFA during in vitro fermentation. Wheat bran and oat fiber produced substantial amounts of butyrate after in vitro fermentation using human feces, whereas arabinoglucan, psyllium, and guar gum produced a high proportion of propionate (*12*). Starch and resistant starch from corn formed high butyrate molar fractions during fermentation (*13*, *14*). The fermentation of β-glucan produced propionate and butyrate in higher amounts than did pectin and resistant starch (*15*). The SCFA formation may depend on the monomeric composition of polysaccharides, the type of linkages between monomers, and their solubility and molecular weight (*6*, *15*, *16*). The type of human feces also could give different amounts and patterns of SCFA during colonic fermentation (*17*, *18*). The interaction of human fecal and β-glucan sources and amount of SCFA formation has not been investigated, and the findings may contribute to understanding in vitro fermentation of dietary fiber.

The objective of this study was to evaluate the impact of β-glucan level in oat flours on SCFA production during in vitro fermentation. Also, the impact of the human fecal flora sources

*To whom correspondence should be addressed. Phone: 1-515-294-9688. Fax: 1-515-294-8181. E-mail: pjwhite@iastate.edu.

Published on Web 07/02/2009

obtained from different healthy individuals on SCFA production was investigated by using these oat flours.

MATERIALS AND METHODS

Oat Grains and Oat Flour Preparation. Three oat (*Avena sativa*) lines were selected for this study: two publicly available oat lines, "Jim" and "Paul" with typical concentrations of β-glucan and one experimental oat line developed at Iowa State University, "N979" with a high level of β-glucan. All oat lines were grown at the Agronomy and Agricultural Engineering Field Research Center in Ames, Iowa, and harvested in 2006. The harvested oat kernels were dried and stored in plastic bags at 4 °C until they were shipped for milling to the Quaker Oats pilot plant in Cedar Rapids, Iowa. Oat grains were dehulled with a Buhler aspirator, steamed for 1 min at 80 °C, and rolled to a flake thickness of 0.61 mm. The flakes were then ground into flour with a hammer mill through a 0.56 mm screen.

Oat Composition. Moisture content of oat flours was determined by AACC method 44−15A (*19*). The β-glucan concentrations in oat flours, digested flours, and extracts were determined enzymatically by AACC method 32−23 by using a mixed β-glucan linkage kit (Megazyme International Ltd., Wicklow, Ireland). Proteins were determined by use of an automatic nitrogen analyzer (Elementar Analyzen system GmbH, Germany) with a nitrogen conversion factor of 6.25. Starch content was analyzed by AACC method 76−13 by using a Total Starch Kit (Megazyme International Ltd., Wicklow, Ireland). Lipids were analyzed by following the gravimetric method after extraction with the mixture of petroleum ether and 2-propanol (3:2) in a Goldfisch system (AACC method 30−25). All analyses were run in triplicate and the average reported on a dry weight basis (db).

Molecular Characterization of β-Glucan. The β-glucan was extracted from each oat line by using water containing heat-stable α-amylase and pancreatin according to the method of Yao et al. (*20*) after removing endogenous enzymes and fats by using 82% ethanol (*21*, *22*). The molecular weight (MW) of the extracted β-glucan was determined by size-exclusion high-performance liquid chromatography (SE-HPLC). The chromatography system consisted of a solvent delivery module (model 210, ProStar, Varian Inc., Rheodyne, CA), an injection valve with a 100 μL loop, a guard column (Ohpak SB-G, Shodex Showa Denko KK, Tokyo, Japan), three serially connected columns (Ohpak SB-806 HQ, Ohpak SB-805 HQ, Ohpak SB-804 HQ, Shodex Showa Denko KK), and a refractive index detector (model 350, ProStar, Varian Inc.). The column temperature was at 40 °C, and the flow rate of the mobile phase, MiliQ water (Milipore, Bedford, MA) containing 0.02% sodium azide, was 0.5 mL/min. Samples were prepared in MiliQ water at the concentration of 5 mg/mL and filtered through a 0.45 μm nylon syringe filter (25 mm i.d., Whatman, NY) before the injection. Five MW standards (Megazyme International Ltd., Wicklow, Ireland) with reported MW values, 3.59×10^5, 2.45×10^5, 1.83×10^5, 1.23×10^5, and 0.4×10^5 g/mol, were used to determine the MW distribution of extracted β-glucan. The peak MW and number average MW (M_n) were obtained by a first-order polynomial curve of log MW versus retention time. M_n was calculated by the equation $M_n = \sum w_i / \sum (w_i/\mathrm{MW}_i)$, where w_i was the weight fraction of time × height derived from the HPLC chromatogram, and MW_i was the MW of the ith species calculated from the standard curve (*23*).

In Vitro Digestion of Oat Flours. An in vitro digestion of oat flours was accomplished according to the method of Sayar et al. (*21*). Oat flours (8 g, db) were mixed with 100 mL of distilled water and heated in a boiling water bath for 5 min. After cooling to room temperature, sodium phosphate butter (50 mM, pH 6.9) was added and stirred slowly at 37 °C for 15 min. Human salivary α-amylase (5 mg/mL in 3.6 mM $CaCl_2$, EC 3.2.1.1.), porcine pepsin (0.5 mg/mL in 0.9% NaCl, EC 3.4.23.1), and pancreatin (from porcine pancreas, 0.5 mg/mL in 50 mM, pH 6.9 sodium phosphate buffer) enzymes (Sigma-Aldrich, St. Louis, MO) were added, respectively. The digestion slurry was stirred slowly at 37 °C for 90 min. The residue was collected by centrifugation and freeze-dried for the in vitro fermentation study. This process just described allows some of the water-soluble β-glucan to be extracted and separated with the digested part by centrifugation. During human digestion, most of β-glucan remains in colon. Thus, to simulate the human digestion system in the current study, the amount of β-glucan in the supernatant after centrifugation was measured and an equal amount of extracted β-glucan from each oat type, which was prepared for MW determination, was added back to the digested residue.

Water Solubility. Water solubility of extracted β-glucan and digested oat flour residues was determined according to the method of Park et al. (*24*). The β-glucan dispersion in water (1%, w/v) was agitated at 37 °C for 24 h and then centrifuged at 1400*g* for 20 min. The supernatant was separated and freeze-dried. The solubility was calculated as: solubility (%) = (weight of β-glucan dissolved in the supernatant)/(initial weight of β-glucan in the dispersion) × 100.

In Vitro Fermentation of Digested Residue. In vitro fermentation of the digested oat flour residues was conducted by a batch fermentation system under strict anaerobic conditions with human fecal flora from three healthy volunteers (sources 1, 2, and 3). The sources, one male and two females, had not received antibiotics for at least 3 months and had not suffered from indigestion problems within the previous week. Fermentation medium (100 mL) was prepared with 3.7 g of brain heart infusion (BHI; Difco Laboratories, Detroit, MI), 93 mL of deionized water, 5 mL of 8% sodium bicarbonate, 2 mL of 1.25% cysteine sulfide (a reducing agent), and 0.1 mL of 1% resazurin (O_2 indicator, Aldrich Chemical, Milwaukee, WI) according to the method of Zheng et al. (*25*). The digested residues (100 mg) were weighed into 50 mL serum bottles. Eight mL of fermentation medium was added to each bottle, and the headspace of the bottle was flushed with CO_2. The serum bottles were sealed with PTFE/silicone septa and aluminum caps (Supelco Inc., Bellefonte, PA), and the materials were hydrated overnight at 4 °C. Lactulose, a completely fermentable substrate, and blank, without any substrate, were prepared as controls.

The inoculums were prepared from fresh feces collected from the volunteers. Feces were immediately homogenized with three parts of CO_2-saturated fermentation medium. The mixture was then filtered through four layers of cheesecloth under continuous CO_2 flow. Two mL of inoculum was added to each sample bottle, and the headspace rinsed with CO_2 for 1 min. The recapped bottles were placed in a shaking water bath at 37 °C and incubated for 0, 2, 4, 8, 12, and 24 h. Total gas production was measured by the overpressure in the headspace of the bottle by using a digital manometer (Fisher Scientific, Pittsburgh, PA). Fermentation was terminated by adding 0.1 mL of saturated mercury chloride solution. The sample slurry was transferred to a centrifuge tube, and pH was measured. After centrifugation at 3100*g* for 10 min, 1 mL of aliquot from the supernatant was taken for the SCFA analysis.

SCFA Analysis. The SCFA (acetate, propionate, iso-butyrate, butyrate, iso-valerate, and valerate) were analyzed as their silyl derivatives by a GC method (*7*). A 1 mL aliquot of the fermentation solution was mixed with 100 μL of 2-ethylbutyric acid as an internal standard. A 500 μL aliquot of hydrochloric acid was added to protonize the SCFA, which were then extracted with diethyl ether. One milliliter of the ether layer was derivatized by 100 μL of *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA; Sigma) to silylate the SCFA at 80 °C for 20 min. After derivatization at room temperature in the dark for 24 h, 1 μL of material was injected into a Hewlett-Packard 5890 GC (Hewlett-Packard, Palo Alto, CA). The column was an SPB-5 (30 m × 0.25 mm; Supelco, Inc., Bellefonte, PA), and helium was used as the carrier gas. The oven temperature was held at 70 °C for 3 min and programmed to increase to 160 at 7 °C/min and stay for 5 min. The injector and detector temperatures were 220 and 250 °C, respectively. SCFA were identified and quantified by comparison with known fatty acid standards (Sigma-Aldrich, St. Louis, MO).

Statistical Analysis. Results were analyzed by using the analysis of variance (ANOVA), followed by Tukey's test to compare the differences among treatments by using SPSS version 11.0 (SPSS Inc., Chicago, IL) at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Composition of Oat Flours and Digested Oat Flours. The contents of β-glucan, starch, protein, and fat in the oat flours and digested oat flours are shown (**Table 1**). The oat lines were selected for their differences in β-glucan concentrations. The traditional oat lines, Jim and Paul, contained 5.17 and 5.31% β-glucan, respectively. The experimental line, N979, contained 7.70% β-glucan, which is greater than in typical domestic

Table 1. Composition of Oat Flours and Digested Oat Flours and Molecular Weight (MW) of the β-Glucan

		composition[a] (%)				β-glucan MW ($\times 10^5$ g/mol)	
	D [b](%)	β-glucan	starch	protein	fat	peak MW	M_n
				Oat Flours			
Jim		5.17 ± 0.2a	54.05 ± 1.7a	14.82 ± 0.1a	7.72 ± 0.1a	8.07a	6.31a
Paul		5.31 ± 0.1a	54.41 ± 0.9a	15.35 ± 0.2b	8.68 ± 0.1a	8.16a	6.81a
N979		7.88 ± 0.4b	50.96 ± 1.7a	15.34 ± 0.1b	7.82 ± 0.4a	11.30b	6.66a
				Digested Oat Flours			
Jim	61.9	5.17 ± 0.2a		15.24 ± 0.7a	7.67 ± 0.1a		
Paul	60.3	5.31 ± 0.1a		14.47 ± 0.3a	8.06 ± 0.1a		
N979	61.3	7.88 ± 0.4b		13.88 ± 0.2b	7.62 ± 0.1a		

[a] Values are means ± standard deviation (n = 3). Values with different letters in the same column and within flour type (oat flour vs digested oat flour) are significantly different ($P < 0.05$). [b] D: Digestibility (%) = (dry weight of sample − dry weight of indigestible pellet)/dry weight of sample × 100.

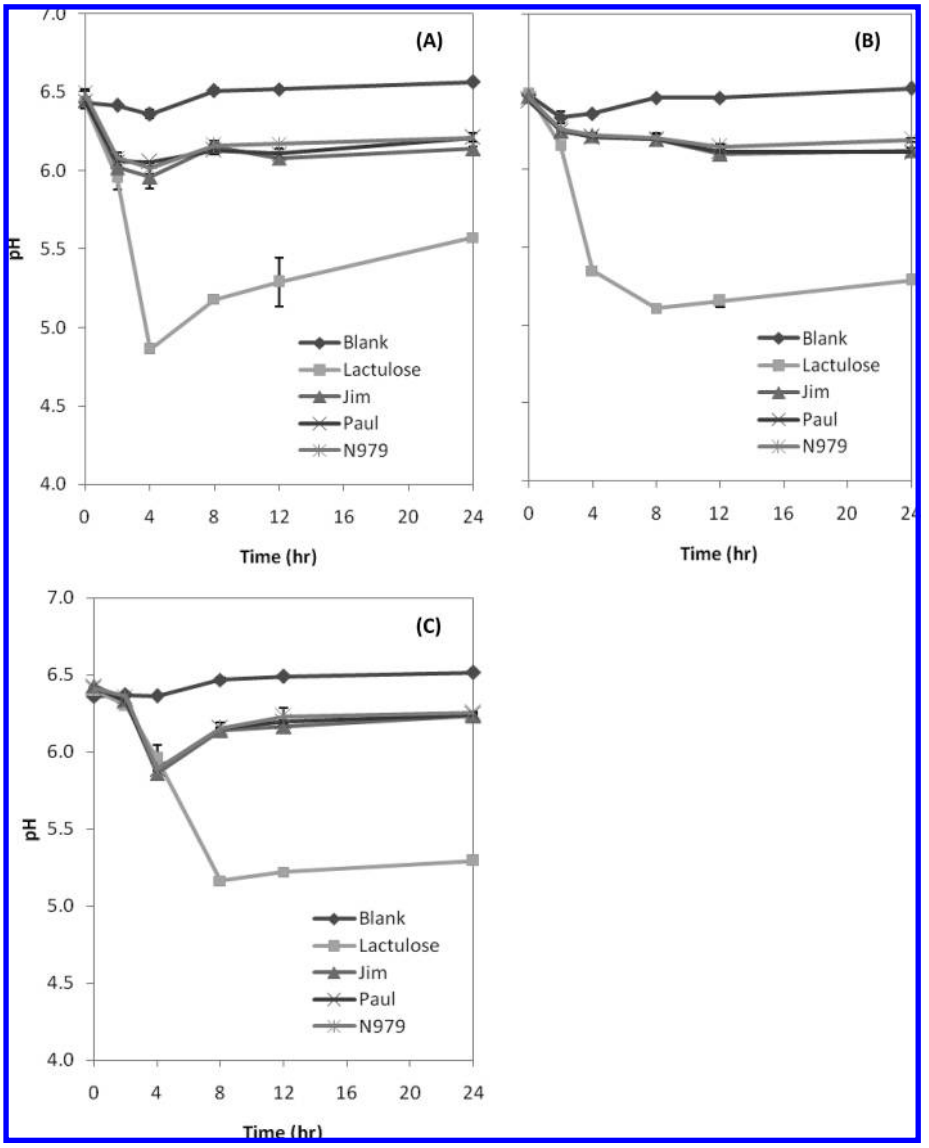

Figure 1. pH changes during in vitro fermentation of blank, lactulose, and digested oat flours by fecal source: (A) source 1, (B) source 2, and (C) source 3. Values are means ± standard deviation.

cultivars. Miller et al. (*1*) reported a range of 3.7−5.0% β-glucan in domestic *A. sativa* cultivars. The starch content of oat lines used in the current study ranged between 51.0 and 54.4%, which was not different among oat lines. The protein concentration of Jim was lower than that of Paul and N979 oats ($P < 0.05$) but within the range of common oats reported (*1*, *20*). The lipid concentrations of the three oat lines were not different from each other.

The digestibility of the oat flours was determined after in vitro digestion, which used the human salivary α-amylase, pepsin, and pancreatin enzymes to simulate the human digestion system. The Jim, Paul, and N979 oat flours were digested to 60.3−61.9% of the original weight with no differences among oat lines ($P > 0.05$). The enzymatic in vitro digestion of the oat flours hydrolyzed 71.8−73.4% of the starch and 47.5−50.3% of the protein. Sayar et al. (*21*) determined the overall digestibility of oat flours of

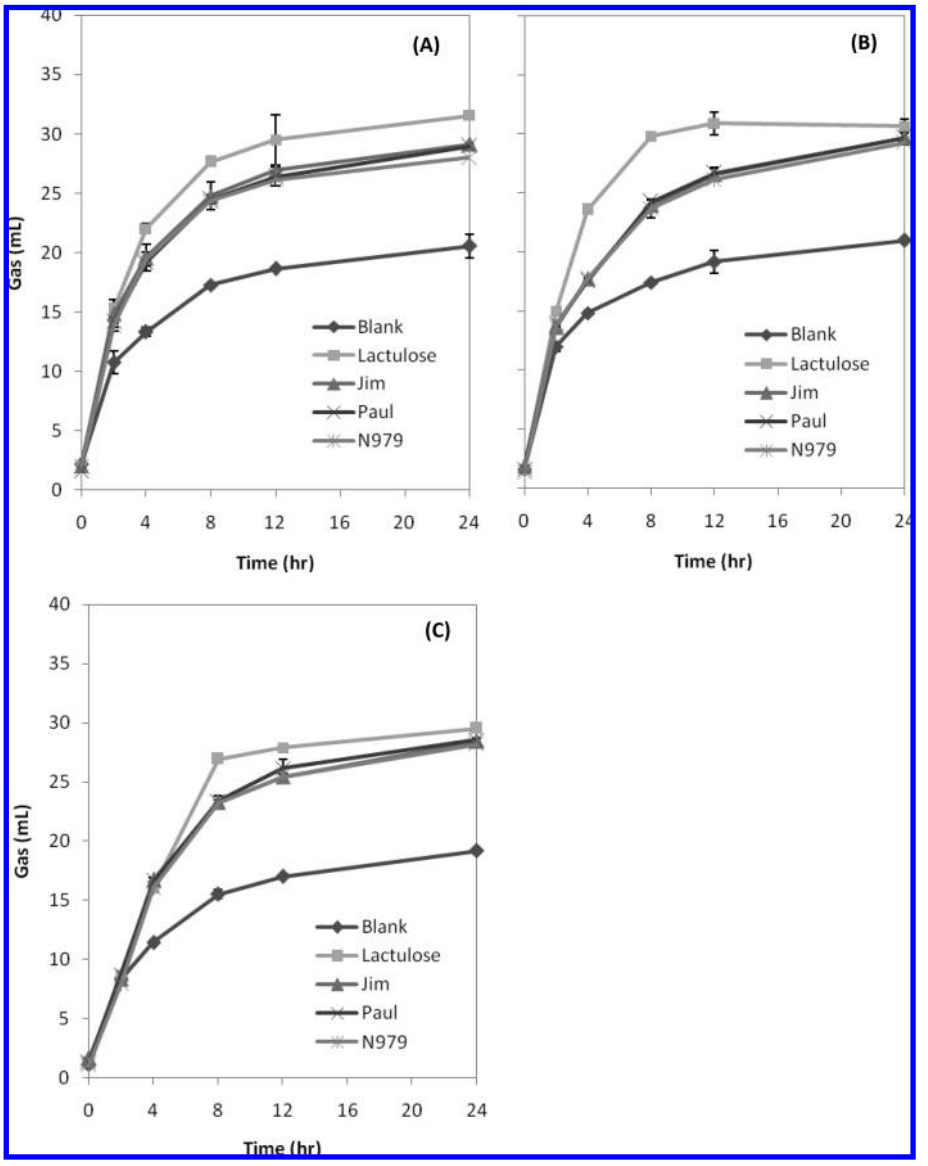

Figure 2. Total gas production during in vitro fermentation of blank, lactulose, and digested oat flours by fecal source: (A) source 1, (B) source 2, and (C) source 3. Values are means ± standard deviation.

Jim, Paul, and N979 varieties, which were grown in 2003 was in the range of 76.9−81.4%. The lower digestibility values (**Table 1**) in the current study might be a result of a lower substrate to enzyme ratio applied to the oat flours.

Water solubility of the digested oat flour residues and the extracted β-glucan were 30% and 83%, respectively (data not shown). The digested oat flour residues before in vitro fermentation were hydrated overnight after the addition of the fermentation medium, and the fermentation was carried out in a shaking water bath at 37 °C. The overnight hydration and the fermentation temperature at 37 °C with shaking helped increase the solubility of substrates during in vitro fermentation.

Molecular Weight of the Extracted β-Glucan. β-Glucan was extracted from each oat line to determine the MW distribution. The extraction yield of β-glucan from oat flours ranged from 60 to 68%. The β-glucan extraction yields from oats reported in the literature varied from 30 to 80% depending on extraction conditions: enzymatic, alkaline, or water extraction with various temperature and incubation times (*21*, *22*, *26*, *27*). The extraction with hot water containing heat-stable α-amylase extracted 60−75% and the yield increased to 75−80% when followed by a dimethyl sulfoxide rinse (*28*). The lack of inactivation of endogenous β-glucanase under mild extraction led to an increased total extraction of β-glucan but decreased molecular weight (*29*). In the current study, the β-glucanase in oat flour was inactivated by treatment with ethanol (*21*, *22*), and starch and protein were removed by using α-amylase and pancreatin (*20*).

The peak MW of extracted β-glucan from N979 (11.3×10^5 g mol^{-1}) was greater than that of Jim (8.4×10^5 g mol^{-1}) and Paul (8.6×10^5 g mol^{-1}) ($P < 0.05$) (**Table 1**). However, the number average MW (M_n) values from the three oat lines did not differ ($P > 0.05$), ranging from 6.3×10^5 g mol^{-1} to 6.8×10^5 g mol^{-1}. The peak MW of Jim, Paul, and N979 oat lines grown in 2003 ranged from 24.0 to 34.5×10^5 g mol^{-1} (*21*), which were greater than those from our current study. The differences might be attributed to the impact of climate on oat composition. During 2003 (April 15−July 31), the weather conditions were wetter and cooler than those during 2006 (*30*). Ajithkumar et al. (*27*) reported that molecular weight of β-glucan seems to be controlled more by environmental factors, which differ in rainfall distribution over the years, rather than by genetic factors. Also, in the current study, the extracted β-glucan was freeze-dried prior to SE-HPLC

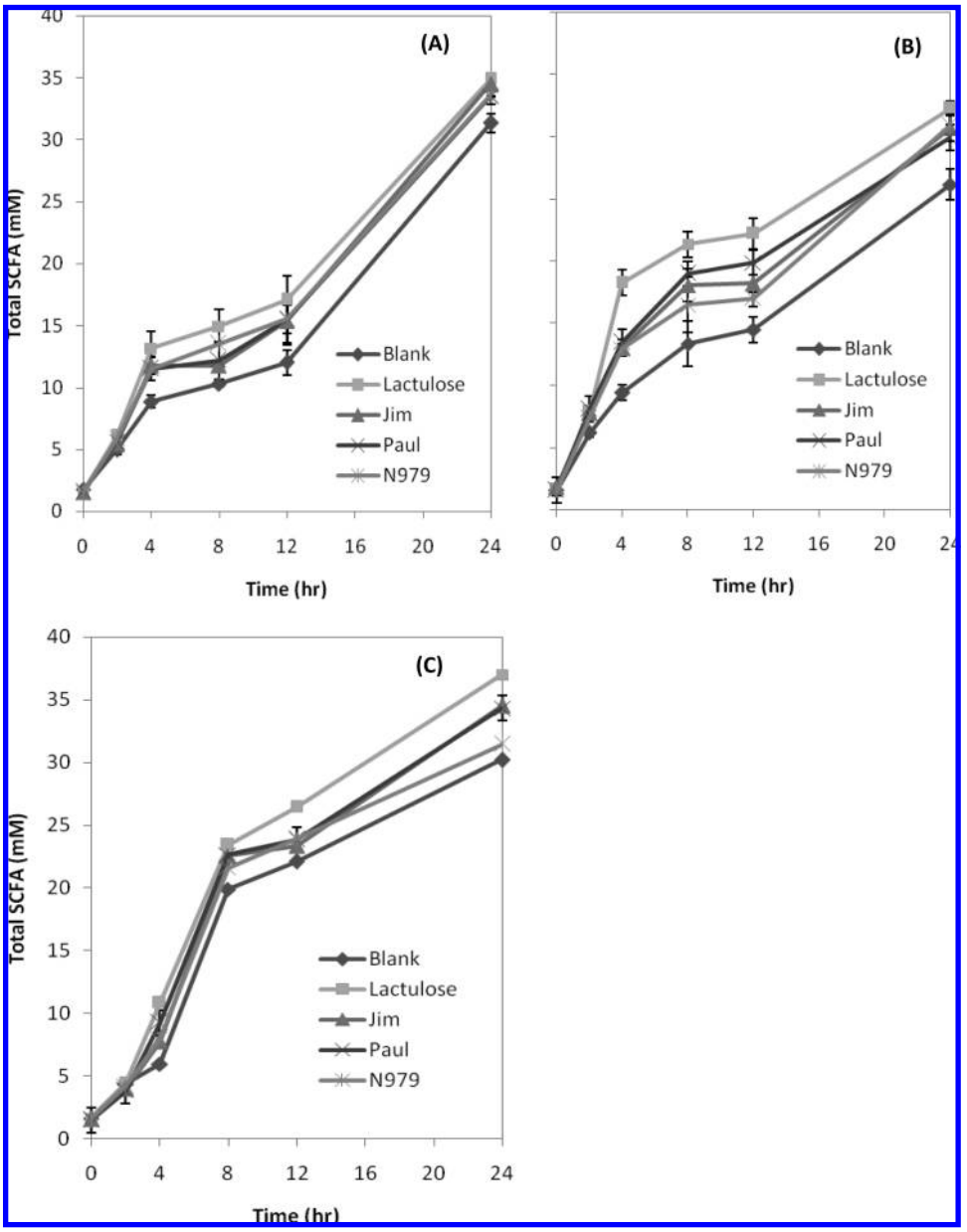

Figure 3. Total short chain fatty acid (SCFA) formation during in vitro fermentation of blank, lactulose, and digested oat flours by fecal source: (A) source 1, (B) source 2, and (C) source 3. Values are means ± standard deviation.

injection. The solution at a concentration of 5 mg extracted and freeze-dried β-glucan/1 mL water was prepared for the injection. In our previous study (*21*), aliquots were not freeze-dried before SE-HPLC analysis, and the extracted β-glucan solution before freeze-drying was used directly for HPLC injection. The freeze-drying process might decrease the solubility of β-glucan when prepared into the solution and lower MW values (*31*).

pH Changes during in Vitro Fermentation. The in vitro fermentation progress of the blank, lactulose, and the three digested oat flours was monitored by measuring pH, gas production, and SCFA production for 0, 2, 4, 8, 12, and 24 h (**Figure 1**). The pH of all samples decreased until between 4 and 8 h, after which it slightly increased until the end of fermentation. These results are consistent with those observed for oat bran and purified β-glucan in other studies (*7*, *29*). The pH of lactulose, which is completely metabolized in the colon by enteric bacteria (*32*), dropped greatly. The pH of Jim, Paul, and N979 digested flours decreased from 6.4−6.5 to 5.8−6.1, with no differences among oat lines ($P > 0.05$). The timing of the pH drop during fermentation, however, differed slightly among the three individual fecal sources (**Figure 1**): the pH for sources 1 and 3 was lowest at 4 h and the pH for source 2 was lowest at 12 h. The digested oat flour treatments produced lower pH during fermentation than did the blank without substrate ($P < 0.05$). The lowering of pH during fermentation is caused by the production of SCFA and is beneficial for colon health. Acidifying the colonic environment can protect against carcinogenic potential and pathogenic bacteria and can help the absorption of minerals (*8*).

Gas Production during in Vitro Fermentation. As the fermentation time of digested oat flours increased from 0 to 24 h, the gas production for all treatments (all fecal sources and all oat lines) increased (**Figure 2**). Lactulose produced the greatest amount of gas during fermentation. Digested Jim, Paul, and N979 oat flours produced greater amounts of gas than did the blank. There were no differences in total gas production among oat lines. Similar to the pH changes, total gas amounts produced from the three individual fecal sources were not different after 24 h of in vitro fermentation. Total amounts of gas after 24 h of fermentation were 29.5−31.6 mL

Table 2. Effect of Feces Flora from Three Healthy Individuals (Sources) on the Formation of SCFA after 24 h of in Vitro Fermentation of Digested Oat Flours

		SCFA[a] (mM)						
source	treatment	total	acetate	propionate	iso-butyrate	butyrate	iso-valerate	valerate
1	blank	31.36 ± 0.76a	13.40 ± 0.22	5.68 ± 0.19a	0.44 ± 0.04	9.66 ± 0.23a	1.00 ± 0.06a	1.18 ± 0.12a
	lactulose	34.89 ± 1.92b	13.59 ± 1.19	4.91 ± 0.30b		16.06 ± 0.41b	0.17 ± 0.02b	0.15 ± 0.01b
	Jim	34.46 ± 0.43ab	14.39 ± 0.20	5.90 ± 0.01a	0.46 ± 0.01	12.29 ± 0.24c	0.82 ± 0.01c	0.60 ± 0.03c
	Paul	33.50 ± 0.05ab	13.84 ± 0.48	6.10 ± 0.07a	0.48 ± 0.02	11.53 ± 0.28c	0.78 ± 0.02c	0.76 ± 0.04c
	N979	33.61 ± 0.75ab	13.95 ± 0.47	6.14 ± 0.04a	0.46 ± 0.03	11.60 ± 0.14c	0.77 ± 0.07c	0.69 ± 0.03c
2	blank	26.16 ± 1.23a	11.00 ± 1.01a	5.14 ± 0.21a	0.25 ± 0.02	6.20 ± 0.14a	0.42 ± 0.02	3.15 ± 0.13a
	lactulose	32.33 ± 0.51b	13.31 ± 0.23b	7.98 ± 0.12b		10.90 ± 0.22b		0.14 ± 0.10b
	Jim	30.71 ± 1.08b	11.88 ± 0.55ab	7.46 ± 0.23bc	0.23 ± 0.01	9.74 ± 0.25c	0.42 ± 0.01	0.98 ± 0.14c
	Paul	29.95 ± 3.03b	11.85 ± 1.11ab	7.20 ± 0.77c	0.26 ± 0.03	9.21 ± 0.99c	0.46 ± 0.05	0.98 ± 0.07c
	N979	30.95 ± 0.74b	12.24 ± 0.44ab	7.91 ± 0.25bc	0.21 ± 0.07	9.36 ± 0.24c	0.45 ± 0.01	0.78 ± 0.01c
3	blank	30.21 ± 0.21a	10.46 ± 0.34a	7.03 ± 0.15a	1.81 ± 0.15a	8.10 ± 0.26a	2.30 ± 0.18a	0.52 ± 0.03a
	lactulose	36.98 ± 0.10b	19.22 ± 0.16b	5.97 ± 0.04b		11.48 ± 0.10b	0.14 ± 0.01b	0.16 ± 0.01b
	Jim	34.59 ± 0.42c	13.26 ± 0.10c	8.37 ± 0.11c	0.69 ± 0.01b	11.15 ± 0.19b	1.01 ± 0.02c	0.12 ± 0.01b
	Paul	34.49 ± 0.15c	13.15 ± 0.32 cd	8.36 ± 0.22c	0.96 ± 0.01c	10.52 ± 0.42bc	1.36 ± 0.01d	0.15 ± 0.01b
	N979	31.45 ± 1.16c	12.55 ± 0.15d	7.47 ± 0.52a	0.79 ± 0.04bc	9.57 ± 0.69c	1.08 ± 0.07c	

[a] Values are means ± standard deviation (n = 4). Values with different letters in the same column and within the source (1, 2, or 3) are significantly different ($P < 0.05$).

for lactulose, 28−29.7 mL for the three oat lines, and 19.2−21.0 mL/100 mg for the blank. Wood et al. (*29*) and Sayar et al. (*7*) reported total gas production of between 30 and 40 mL/100 mg of both purified β-glucan and digested oat flour residues. When considering a dietary fiber source, the production amount and type of gases, which are byproduct of bacterial fermentation of carbohydrates in the large intestine, are important factors (*14*). Uncomfortable bloating can result from rapid gas production during fermentation, whereas a slow and steady rate of gas production would result in less discomfort and bloating (*14*,*33*). Compared to wheat bran, oat bran is reported to induce less discomfort and less formation of gas (*34*). Livesey (*35*) suggested that an upper limit of gas production to avoid abdominal discomfort is no more than 90 g fermentable low digestible carbohydrate per day.

SCFA Production during in Vitro Fermentation. Total SCFA formation from the blank, lactulose, and digested Jim, Paul, and N979 oat flours during in vitro fermentation continuously increased as the fermentation time increased (**Figure 3**). Lactulose produced greater amounts of total SCFA than did digested oat flours during 24 h of fermentation. Total SCFA amounts were not different among the Jim, Paul, and N979 oat flours. High correlations occurred between gas production and SCFA formation ($R^2 = 0.85$). Acetate, propionate, and butyrate, typical metabolites for dietary fiber fermentation (*29*, *36*, *37*), were the main SCFA (**Table 2**). Among all treatments, acetate (40−45 mol %) was produced in the greatest proportion, followed by butyrate (27−37 mol %) and propionate (18−24 mol %). In addition to the three main SCFA, small amounts of iso-butyrate, iso-valerate, and valerate were formed from all treatments. Iso-valerate and valerate are the major products of protein fermentation (*38*).

The average of the total SCFA (mM) was calculated for all fecal sources at the fermentation time of 24 h for the blank, lactulose, and each oat line (**Figure 4**). The digested oat flours produced more acetate, propionate, and butyrate than did the blank and more propionate than did the lactulose. The molar fraction of acetate, propionate, and butyrate for lactulose was 45:18:37 and for the three digested oat flours was 40−43:23−24:34−35. This ratio for a previous in vitro study using human fecal inocula with purified β-glucan was 51:32:17, which is relatively high in propionate (*16*). The difference in these ratios may be attributed to the use of highly purified (>96%) β-glucan for fermentation in their study, compared with the use of oat flours with lower levels of naturally occurring β-glucan in the

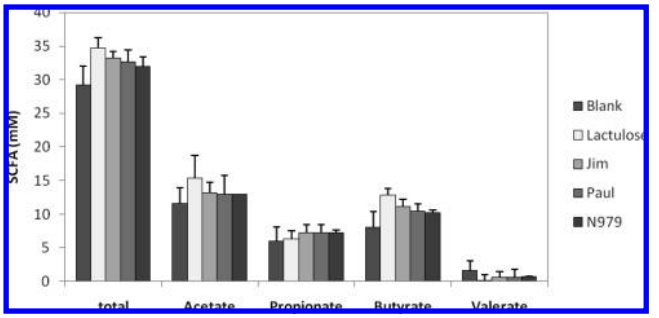

Figure 4. Effect of oat line on the average SCFA production (mM) of acetate, propionate, butyrate, and valerate from all individuals (sources 1, 2, and 3) after 24 h of in vitro fermentation. Values are means ± standard deviation.

present study. The production of propionate and butyrate at high concentrations is physiologically important. Propionate was previously shown to reduce serum cholesterol levels because it is converted to glucose in the liver and to modulate hepatic carbohydrate and lipid metabolism (*9*, *39*). Butyrate, reported as the major energy source for the colonic epithelium, helps to stimulate cell proliferation and protect against colon cancer (*10*, *40*−*42*).

Fermentability and the proportion of the main fermentation products differ according to the dietary fiber source. For example, total gas and SCFA production during in vitro fermentation of oat bran, containing β-glucan, were greater than those of apple pomace and celery, containing pectic substances, and pea hulls, containing cellulose (*37*). β-Glucan from oat dietary fiber produced higher proportions of propionate and butyrate during fermentation than did starch, pectin, and resistant starch (*15*). Starch and resistant starch from corn generally cause proportionally more butyrate production than do other polysaccharides during fermentation (*13*,*14*). It is possible that the structure of the cell wall and the linkages between indigestible compounds changes the fermentability and results in different metabolism into specific SCFA. The chemical compositions of dietary fibers likely also impact their susceptibility to the human fecal flora.

The effect of fecal source on the average mol % of acetate, propionate, butyrate, and valerate during fermentation was evaluated (**Figure 5**). Source 1 formed a greater amount of acetate and butyrate and less propionate than did sources 2 and 3. The total SCFA formation rates (mM SCFA/h) differed in each

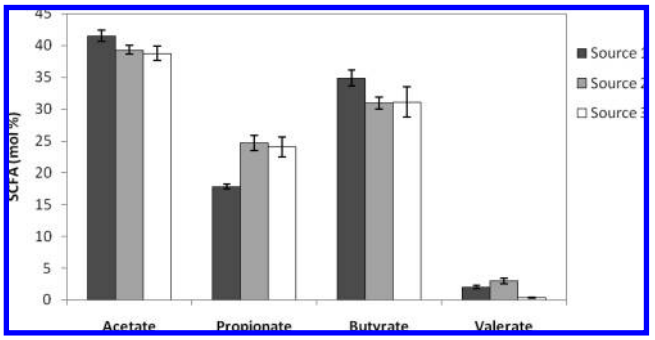

Figure 5. Effect of individual fecal source on the average molar proportion of acetate, propionate, butyrate, and valerate after 24 h of in vitro fermentation of Jim, Paul, and N979 digested oat flours. Values are means ± standard deviation.

Table 3. Total SCFA Formation Rate during in Vitro Fermentation of Blank, Lactulose, Jim, Paul, and N979 Digested Oat Flours for Each Fecal Source

	SCFA formation rate[a] (mM SCFA/h)				
	fermentation				
source	2 h	4 h	8 h	12 h	24 h
1	2.0	2.9	0.3	0.6	1.5
2	2.9	3.0	1.1	0.2	1.0
3	1.3	2.1	3.4	0.5	0.8

[a] Average of total SCFA formed from all treatments for each fecal source and rate were calculated.

source during fermentation (**Table 3**). Sources 1 and 2 mostly produced high amounts of SCFA during the first 4 h, whereas source 3 produced high amounts for up to 8 h of fermentation (**Table 3** and **Figure 3**). The types of human feces obtained from the three healthy individuals provided the different fermentation rates (**Table 3**) and SCFA proportions (**Figure 5**). The total SCFA amounts formed after 24 h of fermentation were not significantly different. Nilsson et al. (*18*) reported that total SCFA concentrations of feces obtained from healthy individuals were similar to that measured in individuals with ulcerative colitis and irritable bowel syndrome, but the concentration of butyrate was higher in the healthy sources than in the patients with ulcerative colitis. Vernia et al. (*43*) also showed that the concentration of butyrate decreased with severity of ulcerative colitis. These studies (*19*, *43*) and the current study indicated that total SCFA formation and its proportions were greatly related to the properties of the fecal microflora applied from different sources. Bourquin et al. (*17*) reported variations in the ability to ferment dietary fiber between the fecal samples obtained from different individuals. However, the human feces collected at different times from the same individual were uniform in producing similar in vitro fermentation products (*44*).

In vitro fermentation of digested oat flours containing different levels of β-glucan lowered the pH after 4 to 8 h of fermentation. The lower pH values formed as a result of the SCFA production during fermentation provide a favorable environment for health of the colon. Acetate, propionate, and butyrate were the major SCFA produced from digested oat flours. High concentrations of propionate and butyrate in the colon are known to have great potential for preventing hypocholesterolemia and tumorogenesis (*3*, *44*). Human fecal floras obtained from three individuals produced SCFA at different rates during fermentation.

LITERATURE CITED

(1) Miller, S. S.; Wood, P. J.; Pietrzak, L. N.; Fulcher, R. G. Mixed linkage beta-glucan, protein content, and kernel weight in *Avena* species. *Cereal Chem.* **1993**, *70*, 231–233.

(2) Lazaridou, A.; Biliaderis, C. G. Molecular aspects of cereal β-glucan functionality: Physical properties, technological applications, and physiological effects. *J. Cereal Sci.* **2007**, *46*, 101–118.

(3) Braaten, J. T.; Wood, P. J.; Scott, F. W.; Wolynetz, M. S.; Lowe, M. K.; Bradley-White, P.; Collins, M. W. Oat beta-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *Eur. J. Clin. Nutr.* **1994**, *48*, 465–474.

(4) Burton-Freeman, B. Dietary fiber and energy regulation. *J. Nutr.* **2000**, *130*, 272S–275S.

(5) Krauss, R. M.; Eckel, R. H.; Howard, B. AHA dietary guidelines. Revision 2000: A statement for healthcare professionals from the Nutrition committee of the American Heart Association. *Circulation* **2000**, *102*, 2284–2299.

(6) Daniels, M.; Wisker, E.; Rave, G.; Feldheim, W. Fermentation in human subjects of nonstarch polysaccharides in mixed diet, but not in a barley fiber concentrate, could be predicted by in vitro fermentation using human fecal inocula. *J. Nutr.* **1997**, *127*, 1981–1988.

(7) Sayar, S.; Jannink, J.-L.; White, P. J. Digestion residues of typical and high β-glucan oat flours provide substrates for in vitro fermentation. *J. Agric. Food Chem.* **2007**, *55*, 5308–5311.

(8) Cummings, J. H. Short chain fatty acids in the human colon. *Gut* **1981**, *22*, 763–779.

(9) Goni, I.; Martin-Carron, N. In vitro fermentation and hydration properties of commercial dietary fiber-rich supplements. *Nutr. Res. (N.Y.)* **1998**, *18*, 1077–1089.

(10) Roediger, W. E. W. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosae in man. *Gut* **1980**, *21*, 793–798.

(11) Young, G. P.; Gibson, R. P. Butyrate and colorectal cancer cell. In *Short Chain Fatty Acids*; Binder, H. J., Cummings, J., Soergel, K. H., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1994; pp 148−160.

(12) Edwards, C. Interaction between nutrition and the intestinal microflora. *Proc. Nutr. Soc.* **1993**, *52*, 375–382.

(13) Fassler, C.; Arrigoni, E.; Venema, K.; Brouns, F.; Amado, R. In vitro fermentability of differently digested resistant starch preparations. *Mol. Nutr. Food Res.* **2006**, *50*, 1220–1228.

(14) Rose, D. J.; Keshavarzian, A.; Patterson, J. A.; Venkatachalam, M.; Gillevet, P.; Hamaker, B. R. Starch-entrapped microspheres extend in vitro fecal fermentation, increase butyrate production, and influence microbiota pattern. *Mol. Nutr. Food. Res.* **2009**, *53*, 121–130.

(15) Casterline, J. L.; Oles, C. J.; Ku, Y. In vitro fermentation of various food fiber fractions. *J. Agric. Food Chem.* **1997**, *45*, 2463–2467.

(16) Hughes, S. A.; Shewry, P. R.; Gibson, G. R.; MacCleary, B. V.; Rastall, R. A. In vitro fermentation of oat and barley derived β-glucan by human fecal microbiota. *FEMS Microbiol. Ecol.* **2008**, *64*, 482–493.

(17) Bourquin, L. D.; Titgemeyer, E. C.; Fahey, G. C. Fermentation of various dietary fiber sources by human fecal bacteria. *Nutr. Res. (N.Y.)* **1996**, *16*, 1119–1131.

(18) Nilsson, U.; Johansson, M.; Nilsson, A.; Bjorck, I.; Nyman, M. Dietary supplementation with β-glucan enriched oat bran increases fecal concentration of carboxylic acids in healthy subjects. *Eur. J. Clin. Nutr.* **2008**, *62*, 978–984.

(19) AACC International. *Approved Methods of the AACC*, 10th ed.; American Association for Clinical Chemistry: St. Paul, MN, 2000; Methods 30−25, 32−23, 44−15A, 76−13.

(20) Yao, N.; Jannink, J.-L.; White, P. J. Molecular weight distribution of (1−3)(1−4)-β-glucan affects pasting properties of flour from oat lines with high and typical amounts of β-glucan. *Cereal Chem.* **2007**, *84*, 471–479.

(21) Sayar, S.; Jannink, J.-L.; White, P. J. In vitro bile acid binding of flours from oat lines varying in percentage and molecular weight distribution of β-glucan. *J. Agric. Food Chem.* **2005**, *53*, 8797–8803.

(22) Skendi, A.; Biliaderis, C. G.; Lazaridou, A.; Izydorczyk, M. S. Structure and rheological properties of water soluble β-glucans from oat cultivars of *Avena sativa* and *Avena bysantina*. *J. Cereal Sci.* **2003**, *38*, 15–31.

(23) Painter, P. C.; Coleman, M. M. Molecular weight and branching. In *Fundamentals of Polymer Science*; Painter, P. C., Coleman, M. M., Eds.; Technomic Publishing: Lancaster, PA, 1997; pp 373−381.

(24) Park, S. Y.; Bae, I. Y.; Lee, S.; Lee, H. G. Physicochemical and hypocholesterolemic characterization of oxidized oat b-glucan. *J. Agric. Food Chem.* **2009**, *57*, 439–443.
(25) Zheng, Y.; Hu, J.; Murphy, P. A.; Alekel, D. L.; Franke, W. D.; Hendrich, S. Rapid gut transit time and slow fecal isoflavone disappearance phenotype are associated with greater genistein bioavailability in women. *J. Nutr.* **2003**, *133*, 3110–3116.
(26) Colleoni-Sirghie, M.; Kovalenko, I. V.; Briggs, J. L.; Fulton, B.; White, P. J. Rheological and molecular properties of water soluble (1,3) (1,4)-β-D-glucans from high β-glucan and traditional oat lines. *Carbohydr. Polym.* **2003**, *52*, 439–447.
(27) Ajithkumar, A.; Andersson, R.; Aman, P. Content and molecular weight of extractable β-glucan in American and Swedish oat samples. *J. Agric. Food Chem.* **2005**, *53*, 1205–1209.
(28) Beer, M. U.; Wood, P. J.; Weisz, J. Molecular weight distribution and (1−3)(1−4)-β-D-glucan content of consecutive extracts of various oat and barley cultivars. *Cereal Chem.* **1997**, *74*, 705–709.
(29) Wood, P. J.; Arrigoni, E.; Miller, S. S.; Amado, R. Fermentability of oat and wheat fractions enriched in β-glucan using human fecal inoculation. *Cereal Chem.* **2002**, *79*, 445–454.
(30) Iowa Environmental Mesonet. http://mesonet.agron.iastate.edu/agclimate (Access date: November 3, 2008). *Department of Agronomy, Iowa State University*: Ames, IA.
(31) Lan-Pidhainy, X.; Brummer, Y.; Tosh, S. M.; Wolever, T. M.; Wood, P. J. Reducing beta-glucan solubility in oat bran muffins by freeze-thaw treatment attenuates its hypoglycemic effect. *Cereal Chem.* **2007**, *84*, 512–517.
(32) Shawcross, D. L.; Jalan, R. Treatment of hepatic encephalopathy: it's not lactulose. *BMJ Clin. Res* **2004**, *329*, 112.
(33) Cummings, J. H; Macfarlane, G. T. Prebiotic digestion and fermentation. *Am. J. Clin. Nutr.* **2001**, *74*, 415S–420S.
(34) Meyer, S.; Calloway, D. H. Gastrointestinal response to oat and wheat milling fractions in older women. *Cereal Chem.* **1977**, *54*, 110–119.
(35) Livesey, G. Tolerance of low-digestible carbohydrates: a general view. *Br. J. Nutr.* **2001**, *85*, S7–S16.
(36) Salvador, V.; Cherbut, C.; Barry, J.-L. Sugar composition of dietary fiber and short-chain fatty acid production during in vitro fementation by human bacteria. *Br. J. Nutr.* **1993**, *70*, 189–197.
(37) Lebet, V.; Arrigoni, E.; Amado, R. Measurement of fermentation products and substrate disappearance during incubation of dietary fiber sources with human fecal flora. *LWT—Food Sci. Technol.* **1998**, *31*, 473–479.
(38) Macfarlane, G. T.; Gibson, G. R.; Beatty, E.; Cummings, J. H. Estimation of short chain fatty acid production from protein by human intestinal bacteria based on branched-chain fatty acid measurements. *FEMS Microbiol. Ecol.* **1992**, *1010*, 81–88.
(39) Chen, W. J.; Anderson, J. W.; Jennings, D. Propionate may mediate the hypocholesterolemic effects of certain soluble plant fibers in cholesterol-fed rats. *Proc. Soc. Exp. Biol. Med.* **1984**, *175*, 215–218.
(40) Bugaut, M.; Bentejac, M. Biological effects of short-chain fatty acids in nonruminant mammals. *Annu. Rev. Nutr.* **1993**, *13*, 27–241.
(41) Malkki, Y.; Virtanen, E. Gastrointestinal effects of oat bran and oat gum. *LWT— Food Sci. Technol* **2001**, *34*, 337–347.
(42) Topping, D. L.; Clifton, P. M. Short-chain fatty acids and human colonic fuctions: roles of resistant starch and nonstarch polysaccharides. *Physiol. Rev.* **2001**, *81*, 1031–1064.
(43) Vernia, P.; Caprilli, R.; Latella, G.; Barbetti, F.; Magliocca, F. M.; Cittadini, M. Fecal lactate and ulcerative colitis. *Gastroenterology* **1988**, *95*, 1564–1568.
(44) McBurney, M. I.; Thompson, L. U. Effect of human faecal inoculum on in vitro fermentation variables. *Br. J. Nutr.* **1987**, *58*, 233–243.

Received March 8, 2009. Revised manuscript received May 20, 2009. Accepted May 26, 2009. This project was supported by the USDA-NRI Competitive Grants Program, award number 2007-02701.