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Abstract

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Keywords

Agronomy, In vitro fermentation, β -glucan, oat, short-chain fatty acid

Disciplines

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Digestion Residues of Typical and High- β -Glucan Oat Flours Provide Substrates for in Vitro Fermentation

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In vitro fermentabilities of the oat flour digestion residues (ODR) from two commercial oat lines with 4.7 and 5.3% β -glucan and from two high- β -glucan experimental lines with 7.6 and 8.1% β -glucan were evaluated and compared with fermentations of lactulose, purified oat β -glucan (POBG), and purified oat starch (POS). Substrates were fermented by using an in vitro batch fermentation system under anaerobic conditions for 24 h. The progress of the fermentation was studied by following the change in pH of the fermentation medium, production of short-chain fatty acids (SCFA) and gases, and consumption of carbohydrates. The substrate from the flour with the greatest amount of β -glucan tended to have the greatest pH decline and the greatest total SCFA production. A significant correlation occurred between gas production and SCFA formation ($R^2 = 0.89$ – 0.99). Acetate was produced in the greatest amounts by all of the substrates except POBG, by which butyrate was produced in the greatest amount. More propionate and butyrate, but less acetate, were produced from high- β -glucan ODR. With the given fermentation conditions, >80% of the total carbohydrate was depleted by the bacteria after 24 h. Glucose was the most rapidly consumed carbohydrate among other available monosaccharides in the fermentation medium. Overall, the high- β -glucan experimental lines provided the best conditions for optimal in vitro gut fermentations.

KEYWORDS: In vitro fermentation; β -glucan; oat; short-chain fatty acid

INTRODUCTION

Oats are a complex carbohydrate food with potentially important metabolic effects in humans (1). Positive health effects generally have been associated with the (1 \rightarrow 3),(1 \rightarrow 4)- β -D-glucan (hereafter referred to as β -glucan), a soluble dietary fiber, in the nonstarch polysaccharide fraction of oat (2–4). Most of the carbohydrates of the fiber fraction can promote a number of positive physiological effects, such as helping to prevent constipation and helping to lower blood cholesterol and/or glucose levels (5). Dietary fiber also increases satiety and may decrease overeating and obesity (5). Additionally, the oligosaccharides and polysaccharides that escape digestion in the small intestine provide a source of carbon and energy for the colonic flora (6). The major fraction of the dietary fiber reaching the colon contains mainly β -glucan, cellulose, hemicellulose, pectin, and some plant gums (7). However, the main substrates for fermentation are the noncellulosic polysaccharides, which may degrade by up to 80% in the colon (7). These carbohydrates are extensively broken down by the microflora when they reach the large intestine; this metabolic process results in

the production of gas and short-chain fatty acids (SCFA), composed essentially of acetate, propionate, and butyrate (8). Furthermore, some SCFA, such as *n*- and isovalerate, are formed by deamination of amino acids as a result of protein fermentation (9).

Several studies have demonstrated that structurally different polysaccharides and fiber constituents produce SCFA in different amounts and proportions (7, 8, 10, 11). Certain SCFA may protect against colon carcinogenesis and aid in cholesterol and glucose metabolism (8, 10). In addition, lower pH resulting from the production of SCFA creates an environment that prevents the growth of harmful bacteria, aids in the absorption of minerals such as calcium and magnesium (8), and may lead to a lower formation rate of secondary bile acids, which may be involved in large-bowel carcinogenesis (13).

Fermentability of the indigestible carbohydrates and production of SCFA can be studied with in vitro batch systems utilizing fecal bacteria. Different in vitro models simulating conditions in the human gastrointestinal tract have been developed. In vivo studies of the fermentation process are difficult, because of the inaccessibility of the colon, interference from other components of the diet, and ethical and financial aspects. However, in vitro studies have the advantage of using single and relatively pure substrates, which simplifies determination of the extent of substrate depletion and SCFA production (12, 14, 15). The

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similarity of the results from in vitro studies to in vivo measurements also provides strong evidence that in vitro systems can be used to model the human colon (15, 16).

Various fiber sources have been used as substrates in different in vitro fermentation studies. Among dietary fiber fractions from fig, oat, soy, pea, apple, corn, wheat, and pear fermented in vitro with human fecal inocula, oat and fig fiber fractions produced the highest amounts of SCFA, with SCFA production being proportional to the fermentability of these fibers (10). Fermentation of the major fiber constituents, resistant starch, β -glucan, and pectin (10) demonstrated that greater amounts of propionate and butyrate were produced from the fermentation of β -glucan than from the fermentation of pectin or resistant starch. In rye, the water- and alkali-extractable rye bran fractions were fermented more quickly than the unextractable fraction and rye bran itself (17). Oat soluble fiber is thought to ferment more rapidly than insoluble fiber (2). However, enzyme-resistant oat starch also could provide a separate source of fermentable material in the colon (1). In addition to solubility, the structural characteristics and associations between cell-wall polysaccharides and lignin also were important determinants of fermentation rate (17).

Therefore, the specific aims of this study were to investigate the impact of oat lines with high levels of β -glucan on the extent and the products of in vitro fermentation and on the depletion of available carbohydrates during in vitro fermentation.

MATERIALS AND METHODS

Oat Grains and in Vitro Digestion. Two experimental oat (*Avena sativa*) lines developed at Iowa State University, IA95111 and N979-5-2-4 (N979), and two publicly available cultivars, 'Jim' and 'Paul', were chosen for this study, because they displayed a broad range of β -glucan concentrations. Lines Jim and Paul had β -glucan levels of 4.8 and 5.3% (dwb), respectively, and IA95111 and N979 had 7.6 and 8.1% (dwb) β -glucan, respectively. Typical values reported for domestic *A. sativa* cultivars in the literature are 3.7–5.0% (18). Detailed information about the oat lines was previously presented (3, 4). The samples, except the naked variety, Paul, were dehulled with an air-pressure dehuller (Codema, Eden Prairie, MN). Kernels from all oat types were ground in an ultracentrifugal mill (ZM-1, Retch GmbH & Co., Haan, Germany) fitted with a 0.5 mm sieve.

An in vitro digestion process, simulating the human digestion system, was applied as described (4). Briefly, oat flours were cooked in boiling water and subjected to human salivary α -amylase, porcine pepsin, and pancreatin enzymes (Figure 1). The in vitro digested solutions were centrifuged, and the digestion residue (insoluble part, pellets) was freeze-dried and used as the substrate, referred to as oat flour digestion residues (ODR) in the current study. This process allows most of the water-soluble β -glucans to be extracted and separated with the digested part (supernatant) via centrifugation. Because the β -glucan is not digestible by the in vitro conditions, it should be in the ODR. The total amounts of β -glucan in the supernatant were determined, and equal amounts from the appropriate oat type were added back to the ODR as pure β -glucan, in order to simulate the human digestion system, where all of the β -glucan reaches the colon for fermentation (1). The described procedure was applied to evaluate the fate of the β -glucan, rather than using precipitation or dialysis techniques, which may not recover all of the β -glucans in the extract (19).

In Vitro Fermentation of the Digestion Residues. In vitro fermentation was carried out by using a batch fermentation system under strict anaerobic conditions for 24 h. The anaerobic fermentation medium was prepared by using brain heart infusion (Difco Laboratories, Detroit, MI) according to the method of Zheng et al. (20). The inoculum was prepared from the fresh feces collected from two healthy volunteers who had not received antibiotics for at least 3 months. Feces were immediately pooled, mixed with three parts of the fermentation medium, and filtered through four layers of cheesecloth. The filtrate was kept in an Erlenmeyer flask under continuous CO₂ flow. It was previously

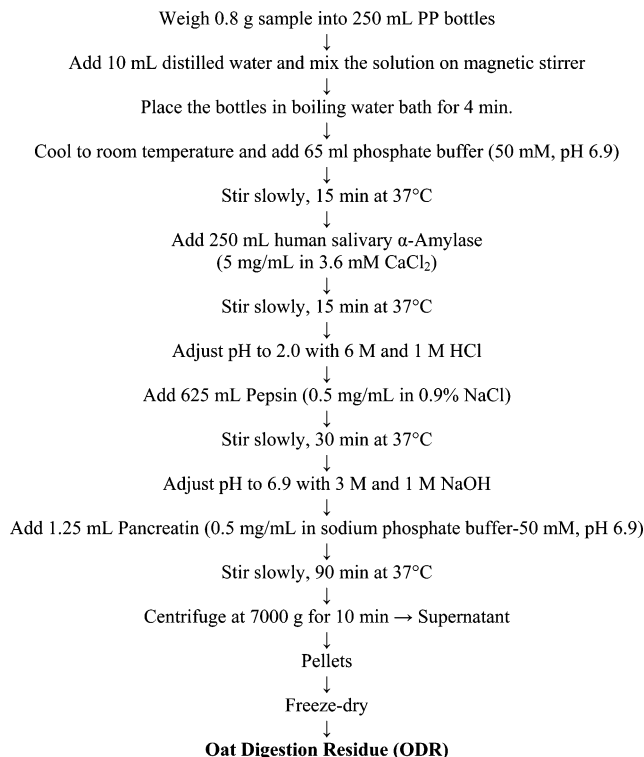


Figure 1. In vitro digestion procedure for oat flour.

noted that total bacterial counts, number of species, and the distribution of species in the feces collected on different occasions were sufficiently uniform to yield similar in vitro fermentation findings among collections (16).

The ODR substrates (100 mg) were weighed into 50 mL serum bottles, and 8 mL of fermentation medium was added to each bottle. After the headspace had been rinsed with CO₂ for 1 min, the bottles were sealed by using PTFE/silicone septa with aluminum caps (Supelco Inc., Bellefonte, PA) and the substrates hydrated at 4 °C for 16 h. Two milliliters of inoculum was added to each bottle, and the incubation was carried out in a shaking water bath at 37 °C. Lactulose was tested as a completely fermentable substrate (21), and blanks without any substrate were used as controls. Purified oat β -glucan (POBG) and purified oat starch (POS), the main constituents of the ODR, also were fermented. Extraction conditions and characterization of these components are presented in Sayar et al. (4) for POBG and in Sayar et al. (3) for POS. Individual bottles were prepared for each time data point (2, 4, 8, 12, and 24 h) to prevent pressure loss or a break in the anaerobicity during sampling. Total gas production was measured by the overpressure in the bottles with a digital manometer (Fisher Scientific, Pittsburgh, PA). Fermentation was stopped by adding 0.1 mL of saturated mercury chloride solution (14). The slurry was transferred to a centrifuge tube, the pH measured, and the slurry centrifuged at 4200g for 10 min, and a 1 mL aliquot of the supernatant was taken for the SCFA analysis. The fermentation residues (pellets) were freeze-dried and used for the neutral sugar analysis.

Chemical Analysis. Moisture, β -glucan, protein, and starch contents of the ODR were analyzed as previously described (3, 4).

The SCFA (acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate) were analyzed as their silyl derivatives by a GC method (22). Briefly, 1 mL of the fermentation solution was mixed with 100 μ L of 2-ethylbutyric acid (internal standard). Hydrochloric acid was added to protonize the SCFA, which were then extracted with diethyl ether and silylated with *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA; 22). After standing for 24 h for complete derivatization, samples were injected onto an SPB-5 column (30 m \times 0.25 mm; Supelco, Inc.). The column temperature was held at 70 °C for 4 min and then programmed to rise to 160 °C at 7 °C/min. The injector and detector temperatures were 220 and 250 °C, respectively. Helium was the carrier gas, and the injections (1 μ L) were made in the

Table 1. Main Composition of Oat Flours before and after in Vitro Digestion and Yield after Digestion^a

Oat type/ component	Substrate amount, mg		Digestion residue, mg
Jim	100^b		25.4±3.3
β-glucan ^c	4.8±0.1	→	4.8±0.1
Starch	63.3±0.2		11.7±0.2
Protein	15.5±0.4	→	4.3±0.2
Paul	100^b		23.7±2.1
β-glucan ^c	5.3±0.1		5.3±0.1
Starch	57.6±0.9		8.5±0.1
Protein	17.8±0.3		4.6±0.3
IA95	100^b		29.3±4.0
β-glucan ^c	7.6±0.3		7.6±0.3
Starch	56.8±1.4		9.9±0.1
Protein	19.1±0.4	→	6.2±0.5
N979	100^b		25.5±1.9
β-glucan ^c	8.1±0.2	→	8.1±0.2
Starch	54.4±0.9		8.0±0.1
Protein	18.6±0.9		4.6±0.2

^a Values are the average of three determinations ± standard deviation. ^b Amount of substrate taken to digestion (dwb). ^c Amount of extracted β-glucans in the supernatant at the end of the digestion was added back to the ODR substrates (explained under Materials and Methods).

split mode. Neutral sugar residues arising from acid hydrolysis were analyzed from the unfermented portion of the substrates as their alditol acetates by GC, with an SP-2330 column (15 m × 0.25 mm; Supelco), as previously described (23). Acid hydrolysis was achieved by hydrolyzing the fermentation residues in 12 M H₂SO₄ for 1 h at 30 °C followed by dilution to 2 M H₂SO₄ and autoclaving at 125 °C for 1 h (23). Uronic acid (as galacturonic acid) and Klason lignin contents also were determined (23).

Statistical Analysis. All analyses were conducted at a minimum in triplicate: *n* values are reported. Results were analyzed by using a statistical analysis software system (SPSS version 12.0, SPSS Inc., Chicago, IL). Differences among substrates were compared by using a least significant difference (LSD) test with a probability level (α) of 0.05.

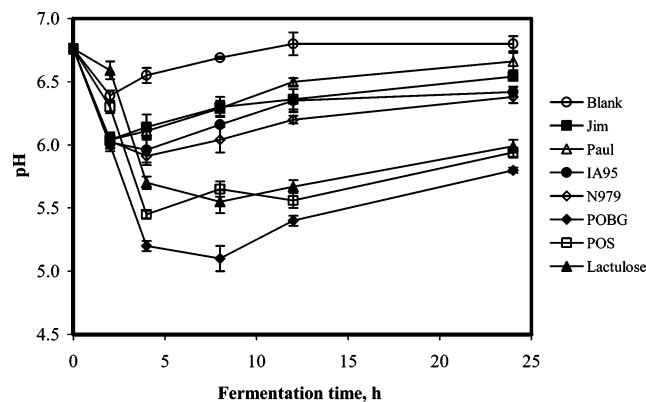
RESULTS AND DISCUSSION

In Vitro Digestion of Oat Flours and Composition of Digestion Residues. In vitro enzymatic digestibilities of the whole oat flours were 70.7–76.3%, leaving digestion residues of 23.7–29.3% (Table 1). Enzymatic digestion of the oat flours hydrolyzed 81.5–85.3% of the starch and 67.5–72.3% of the proteins (Table 1), with no significant correlations ($P > 0.05$) based on β-glucan content or oat type. Karppinen et al. (24) measured total digestibilities of 60% for oat bran, 42% for rye bran, and 29% for the wheat bran, with starch being the most highly (between 90 and 92%) digested component from the brans. The β-glucan digestibility, measured as the percentage of β-glucan in the hydrolysate (extract) after enzymatic hy-

Table 2. Total Dietary Fiber Composition (Percent) of Oat Flour Digestion Residues (ODR) Determined as Neutral Sugar Residues, Uronic Acid, and Klason Lignin^{a-c}

source of digestion residue	Ara	Xyl	Glu	Rha + Fuc + Man + Gal	Uronic acid	Klason lignin
Jim	3.9 b	4.9 a	75.6 b	5.2 c	7.3 a	3.1 a
Paul	8.2 c	13.3 c	58.0 a	4.0 b	11.9 c	4.6 b
IA95	2.6 a	8.6 b	72.1 b	3.6 a	9.9 b	3.1 a
N979	2.9 a	8.0 b	72.4 b	3.6 a	9.9 b	3.2 a

^a Values are means of three measurements. Values within a column followed by a common letter (a–d) are not significantly different ($P > 0.05$). ^b Ara, arabinose; Xyl, xylose; Glu, glucose; Rha, rhamnose; Fuc, fucose; Man, mannose; Gal, galactose ^c Data normalized to 100%.

**Figure 2.** Changes in pH during in vitro fermentation of oat flour digestion residues (ODR), purified oat β-glucan (POBG), purified oat starch (POS), and lactulose (*n* = 3).

drolysis and dialysis, was 2–17%, measured by using dialysis to remove the digestion products (24). In the current study, the digestion steps were not applied to the lactulose, POBG, and POS: they were used directly as substrates for fermentation. POBG and POS were separately fermented to determine the impact of β-glucan and starch, the main components of the ODR, on the extent and products of the in vitro fermentation process.

Glucose, the monomer of both β-glucan and starch, was the most abundant anhydrosugar found in the neutral sugar residues of the ODR (Table 2). Arabinose, xylose, and lignin, the main components of the insoluble dietary fiber, were greater from the Paul ODR than from Jim, IA95, and N979 ODR. The greater insoluble dietary fiber concentration of Paul compared to Jim, IA95, and N979 oats was previously noted (4). Rhamnose, fucose, mannose, and galactose contents of the substrates were small, with the sum of these four anhydrosugars ≤5.2% (Table 2).

Change in pH during in Vitro Fermentation. Fermentation progress was followed by measuring changes in pH, gas production, and SCFA production, as a function of time. The greatest drop in pH occurred in the POBG treatment (Figure 2). The pH drops for lactulose and starch were greater than for the substrates from ODR. Differences in pH drop among the substrates from ODR mostly were observed between 4 and 8 h of fermentation, when the total gas production generally reached its maximum (Figure 3). The lowest pH values also were observed at that time, after which they slightly increased until the end of the fermentation process (Figure 2). The slight increase in the pH values after 4–8 h may be attributed to the production of different metabolites in the media, such as

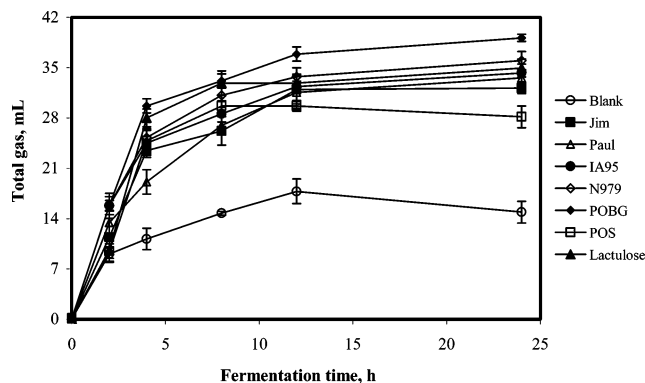


Figure 3. Total gas formation during in vitro fermentation of oat flour digestion residues (ODR), purified oat β -glucan (POBG), purified oat starch (POS), and lactulose ($n = 3$).

ammonia (11), an observation also made elsewhere after 4 h of fermentation of purified β -glucan and oat bran (2).

The decrease in pH was from 6.8 to 5.8 for POBG and from 6.8 to 6.4 for the substrates from high- β -glucan oat flours (IA95 and N979) in the current study. However, the decrease was only from 6.7 to 6.5 and to 6.2 for oat bran and purified oat β -glucan, respectively, in the study by Wood et al. (2). The differences between the pH drops for similar samples in our study versus the referenced study (2) may be attributed to differences in the nature of the fecal inocula used. During a 24 h fermentation of rye bran and its fractions (water-extractable, alkali-extractable, and unextractable fractions), the pH decreased from 7.8–8.0 to 6.6–7.2 (17). Lower pH caused by the production of SCFA can promote a number of positive physiological effects in the colon. Using a rat–dimethylhydrazine colon carcinogenesis model, Samuelson et al. (25) found that rats with more acidic stool pH, produced by consumption of lactulose, had significantly fewer colon tumors after injections of dimethylhydrazine than rats treated with dimethylhydrazine alone. A lower pH is thought to depress the conversion rate of primary to secondary bile acids and lower their carcinogenic potential (26). Furthermore, a low intestinal pH may protect against pathogenic bacteria and aid in the absorption of minerals, such as calcium and magnesium (8).

Production of Gas during in Vitro Fermentation. After 24 h of fermentation, the amount of total gas produced was the greatest with POBG (Figure 3). There were no significant differences ($P > 0.05$) in gas production among the ODR substrates. Fermentation of POS had the lowest gas production, although this substrate was among the greatest in pH drop and SCFA production. Perhaps the POS promoted growth of anaerobic microorganisms that produce high amounts of SCFA but low amounts of gas. Compared with Paul flours, all other oat flour substrates produced gases at higher rates until 4 h of fermentation. Perhaps the higher arabinoxylan (2, 11), uronic acid, and lignin contents of Paul compared to the other substrates (Table 2) caused slower fermentation and, therefore, lower gas production rates. Very high correlations were found between gas production and SCFA formation ($R^2 = 0.89–0.99$). Total amounts of gas produced were 39.2, 28.2, and 34.9 mL/100 mg of POBG, POS, and lactulose, respectively. They were between 32.2 and 36.0 mL/100 mg for the ODR substrates. Wood et al. (2) also reported total gas production of between 30 and 40 mL/100 mg for lactulose and purified oat β -glucan and around 25 mL/100 mg for oat bran.

Production of SCFA during in Vitro Fermentation. Total in vitro SCFA production for each substrate increased by different amounts as fermentation time proceeded (Figure 4).

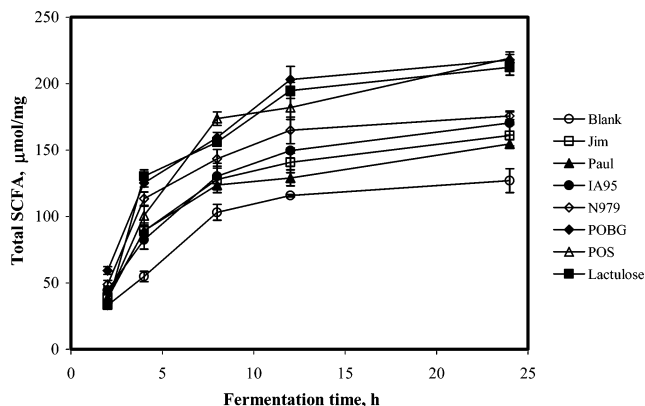


Figure 4. Total short-chain fatty acid (SCFA) formation during in vitro fermentation of oat flour digestion residues (ODR), purified oat β -glucan (POBG), purified oat starch (POS), and lactulose ($n = 3$).

Table 3. Molar Proportions of Acetate, Propionate, and Butyrate after 24 h of Fermentation of the Substrate^a

substrate	acetate	propionate	isobutyrate	butyrate	isovalerate	valerate
Jim	47 d	13 a	4 b	22 b	4 b	9 ab
Paul	44 c	14 ab	4 b	22 b	4 b	11 b
IA95	43 bc	16 b	4 b	23 bc	4 b	10 b
N979	41 b	16 b	4 b	24 c	4 b	11 b
POBG ^b	33 a	23 d	2 a	29 d	3 ab	10 b
POS ^c	51 e	19 c	2 a	18 a	2 a	7 a
Lactulose	49 de	12 a	2 a	25 c	4 b	8 a

^a Values are means of three measurements. Values within a column followed by a common letter (a–d) are not significantly different ($P > 0.05$). ^b POBG, purified oat β -glucan. ^c POS, purified oat starch.

POBG, POS, and lactulose produced higher amounts of SCFA than the substrates from ODR. The substrate from the high- β -glucan oat flours (IA95 and N979) tended to have greater total SCFA production than Jim and Paul, up to 24 h of fermentation. Both total amount and pattern of fermentation varied among substrates. Acetate, propionate, and butyrate were the main SCFA for all of the substrates (Table 3). The total of these three contributed 81–89 mol % of the total SCFA produced. In addition to these three main SCFA, isobutyrate, isovalerate, and valerate also were detected in low proportions (Table 3). Acetate was produced in the greatest amounts by all of the substrates except for POBG, which produced butyrate in the greatest amount. Of the ODR substrates, those with high β -glucan tended to have greater propionate and butyrate, but lower acetate, production. POS had the greatest acetate and the lowest butyrate production, whereas lactulose had the lowest propionate production. Casterline et al. (10) also observed that fermentation of β -glucan produced propionate and butyrate in greater amounts than did starch, pectin, and resistant starch. In contrast, Brouns et al. (27) noted that starch was butyrogenic. Possibly, the microbial population and composition of the inoculum in the current study and the physicochemical properties of oat starch might have affected the results, or starch may be just comparatively less butyrogenic than the substrates used in the current study. Different sources of fiber produce different ratios of SCFA upon fermentation (8, 28, 29). The architecture of the cell wall and the linkages between indigestible compounds may change the fermentability and turn the metabolism products into specific SCFA (28) with particular benefits. For example, propionate has been proposed to inhibit hepatic cholesterol synthesis (30), raise high-density lipoprotein cholesterol and triglyceride, and lower blood glucose and insulin

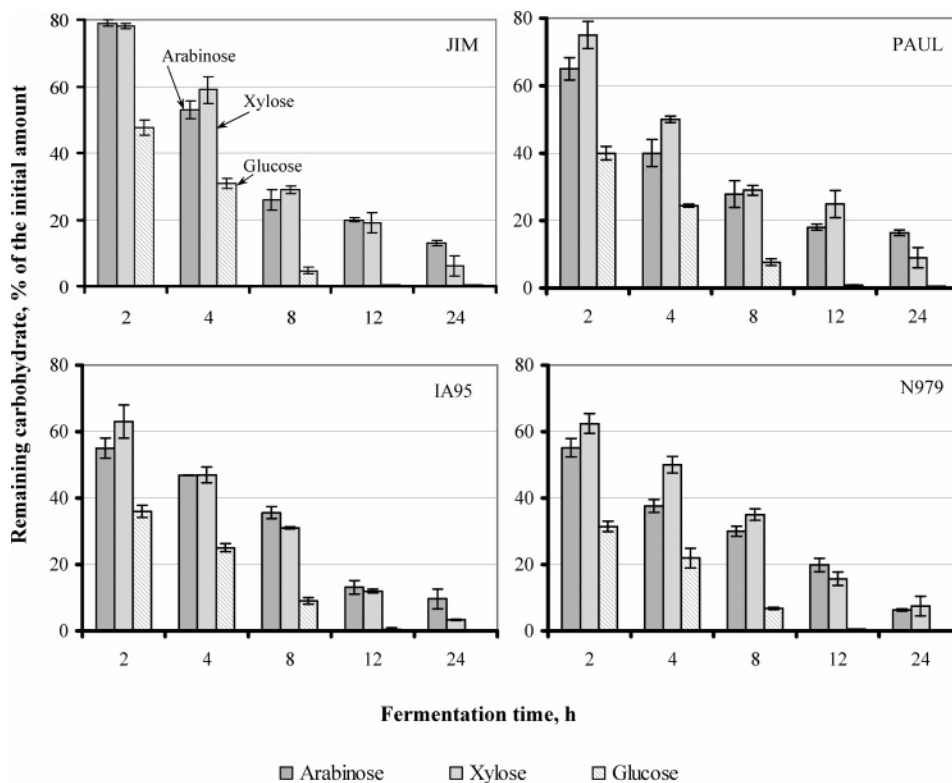


Figure 5. Disappearance of the main neutral sugars (arabinose, xylose, glucose) during in vitro fermentation of oat flour digestion residues (ODR). Disappearance (percent) is expressed as percentage of the initial value ($n = 3$).

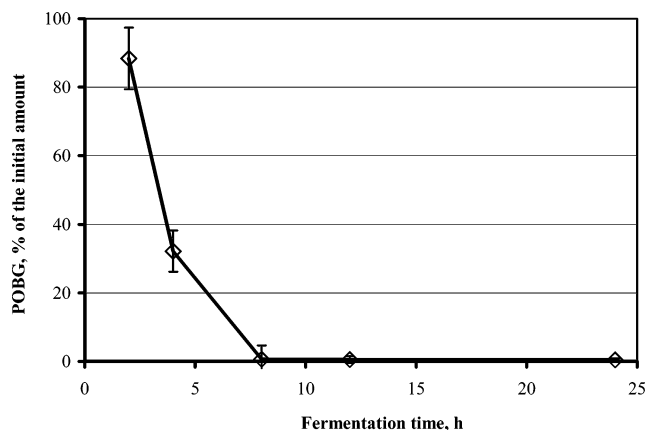


Figure 6. β -Glucan disappearance from purified oat β -glucan (POBG). Disappearance (percent) is expressed as percentage of the initial value ($n = 3$).

levels (31, 32). Also, butyrate is considered to be the major energy source for the colonic epithelium and to have a potential preventive role against colon cancer (32–34).

If one recalculates the results from **Table 3** by considering acetate, propionate, and butyrate as the only three SCFA produced at 24 h, the molar fractions are 39:27:34, 57:14:29, and 58:22:21 for POBG, POS, and lactulose, respectively. The values for the ODR are 50–57% for acetate, 16–20% for propionate, and 27–30% for butyrate, results well within the range of data from the literature. For example, the molar proportions of the major SCFA (acetate, propionate, and butyrate) produced by fermentation of different soluble dietary fiber, insoluble dietary fiber, or pure carbohydrate sources (such as β -glucan, starch, or resistant starch) fell in a broad range: 41–80% acetate, 12–31% propionate, and 9–35% butyrate (2, 10, 14, 17, 34, 35). The proportions of SCFA found in our study for ODR are close to the values of 56:22:22 (acetate/

propionate/butyrate) reported by Karpainen et al. (24) and 57:21:22 (acetate/propionate/butyrate) reported by McBurney and Thompson (16) for oat bran, except the molar percentages of butyrate in our study were slightly greater. McBurney and Thompson (16) noted that both total SCFA production and the molar ratios of SCFA produced in a microbial fermentation can be influenced by substrate. The slightly greater butyrate concentration in the current study may be attributed to the higher β -glucan levels in the ODR.

More than 80% of the available carbohydrates in the fermentation media of the oat flour substrates was depleted (**Figure 5**). Glucose was the most quickly consumed carbohydrate, having almost completely disappeared after 12 h (**Figure 5**). The higher depletion rate of glucose was likely caused by the degradation of β -glucans and starch, which are easily fermentable by colonic bacteria (2, 34). The extent of the POBG depletion during in vitro fermentation also provides evidence that almost all of the β -glucan had been fermented by 8 h (**Figure 6**). Arabinose and xylose were depleted to a lesser extent relative to glucose (**Figure 5**), verifying that arabinoxylans show some resistance to fermentation. These results agree with previously published results (2, 11, 14, 34). Amerin et al. (34) noted that arabinoxylans can be cross-linked by various dehydrodimers of ferulic acid, helping to stabilize cell walls and limit their biodegradability by microorganisms.

Conclusions. Overall, in vitro fermentation of the substrate from oats with greater amounts of β -glucan caused greater drops in pH after 4 h, creating a more favorable environment for the absorption of minerals and prevention of the overgrowth of pH-sensitive pathogenic bacteria. Substrates from the flours with the greatest amounts of β -glucan also tended to produce the greatest relative percent of propionate and butyrate, the SCFA believed to have the greatest potential to reduce the risk of tumorigenesis and hypercholesterolemia (12). Moreover, pentosans were fermented less quickly than glucose, suggesting that

the β -glucans (soluble dietary fiber) would have a better impact on colon health than arabinoxylans (insoluble dietary fiber).

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