Journal of Food and Nutrition Research, 2019, Vol. 7, No. 1, 6-11
Available online at http://pubs.sciepub.com/jfnr/7/1/2
Published by Science and Education Publishing
DOI:10.12691/jfnr-7-1-2

In vitro Fermentation Characteristics of Dietary Fibers from Yellow Millet and Oats

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Received November 07, 2018; Revised December 18, 2018; Accepted January 09, 2019

Abstract The main dietary fibers of yellow millet and oats were isolated. Results from Infrared Spectroscopy (IR) and High Performance Liquid Chromatography (HPLC) revealed that the dietary fibers have the absorption peaks characteristic of sugar ester, and monosaccharide compositions mostly were mainly containing glucose, galactose, rhamnose and arabinose. In order to study the fermentation characteristics of dietary fibers, fresh fecal specimens extracted from healthy adults and children were used as a fermentation model of the human large intestine *in vitro* (anaerobic and 37°C). The concentration of short chain fatty acids (SCFAs) in the system was determined. The results showed that the addition of isolated dietary fibers to the fermentation system of the simulated intestinal environment increased SCFAs content in both adult and child fecal extracts. However, the yield of SCFAs in the child fecal extract fermentation system was substantially higher than that of the adults. This suggests that the model of acids produced from dietary fibers was affected by the fermentation extract sources.

Keywords: *intestinal flora, structural characterization, short chain fatty acids*

Cite This Article: Guo Wen-kui, and Zhao Feng, "*In vitro* Fermentation Characteristics of Dietary Fibers from Yellow Millet and Oats." *Journal of Food and Nutrition Research*, vol. 7, no. 1 (2019): 6-11. doi: 10.12691/jfnr-7-1-2.

1. Introduction

In recent years, with increases in health awareness, the physiological functions and processing characteristics of dietary fibers are being valued more and more by the public [1]. Dietary fiber has an important physiological functions and benefits to the human body, such as the prevention of constipation and rectal cancer, lowered serum cholesterol, regulation of blood sugar levels, prevention of gallstones, adsorption of sodium ions to reduce blood pressure and weight loss [2].

Short chain fatty acids (SCFAs) are products of dietary fiber fermentation in the colon, and includie formic acid, acetic acid, propionic acid, butyric acid, and succinate acid [3,4] SCFAs also have an important roles in human health, affecting colon epithelium, the promotion of cell transportation and the metabolism and growth of cells in the colon and small intestine [5].

Millet and oats are high in nutritional value, contain essential amino acids and are rich in dietary fiber. In this study, we focused on the structural characterization and *in vitro* fermentation characteristics of the main dietary fibers isolated from millet and oats.

2. Materials and Methods

2.1. Materials

Yellow millet and oats were purchased from a supermarket in Harbin, China; SCFA standards, including formic acid, acetic acid, propionic acid, and butyric acid, were purchased from the Tianjin Guangfu Fine Chemical Research Institute (China). All other chemicals were of reagent grade. HPLC was performed on a Waters Corp. (USA) instrument equipped with a 2695-2487 ultra-violet detector (UV) and a 2695-2414 refractive index detector (RID). Fourier transform-infrared spectra (FT-IR) were recorded using an 8400S Shimadzu, Inc. spectrophotometer, (Japan).

2.2. Extraction of Dietary Fiber

Millet and oats were crushed and passed through a 60 mesh sieve, after which, 4 volumes of ethyl acetate was added to the powder and the fatty skimmed for 3 h. Then washed the powder with distilled water to tasteless, oven drying to constant weight. Dietary fibers were extracted by combining millet and oats powders with hot water (powder: water = 1: 8), and adding 0.2% mixed enzymes

(α- amylase: glucoamylase = 1:3) at 68 °C for 30 min, 0.3% protease enzyme was then added to the hydrolyzed samples at 60°C for 60 min, following which the enzyme was inactivated at 100°C for 10 min; Using a vacuum suction filter, the samples were concentrated to 1/3 of their original volume, after which, 4 volumes of ethanol were added and allowed to sit for 12 h. The sample was then filtered and oven dried to yield water-soluble dietary fibers of millet and oats [6].

2.3. Chemical Structure Analysis of Dietary Fiber

IR spectra were recorded using the KBr-disk method with FTIR 8400S. Monosaccharide structural identification of dietary fibers was determined by adding water (1 mL) and H_2SO_4 (2 mL, 2 mol/L) to a dried sample of dietary fiber (20 mg). The reaction mixture was oven at 110°C for 8 h for hydrolysis. After cooling the reaction mixture to room temperature, excess acid was neutralized by the addition of NaOH (8 mol/L), and the total volume of the reaction mixture was adjusted to 5 mL. Pyridoximine-5'-phosphate (PMP) (200 uL, 0.5 mol/L) and NaOH (200 uL, 0.3 mol/L) were added to each of the monosaccharide standards (200 uL) and dietary fiber samples (200 uL). The mixtures were heated in a bath at 70 °C for 30 min. After cooling the reaction mixtures down to room temperature, 200 uL HCl (0.3 mol/L) was added. Monosaccharides were extracted with chloroform (1 mL), while the volume of the water phase remained constant at 1 mL. As presented in other studies, the following HPLC procedure was used HPX-87H column, column temperature of 55°C, flow rate of 0.5 mL/min, injection volume of 20 uL, and a running time of 30 min [7].

2.4. *In vitro* Fermentation of Dietary Fiber

Human faces were anaerobically collected from 5 healthy adults (28-35 years old) and 5 healthy children (2-4 years old) who had received no antibiotic treatment for the past month and experienced no abdominal diarrhea or enteritis. About 40 g fecal samples were dissolved in 200 mL of phosphate buffer (0.1 mol/L, pH 6.5) and homogenized for 1 min to produce a 200 g/L suspension. The suspension was filtered through four layers of nylon sieve to removed particulate matter. The collection and preparation were completed within 1 h [8]. Approximately 5 mL of the fecal suspension from healthy adults and healthy children was transferred to 5 mL of phosphate buffer containing 2.0 g/L, 4.0 g/L, and 8.0 g/L dietary fibers, respectively. Phosphate buffer without dietary fibers was used as a blank control. The mixtures were anaerobically cultured with continuous shaking (100 r/min) at 37 °C for 6, 12, 24 and 36 h. Following fermentation, the samples were mixed and centrifuged (3000 r/min, 10 min, 4 °C) and the supernatants stored at 20°C prior to analysis and determination of SCFAs content [8].

2.5. Analysis of Short Chain Fatty Acids

The supernatants were filtered through 0.45um film for further HPLC analysis. The HPLC conditions used were as follows: HPX-87H column; column temperature: 55 °C; flow rate of 0.5 mL/min; injection volume: 20 uL; running time of 30 min; UV detector, wavelength of 210 nm.

2.6. Statistical Analyses

All experiments were performed in triplicate, and the data were expressed as mean value ± standard error. Statistical analysis was performed using Origin 8.5 software. Data that presented $p < 0.05$ were considered statistically significant.

3. Results and Discussion

3.1. FT-IR Analysis and Structural Identification of the Monosaccharide Composition of Dietary Fiber

From the infrared absorption spectra results, shown in Figure 1 and Figure 2, we can see that the dietary fiber samples have the characteristic absorption peaks of sugar ester. In the IR spectra of the sample of dietary fiber samples from millet, we can see that the characteristic absorption peaks of polysaccharides are as follows: the absorption at 3402 cm^{-1} was the peak of the O-H stretching vibration bond; the absorption at 2926 cm^{-1} was based on a sugar methyl and methylene contraction vibration of C-H ; the absorption peak at 2340 cm^{-1}-2358 cm^{-1} was C≡C; the peak at 1667 cm^{-1} was a characteristic phenyl absorption peak; the dietary fiber of millet had peaks at 1024 cm^{-1} -1080 cm^{-1}, implying that they were composed of C-O-C ring ether and C-O stretching vibration and had the C-O-H of O-H variable angle vibration; a weak absorption peak at 847 cm^{-1} was a α-configuration C-H bond [9,10]; all of the peaks were characteristic absorption peaks of polysaccharide.

Similar to the infrared spectrum of dietary fiber from millet, the IR spectra (Figure 2) of the dietary fiber from oats also showed the characteristic absorption peaks of a sugar ester: the absorption at 3421 cm^{-1} was the peak of the O-H stretching vibration bond; the absorption at 2974 cm^{-1}-2926 cm^{-1} was based on a sugar methyl and methylene contraction vibration of C-H; the absorption peak at 2340-2358 cm^{-1} was C≡C; the dietary fiber of oats had a peak at 1049cm^{-1}, implying that they are composed of C-O-C ring ether and C-O stretching vibration and had the C-O-H of O-H variable angle vibration; a weak absorption peak at 876 cm^{-1} was a α-configuration C-H bond.

The HPLC results revealed that the monosaccharide compositions of dietary fiber from millet and oats consist mostly of glucose, galactose, rhamnose and arabinose, as shown in Figure 3. The relative monosaccharide composition percentage content of the dietary fiber of millet was 54.46%, 0.06%, 40.70%, 4.78%, while the relative percentage content of the dietary fiber of oats was 51.38%, 0.09%, 43.47% and 5.06%, respectively. The results showed that the composition of the monosaccharide composition in the water-soluble dietary fiber of millet and oats were had high similarity.

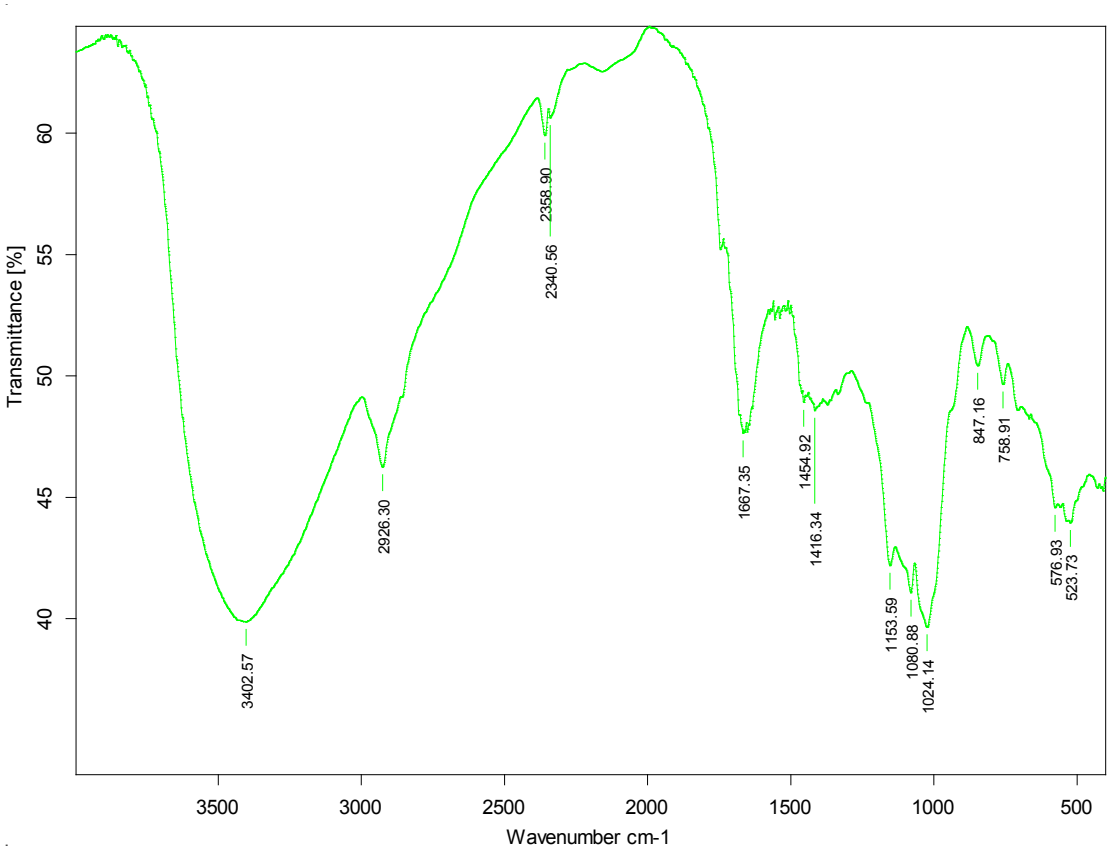

Figure 1. The IR spectrum of the dietary fiber extracted from millet

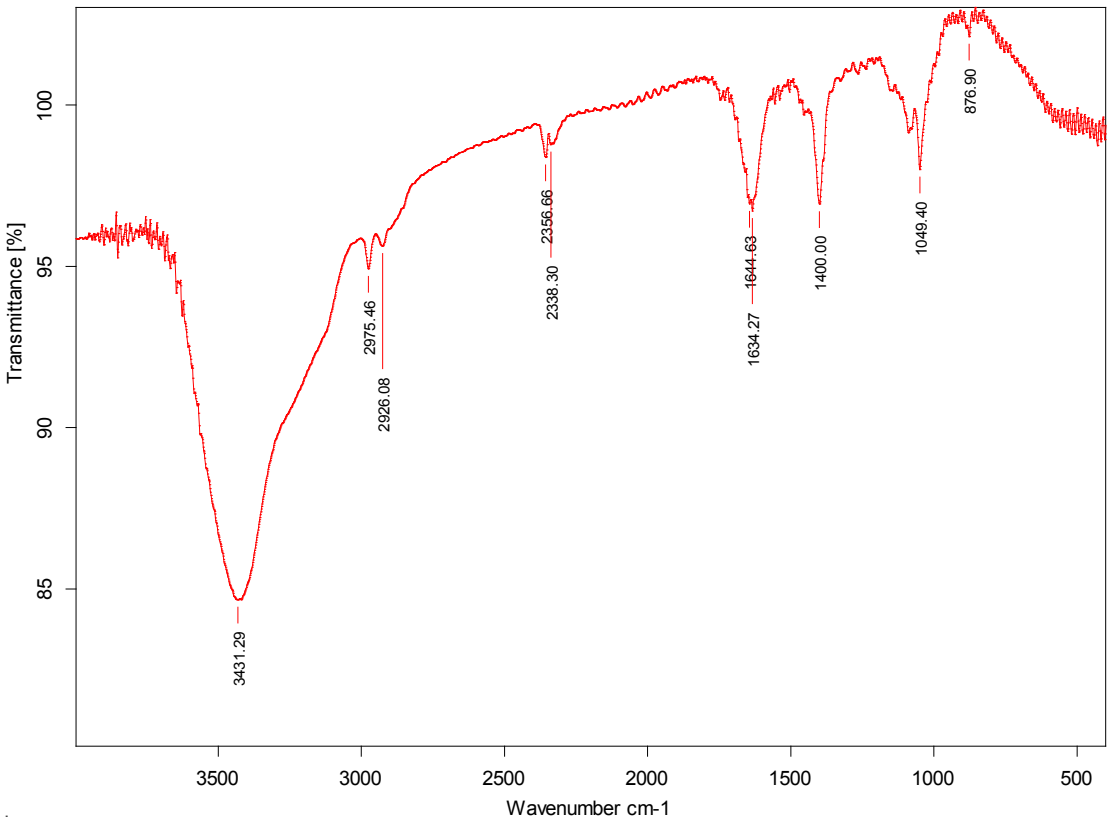

Figure 2. The IR spectrum of the dietary fiber extracted from oats

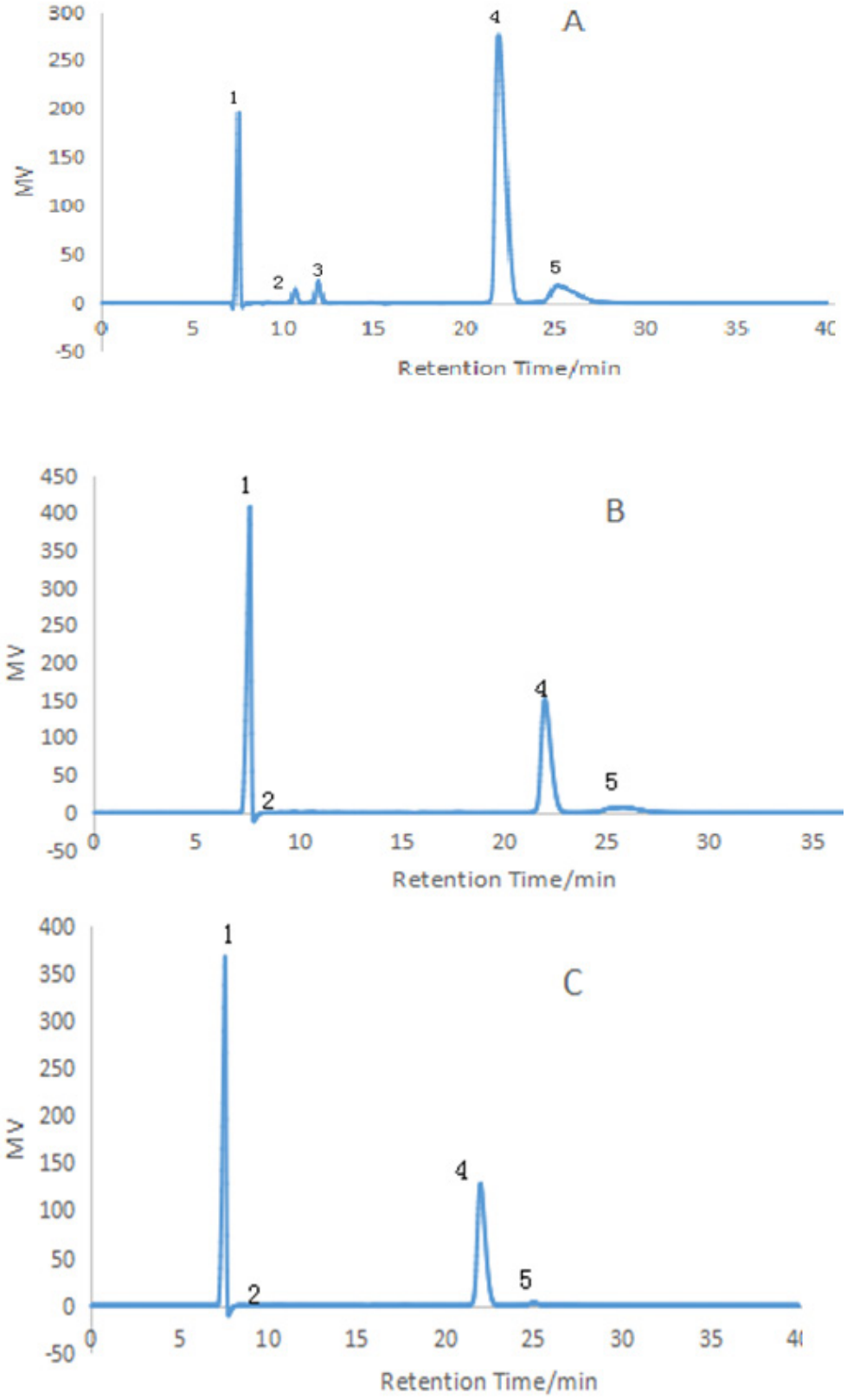

Figure 3. The HPLC chromatogram presenting the monosaccharide composition of the SDFs extracted from millet and oats(A: monosaccharide standards, B: monosaccharide composition of the SDF extracted from millet, C: monosaccharide composition of the SDF extracted from oats; 1, rhamnose; 2, arabinose; 3, mannose; 4, glucose; 5, galactose.)

3.2. Analysis of SCFAs Produced during *in vitro* Fermentation

The production of SCFAs from millet and oats dietary fiber fermentation in the simulated adult intestinal environment, is shown in Table 1 and Table 2. In Table 1, we can see that the SCFAs in the fermentation products gradually increased with increased fermentation time, and also gradually increased with the increased dietary content of millet. All SCFAs, except formic acid, increased between 12 h and 24 h of fermentation, but this increase was smaller after 24 h. Formic acid was gradually

increased between 24 h and 36 h of fermentation. The results showed that most SCFAs were produced within 12 h to 24 h in the fermentation process.

In Table 2, we can see that the production of formic acid, acetic acid and propionic acid were increased between 6 h to 12 h of fermentation.; Butyric acid, different from other SCFAs, increased from 24 h to 36 h. Similarly, SCFAs in the fermentation process also gradually increased with the increased dietary fiber content of oats.

As shown in Table 1 and Table 2, formic acid, acetic acid and propionic acid in the fermentation products increased with increased fermentation time and dietary fiber content, and had similar rates of production. However, the increasing rate of butyric acid production was slower than that of other SCFAs, only increasing from 0.085 mol/L to 0.102 mol/L in the content at 2.0 g/L after 6 h of millet fermentation; and increasing from 0.068 mol/L to 0.174mol/L in the content at 2.0 g/L of oats after 6 h of fermentation. A concentration of butyric acid was at 0.196 mol/L was found in all the fermentation systems, only increasing 1.33 and 2.39 times in the content of 8.0 g/L after 36 h of fermentation.

Similarly to the results obtained from the adult fecal fermentation system, SCFAs also increased with the extension of the fermentation time and increase in dietary fiber content, in the child fecal fermentation system, as shown in Table 3 and Table 4. Interestingly, SCFAs, including formic acid, acetic acid, propionic acid, and butyric acid, were all higher in the child fecal extracts system than in the adult fecal extracts. For example, butyric acid concentrations reached 1.103 mol/L and 0.919 mol/L with 8.0 g/L of millet and oats fiber, respectively, after 36 h in healthy child fecal extract fermention systems.

Table 1. Production of short-chain fatty acids (SCFAs) in millet fermentation system (adults)

Concentration of dietary fibers from millet (g/L)	Time (h)	Organic acids (mol/L)			
		Formic acid	Acetic acid	Propionic acid	Butyric acid
2.0	6	0.015±1.25	0.345±0.47	0.199±1.98	0.085±1.43
	12	0.104±0.37	0.505±0.48	0.145±2.70	0.093±0.43
	24	0.209±0.74	0.308±0.36	0.487±0.28	0.101±0.13
	36	0.286±0.29	0.407±0.94	0.573±0.37	0.102±0.04
4.0	6	0.156±0.05	0.448±1.46	0.241±0.18	0.095±0.36
	12	0.118±0.36	0.619±1.96	0.392±0.43	0.111±0.24
	24	0.211±0.47	0.767±0.37	0.694±0.33	0.121±0.54
	36	0.509±0.18	0.703±0.96	0.755±0.27	0.130±0.23
8.0	6	0.384±0.28	0.513±1.47	0.292±0.94	0.147±0.34
	12	0.674±1.47	0.793±1.98	0.449±1.47	0.197±1.93
	24	0.498±1.97	0.926±0.36	0.263±0.86	0.129±0.34
	36	0.861±0.95	1.006±0.06	0.815±0.36	0.196±0.22

Data were expressed as x±SD (n = 3).

Table 2. Production of short-chain fatty acids (SCFAs) in oats fermentation system (adults)

Concentration of dietary fibers from oats (g/L)	Time (h)	Organic acids (mol/L)			
		Formic acid	Acetic acid	Propionic acid	Butyric acid
2.0	6	0.101±0.34	0.035±0.45	0.103±1.02	0.068±1.43
	12	0.118±0.47	0.493±0.44	0.108±0.34	0.074±0.45
	24	0.151±0.33	0.144±0.24	0.284±0.95	0.109±0.63
	36	0.278±0.47	0.200±0.67	0.608±0.74	0.174±0.64
4.0	6	0.176±1.48	0.359±1.94	0.277±0.34	0.074±0.34
	12	0.179±0.58	0.599±2.54	0.335±0.55	0.084±0.75
	24	0.398±0.34	0.182±0.34	0.477±0.19	0.126±0.66
	36	0.303±0.22	0.332±0.76	0.658±1.47	0.186±0.93
8.0	6	0.197±0.98	0.486±0.77	0.293±0.45	0.082±0.94
	12	0.239±0.34	0.746±0.13	0.517±0.43	0.096±0.45
	24	0.349±1.44	0.668±0.45	0.543±0.95	0.145±1.76
	36	0.331±0.45	0.838±1.49	0.713±0.03	0.196±0.45

Data were expressed as x±SD (n = 3).

Table 3. Production of short-chain fatty acids (SCFAs) in millet fermentation system (children)

Concentration of dietary fibers from millet (g/L)	Time (h)	Organic acids (mg/L)			
		Formic acid	Acetic acid	Propionic acid	Butyric acid
2.0	6	0.063±1.46	0.066±0.65	0.125±0.84	0.238±1.65
	12	0.109±0.34	0.229±0.93	0.238±0.56	0.371±0.75
	24	0.172±0.46	0.493±1.34	0.310±0.84	0.739±0.43
	36	0.297±0.85	0.793±1.06	0.385±0.33	0.769±0.27
4.0	6	0.089±0.03	0.640±0.45	0.371±0.75	0.241±0.37
	12	0.145±0.35	0.735±0.87	0.415±1.56	0.600±0.26
	24	0.184±0.75	0.906±0.77	0.552±0.65	0.739±0.85
	36	0.245±0.74	0.971±1.67	0.721±0.45	0.799±0.36
8.0	6	0.196±1.54	0.743±0.67	0.562±0.75	0.244±1.67
	12	0.230±0.45	1.660±0.33	0.575±0.33	0.859±2.66
	24	0.490±0.23	1.699±0.60	0.686±0.75	1.050±0.64
	36	0.584±0.45	1.921±0.37	0.873±0.85	1.103±0.75

Data were expressed as x±SD (n = 3).

Table 4. Production of short-chain fatty acids (SCFAs) in oats fermentation system (children)

Concentration of dietary fibers from oats(g/L)	Time (h)	Organic acids (mg/L)			
		Formic acid	Acetic acid	Propionic acid	Butyric acid
2.0	6	0.173±0.54	0.525±0.35	0.218±1.56	0.124±1.68
	12	0.196±0.56	0.623±0.56	0.317±0.46	0.300±0.54
	24	0.198±0.54	0.638±0.96	0.389±0.33	0.387±0.66
	36	0.271±1.54	0.701±0.77	0.412±0.75	0.395±0.34
4.0	6	0.198±0.65	0.448±0.34	0.264±0.56	0.415±0.37
	12	0.286±0.76	0.619±0.56	0.368±0.37	0.346±0.55
	24	0.288±0.65	0.767±0.76	0.442±0.74	0.409±0.39
	36	0.358±0.04	0.703±0.30	0.519±1.35	0.854±0.97
8.0	6	0.207±1.65	0.647±1.58	0.329±0.86	0.442±1.58
	12	0.306±0.75	1.275±0.43	0.418±0.43	0.498±2.66
	24	0.298±0.54	1.398±0.85	0.604±0.64	0.658±0.54
	36	0.478±0.86	1.373±0.87	0.986±0.85	0.919±0.32

Data were expressed as x±SD (n = 3).

4. Conclusions

We extracted fresh feces from healthy adults and children, and used the native microbial flora from these adults and children to simulate the intestinal environmentin to ferment dietary fibers *in vitro*. The dietary fiber samples have the characteristic absorption peaks of sugar ester, and the monosaccharide composition percentage content of the dietary fiber of millet was 54.46%, 0.06%, 40.70%, 4.78%, while the relative percentage content of the dietary fiber of oats was 51.38%, 0.09%, 43.47% and 5.06%. We analyzed these extracts for SCFAs resulting from fermentation and found that these gradually increased with increasing fermentation time and dietary fiber content. However, levels of SCFAs were higher in the children fecal extracts system than in the adult fecal extracts system. We believe that the changes in the levels of SCFAs were mainly due to differences in microbial metabolism and the composition in the fermentation system.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (Grant No. 30671700) and the Natural Science Foundation of the Heilongjiang Province (C2016006).

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