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ORIGINAL ARTICLE

Targeted Metabolomic Analysis of Seven Short-Chain Fatty Acids in Feces of Rats with Spleen-Deficiency Syndrome after Administering Raw and Bran-Fried Atractylodis Rhizoma by Gas Chromatography-Mass Spectrometer

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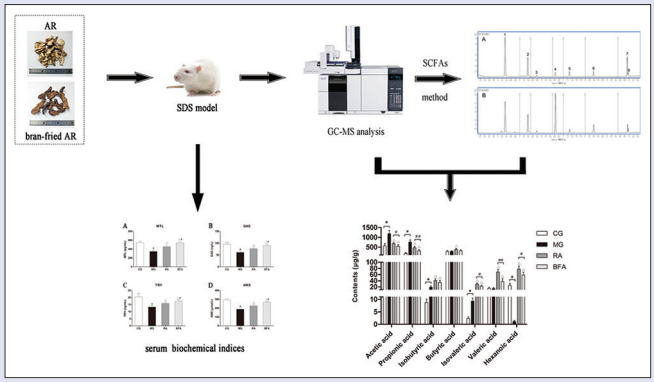
ABSTRACT

Background: For treating spleen-deficiency syndrome (SDS), atractylodis rhizoma (AR) is usually treated with bran, which is measured to enhance efficacy. Newly, we found that bran-fried AR (BFA) had benefits over raw AR in refining gut flora. However, the change about metabolism of gut flora caused by raw and BFA remains unclear. **Aim:** The aim of this study was to compare the difference between raw and BFA acting on short-chain fatty acid (SCFA) metabolism in SDS rats, a metabolic pathway comprising gut flora. **Materials and Methods:** The model of SDS rats was recognized and then given raw and BFA by gavage. The serum was collected for the determination of motilin (MTL), gastrin (GAS), trypsin (TRY), and amylase (AMS) by enzyme-linked immunosorbent assay. The feces were collected for the determination of 7 SCFAs under selective ion monitoring mode by gas chromatography-mass spectrometer. **Results:** Raw and BFA can upsurge the levels of MTL, GAS, TRY, and AMS in the serum of SDS rats, diminution the levels of acetic acid and propionic acid, and rise the level of hexanoic acid in the feces of SDS rats, and BFA showed noteworthy activity than raw AR. **Conclusion:** Raw and BFA can improve the acetic acid, propionic acid, and hexanoic acid metabolism in SDS rats, and BFA was more potent than raw AR.

Key words: Atractylodis rhizoma, processing of traditional Chinese medicine, short-chain fatty acids, spleen-deficiency syndrome, targeted metabolomics

SUMMARY

- A rapid method was recognized for concurrently decisive seven short-chain fatty acids (SCFAs) in rat feces by gas chromatography-mass spectrometer, which was simple and accurate with short time, high sensitivity, and satisfactory recovery
- This method was applied to analyze the metabolic effect caused by raw and bran-fried atractylodis rhizoma (AR) on SCFAs in spleen-deficiency syndrome (SDS) rats
- The mechanism of AR-treated SDS may be related with the development of metabolism of SCFAs
- The enhanced-efficacy mechanism of bran-fried AR could be related to its better enhancement on acetic acid, propionic acid, and hexanoic acid metabolism.



Abbreviations used: AR: Atractylodis rhizoma; SDS: Spleen-deficiency syndrome; SCFA: Short-chain fatty acid; MTL: Motilin; GAS: Gastrin; TRY: Trypsin; AMS: Amylase; GC-MS: Gas chromatography-mass spectrometer; IS: Internal standard; SPF: Specific pathogen free; CG: Control group; MG: Model group; RA: Raw AR; BFA: Bran-fried AR; ELISA: Enzyme-linked immunosorbent assay; SIM: Selective ion monitoring; TIC: Total ion chromatogram; QI: Quantitative ion; AQI: Auxiliary qualitative ion; QC: Quality control; RSD: Relative standard deviation; MC: Mass concentration; LOD: Limit of detection; LOQ: Limit of quantitation.

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INTRODUCTION

Atractylodis rhizoma (AR) was the dried rhizome of *Atractylodes lancea* (Thunb.) DC. or *Atractylodes chinensis* (DC.) Koidz. It has good effectiveness in clearing clammy and tonifying spleen.[1] Bran-fried AR (BFA) is the only treated product of AR recorded in Chinese Pharmacopoeia. In view of traditional Chinese medicine, BFA is thought to have distinct benefits in relieving dryness[2] and establishment the spleen and stomach[3] than raw AR. Therefore, bran fried is often employed to treat spleen-deficiency syndrome (SDS) in traditional Chinese medicine.

There have been many earlier reports regarding the enhanced-efficacy mechanism of BFA against SDS in our team,[4-7] which have providing some help for our understanding of the processed mechanism of BFA. However, they were still inadequate as a whole. In a recent study, we

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found that BFA was better than raw AR in regulating gut flora in SDS rats.[8] Lots of investigates have found that gut flora not only includes in immune response and serves as a physiological barrier but also has a significant influence on the host's endogenous metabolism.[9] Short-chain fatty acids (SCFAs) are volatile fatty acid with 2–6 carbon atoms, metabolites closely related to gut flora.[10] Straight-chain SCFAs are mostly produced from unabsorbed carbohydrates in food fermented by gut flora, comprising acetic acid and propionic acid, etc.[11] Branched-chain SCFAs are mostly produced from gut flora through metabolizing branched-chain amino acids, comprising isobutyric acid and isovaleric acid, etc.[12] SCFAs play a variety of purposes *in vivo* such as preserving the homeostasis of gut flora and electrolyte, and regulating cell proliferation and gene expression,[13-15] whose insufficient secretion or excessive secretion can cause physiological ailments. Therefore, it will be supportive for our sympathetic of the enhanced-efficacy mechanism of BFA from a new perspective through reviewing the effect caused by raw and BFA on SCFA level in SDS rats.

The common quantitative methods of SCFAs comprise high-performance liquid chromatography,[16] gas chromatography (GC),[17] capillary electrophoresis,[18] and so on. GC is the most normally used method. In this study, a quantitative method was recognized to regulate 7 SCFAs in rat feces by gas chromatography-mass spectrometer (GC-MS), which was simple and precise with short time, and had high sensitivity and a satisfactory retrieval. We applied this method to analyze the metabolic effect caused by raw and BFA on SCFAs in SDS rats, which can happen the quantitative requirements of SCFAs in rat feces.

MATERIALS AND METHODS

Materials of raw and bran-fried atractylodis rhizoma

AR was procured from Luotian Chinese medicine planting base (Hubei, China). It was the dried rhizome of *A. lancea* (Thunb.) DC. recognized by Prof. Feng Li (Liaoning University of Traditional Chinese Medicine, Dalian, China). BFA was treated by ourselves from the same batch of raw AR according to the method recorded in Chinese Pharmacopoeia. The processed method was as trails: the bran was put into a heated pot firstly, and then, the raw AR was added to the pot and stir fried rapidly until the surface was dark yellow (weight ratio of wheat bran and raw AR was 1:10). The BFA was acquired after the bran was shifted out.

Materials of senna leaf

Senna leaf (batch number: 20190421) was procured from Dalian Haiwang Xingchen Pharmacy (Dalian, China), heated for 30 min, filtered, and then concentrated into 500 mg/mL decoction for usage.

Preparation of raw and bran-fried atractylodis rhizoma

Raw and BFA were all powdered and approved through a 160 mesh sieve and mixed to 375 mg/mL powder suspension with water correspondingly before being used.

Chemicals

Acetic acid (batch number: 167834), propionic acid (batch number: 833588), isobutyric acid (batch number: 170,589), butyric acid (batch number: 50504), valeric acid (batch number: G148888), and hexanoic acid (batch number: G147661) were procured from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Isovaleric acid (batch number: A05M10 L87327) was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Hexanoic acid-d3 (batch number: 1-AAW-38-1) was procured from Toronto Research Chemicals Inc. (Toronto, Canada). The purity of all SCFA standard products was above 99% by GC.

Motilin (MTL), gastrin (GAS), trypsin (TRY), and amylase (AMS) kits were all procured from Shanghai Kexing Trading Co., Ltd. (Fankew, Shanghai, China).

Ethyl ether (batch number: 20170109), chromatographic purity, was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Phosphoric acid (batch number: 20170402), analytical purity, was procured from Tianjin Komeo Chemical Reagent Co., Ltd. (Tianjin, China).

Preparation of standard solution

According to individual densities of 7 targeted SCFAs and hexanoic acid-d3 (internal standard [IS]), the corresponding volumes were exactly transferred into the volumetric flask and dissolved by ethyl ether to mixed standard stock solutions of 30 mg/mL and IS solution of 1 mg/mL. The standard solutions were prepared and refrigerated at 4°C to avoid the volatilization of SCFAs.

Animals

Forty specific pathogen-free male Sprague Dawley rats (200 ± 20 g) were procured from Liaoning Changsheng Biotechnology Co., Ltd. (license number: SCXK [Liao] 2010-0001, Benxi, Liaoning, China). The rats were accommodated at a certified animal experimental laboratory and permitted free admittance to food and water for 1 week's adaptation. All the procedures were permitted by the Animal Ethics Committee of the Affiliated Hospital of Liaoning University of TCM, Shenyang, China (approval number: 2019YS [DW]-028-01).

Experimental design

The rats were randomly separated into four groups (n = 10): (1) the control group (CG), (2) the model group (MG), (3) the raw AR (RA) group, and (4) the BFA group. Rats in all groups except the CG were recognized a SDS model by compounding factor method, which contained irregular diet, excessive fatigue, oral bitter, and cold Chinese medicine. The model formation referred to Xue[5] and Ma.[8] During the first 2 weeks (days 1–14), the rats were given lard (10 mL/kg) by intragastric in odd days, forced to swim, and fed only Chinese cabbage easily in even days. During the 3rd week (days 15–21), the rats were given senna leaf decoction (10 mL/kg) by intragastric. The establishment of SDS model lasted for 3 weeks, when the wetted bedding was employed for rats during this process. During the 4th week (days 22–28), the rats in the RA and BFA groups were given the corresponding powder suspension (3750 mg/kg) by intragastric, respectively. The administration time was in the morning, once a day, and the rats in CG and MG were gavaged with normal saline everyday.

Collection of biological samples

Fresh feces of four groups were collected into the sterile centrifuge tubes from the same time point on the afternoon of the 28th day, frozen by liquid nitrogen, and transferred to −80°C refrigerator for storage. All rats were abstained for one night and anesthetized with 10% urethane (1 g/kg) intraperitoneally. Blood samples of the abdominal aorta were composed on the morning of the 29th day. The upper serum was found after the centrifugation for 5 min (4000 rpm, 4°C). The rats were euthanized after the collection of samples.

Biochemical analysis

The enzyme-linked immunosorbent assay (ELISA) was employed to regulate the content of serum MTL, GAS, TRY, and AMS in rats of

each group. All the assays were carried out according to the manual of directives provided by the manufacturers.

Blank fecal matrix preparation

The fresh feces in the CG were collected from the same time point, frozen by liquid nitrogen, then ground, and mixed evenly. The feces were exactly separated into 100 mg in EP tubes and stored at −80°C.

Fecal sample pretreatment

The method of fecal sample pretreatment denoted to Jia *et al*.[17] and improved by our team. 100 mg feces were evaluated exactly, added with 100 μL 15% phosphoric acid, 10 μL IS solutions (1 mg/mL), and 500 μL ethyl ether, respectively. The fecal samples were homogenized for 0.5 min and carried out by ultrasonic for 1 min. The supernatants were acquired after centrifugation for 10 min (12,000 rpm, 4°C) and then added with a little anhydrous sodium sulfate powder and hesitated to eliminate trace water in the samples. The supernatants were found after centrifugation for 10 min (12,000 rpm, 4°C) and then transferred into an injection vial for analysis. The above pretreatment steps were carried out at 4°C to avoid the volatilization of SCFAs.

Gas chromatography-mass spectrometry of short-chain fatty acids

The method of GC-MS analysis of SCFAs mentioned to Jia *et al*.[17] and improved by our team. The experiment was carried out on an Agilent 7890B gas chromatograph coupled with an Agilent 5977B mass spectrometric detector (Santa Clara, CA, USA). Chromatographic experiment was carried out on an HP-INNOWax capillary column coated with cross-linked polyethylene glycol (30 m × 0.25 mm i. d., 0.25 μm film thickness, Agilent, Folsom, CA, USA). The injection volume was 1 μL, and the ratio of shunt was 10:1. The solvent delay time was set at 3 min. Helium was employed as carrier gas with a constant flow rate of 1 mL/min. The initial oven temperature was set at 90°C, to 120°C at a rate of 10°C/min, to 150°C at a rate of 5°C/min, to a final temperature of 250°C at a rate of 25°C/min, and held for 2 min. The temperatures of the front inlet, transfer line, quadrupole, and electron impact ion source were set at 250°C, 250°C, 150°C, and 230°C, respectively. The electron energy was 70 eV. The scan mode (*m/z* 30–600) and selective ion monitoring (SIM) mode were employed to collect mass spectral data.

Method validation of gas chromatography-mass spectrometer

Selectivity

Seven SCFA standard solutions and fecal samples were analyzed in the scan mode, and total ion chromatogram (TIC) was recorded. According to the NIST 11.0 library and standard reference, 7 SCFAs in the samples were recognized qualitatively and the peak attribution was determined. According to the strength of fragment peaks in mass spectrometry and base peaks were selected as the quantitative ions (QIs) of target compounds, excimer ion peaks, and unique fragment ions of target compounds were designated as the auxiliary QIs. In the mode of SIM, ion profiling and quantitative analysis of 7 SCFAs were carried out.

Linearity

30 mg/mL mixed SCFA standard stock solution was slowly diluted into a series of concentration of 15, 7.5, 1.5, 0.3, 0.03, and 0.003 mg/mL by ethyl ether. 10 μL of a series of concentration of 30, 15, 7.5, 1.5, 0.3, 0.03, and 0.003 mg/mL was, respectively, taken and added to each equal 100 mg blank fecal matrix and then analyzed after being pretreated according to the method under 2.8 items. Taking the standard concentration as abscissa and the peak area ratio of the standard to IS (excluding peak areas ratio of blank fecal matrix) as ordinate, the standard curve was conspired to fit the regression equation.

Intra- and inter-day precision

The low, medium, and high concentrations (0.25, 5, and 400 μg/mL) of quality control (QC) samples were prepared according to the method under 2.8 items by adding different concentrations of mixed SCFA standard solution to blank fecal matrices and then injected and analyzed. Intra-day precision was assessed by 5 successive determinations of analytes. Inter-day precision was assessed by analyzing the samples for 5 following days. The precision value was found by calculating the relative standard deviation (RSD) of the peak area ratio of each targeted SCFA to IS (excluding peak area ratio of blank fecal matrix).

Stability

The stability of fecal samples was appraised when stored at a low temperature in −80°C refrigerator. Fecal samples stored in −80°C refrigerator for 1, 2, 4, 7, and 14 days were added with mixed SCFA standard solution of different concentrations to prepare the low, medium, and high concentrations (0.25, 5, and 400 μg/mL) of QC samples according to the method under 2.8 items and then injected and analyzed. The ratios of SCFA peak areas to IS peak areas (excluding the peak area ratio of blank fecal matrix) were recorded. The mass concentration (MC) of each SCFA was planned according to the working curve of the day. The stability was assessed by the RSD of MC.

The stability of SCFA solution was assessed when stored at the room temperature. The mixed standard solutions of SCFAs with low, medium, and high concentrations (0.25, 5, and 400 μg/mL) were injected and analyzed after being stored at the room temperature for 0, 4, 8, 12, and 24 h. The peak area ratios of SCFAs to ISs were recorded. The MC of each SCFA was calculated according to the working curve of the day. The stability was gauged by the RSD of MC.

Extraction recovery and matrix effect

The sample analysis was carried out as the following four groups: I: Blank fecal matrix samples were pretreated and then injected and analyzed directly; II: Blank matrix samples were added low, medium, and high (0.25, 5, and 400 μg/mL) concentrations of mixed SCFA standard solutions after pretreatment and then injected and analyzed; III: Blank matrix samples were added low, medium, and high (0.25, 5, and 400 μg/mL) concentrations of mixed SCFA standard solutions, pretreated, and then injected and analyzed; and IV: Low, medium, and high (0.25, 5, and 400 μg/mL) concentrations of mixed SCFA standard solutions were added the same concentration of IS solution and then injected and analyzed directly. The ratios of peak areas of each SCFA to IS peak areas were verified and the MC of each SCFA was calculated according to the working curve of the day. The calculation formula of extraction recovery rate employed was: (III MC–I MC)/(II MC–I MC). The calculation formula of matrix effect used was: (II MC–I MC)/IV MC.

Quantitative determination of short-chain fatty acids in feces of rats among diverse groups

100 mg feces of each sample from four groups were considered precisely, pretreated according to the method under 2.8 items, and then injected and analyzed according to the method under 2.9 item. SIM mode was

selected to quantitatively quantity 7 SCFAs of four groups. Response values were shown and MC was calculated according to the working curve of the day.

Statistical analysis

ELISA and SCFA results were showed in the form of mean ± standard deviation. Statistical analysis was carried out using SPSS 17.0 software (SPSS Inc., Chicago, USA). Differences were evaluated by one-way ANOVA with Bonferroni method, and $P < 0.05$ meant that the two groups had statistical difference.

RESULTS

Effect of raw and bran-fried atractylodis rhizoma on behaviors of spleen-deficiency syndrome rats

During the research, the rats in the CG acted normally, comprising normal diet, activities, and evacuation. In sharp disparity, the rats in the MG performed abnormally. For example, the SDS rats displayed decreased activity, depression, and clear loose stool. After administering raw and BFA, these abnormal actions in SDS rats progressively improved.

Effect of raw and bran-fried atractylodis rhizoma on the serum levels of biochemical indices of spleen-deficiency syndrome rats

Compared with the CG, the contents of MTL, GAS, TRY, and AMS in the MG significantly decreased ($P < 0.01$). Compared with the MG, the contents of MTL, GAS, TRY, and AMS in the RA and BFA groups meaningfully augmented ($P < 0.01$). Compared with the RA group, the contents of MTL, GAS, TRY, and AMS in the BFA group suggestively improved ($P < 0.01$) [Figure 1].

Method validation of gas chromatography-mass spectrometer

Selectivity

The retention time and fragment ion information of 7 SCFAs and IS are revealed in Table 1. The TIC of fecal samples and mixed SCFA standard solution in SIM mode displayed that the peaks of 7 SCFAs were in outstanding shape and the separation degree with contiguous peaks was up to the necessities [Figure 2]. There were no impurity peaks near the IS peaks. All of these presented that this method had an excellent discernment.

Linearity

All the linear regressions of 7 SCFAs had an outstanding linear within their respective ranges of MC ($r > 0.995$). Weighted (1/x) least-squares linear regression was employed to calculate the slope and intercept of calibration curves. Limit of detection and limit of quantitation were found consequently [Table 2].

Table 1: The retention time and fragment ion information of seven short-chain fatty acids and internal standard

n	Name	RT (min)	QI (*m/z*)	AQI (*m/z*)
1	Acetic acid	4.34	43	45, 60
2	Propionic acid	5.39	74	45, 57
3	Isobutyric acid	5.79	43	41, 73
4	Butyric acid	6.65	60	42, 73
5	Isovaleric acid	7.29	60	41, 43
6	Valeric acid	8.41	60	45, 73
7	Hexanoic acid-d3 (IS)	9.92	60	45, 87
8	Hexanoic acid	9.99	60	41, 73

RT: Retention time; SCFAs: Short-chain fatty acids; IS: Internal standard; QI: Quantitative ion; AQI: Auxiliary qualitative ion

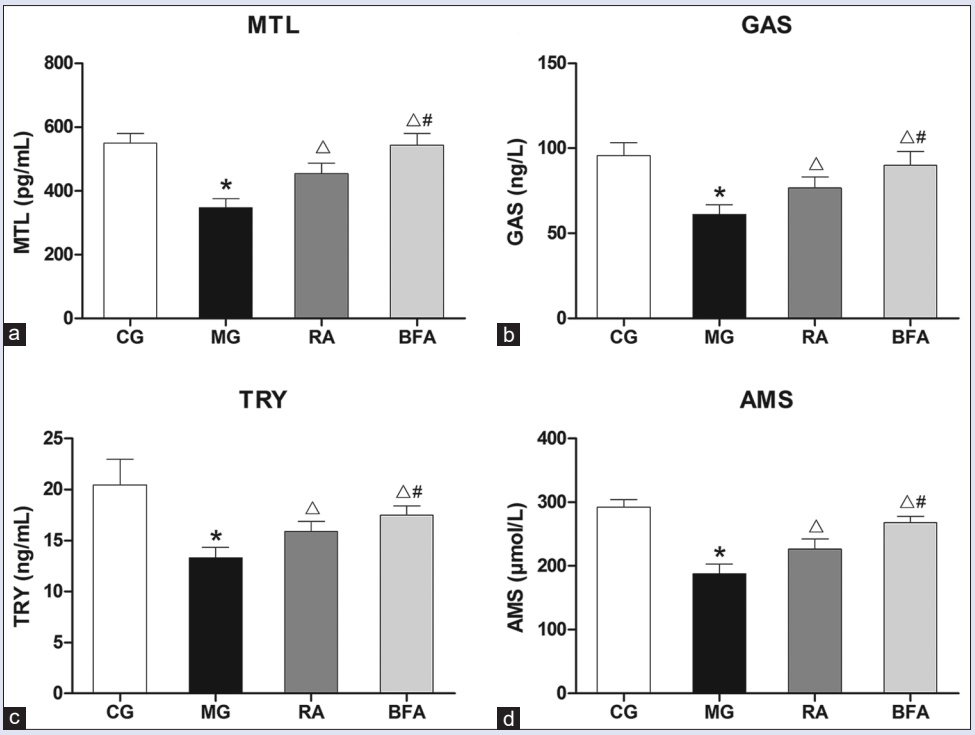

Figure 1: Column charts of the serum levels of biochemical indices among four groups ($n = 10$): (a) motilin level; (b) gastrin level; (c) trypsin level; (d) amylase level. *$P < 0.01$ when compared with the control group; △$P < 0.01$ when compared with the model group; #$P < 0.01$ when compared with the raw atractylodis rhizoma group

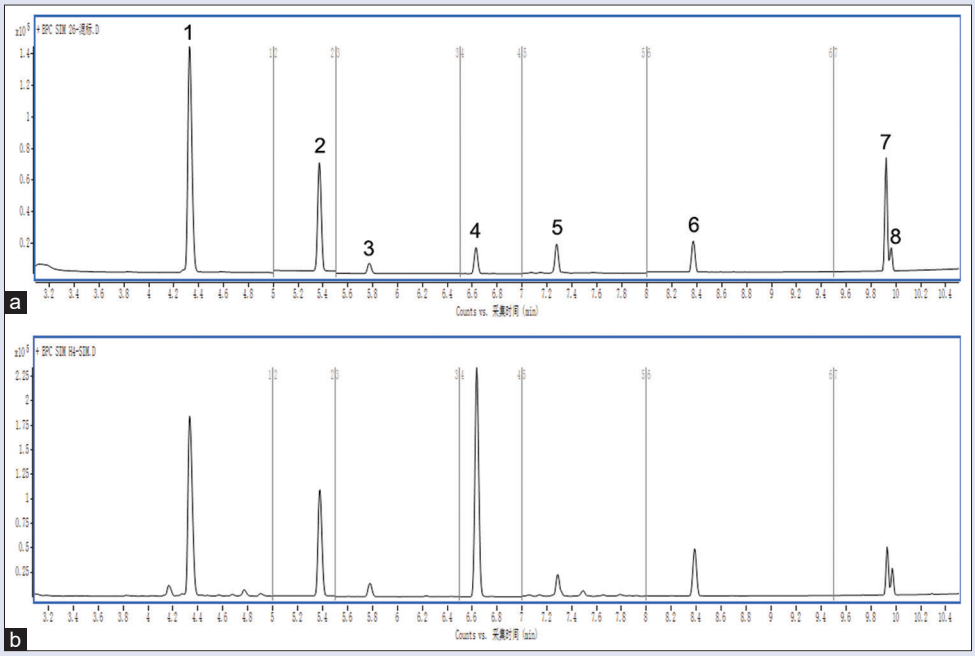

Figure 2: Total ion chromatogram of mixed short-chain fatty acid standard solution (a) and fecal sample (b) in selective ion monitoring mode (1, acetic acid; 2, propionic acid; 3, isobutyric acid; 4, butyric acid; 5, isovaleric acid; 6, valeric acid; 7, hexanoic acid-d3 [internal standard]; and 8, hexanoic acid)

Table 2: Regression, linear range, limit of detection, and limit of quantitation of seven short-chain fatty acids

n	Name	Regression	Correlation coefficient (r)	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
1	Acetic acid	*Y*=0.0573X+0.0047	0.9956	0.5-500	0.05	0.5
2	Propionic acid	*Y*=0.0399X+0.0012	0.9969	0.05-500	0.01	0.05
3	Isobutyric acid	*Y*=0.0435X+0.0041	0.9954	0.05-500	0.01	0.05
4	Butyric acid	*Y*=0.1049X+0.0032	0.9952	0.05-500	0.025	0.05
5	Isovaleric acid	*Y*=0.1159X+0.0013	0.9953	0.05-500	0.025	0.05
6	Valeric acid	*Y*=0.1237X+0.0051	0.9957	0.05-500	0.025	0.05
7	Hexanoic acid	*Y*=0.077X+0.0046	0.9956	0.05-500	0.025	0.05

LOD: Limit of detection; LOQ: Limit of quantitation; SCFAs: Short-chain fatty acids

Intra- and inter-day precisions

The RSD of intra- and inter-day precisions was <4.68% and 13.41%, respectively, which displayed an excellent precision of the instrument. The outcomes are shown in Table 3 in detail.

Stability

Seven SCFAs in fecal samples presented an excellent stability within 14 days stored at −80°C refrigerator with RSD values of <9.74%. Seven mixed SCFA solutions displayed an excellent stability within 24 h stored at the room temperature with RSD values of <13.40% [Table 3].

Extraction recovery and matrix effect

The extraction recoveries about each SCFA were no >85.22%, meeting the detection necessities of biological samples. The matrix effects of each SCFA in fecal samples were satisfactory, ranging from 76.41% to 112.37%, which specified that the matrix had no obvious influence on the recognition of each SCFA [Table 3].

Effect of raw and bran-fried atractylodis rhizoma on the short-chain fatty acids of spleen-deficiency syndrome rats

Compared with the CG, the contents of acetic acid, propionic acid, isobutyric acid, and isovaleric acid in the MG expressively increased ($P < 0.01$) and the content of hexanoic acid in the MG significantly reduced ($P < 0.01$). Compared with the MG, the acetic acid and propionic acid contents in the RA and BFA groups considerably decreased ($P < 0.01$) and the content of hexanoic acid in the RA and BFA groups knowingly increased ($P < 0.01$). Compared with the RA group, the contents of acetic acid ($P < 0.05$) and propionic acid ($P < 0.01$) in the BFA group significantly diminished. In regulating hexanoic acid, although the RA and BFA groups showed a cumulative effect due to their excessive regulation, the accumulation effect of the BFA group was less than that of the RA group significantly ($P < 0.05$). As for the regulation of isobutyric acid and isovaleric acid, the RA and BFA groups displayed no regulation effect but a certain accumulation effect, however, the accumulation effect of the BFA group was less than that of the RA group. Compared with the CG, although the MG presented no significant variations in the contents of butyric acid and valeric acid, of which the RA and BFA groups indicated a cumulative effect and the accumulation effect of the BFA group was correspondingly less than that of the RA group [Figure 3].

DISCUSSION

SDS is thoroughly related to many gastrointestinal illnesses, so the damage of gastrointestinal function is the typical distinctive of SDS. Therefore, we selectively dignified the contents of MTL, GAS, AMS, and TRY in serum to evaluate the model of SDS in the study. MTL and GAS

Table 3: Precision, stability, extraction recovery, and matrix effect of seven short-chain fatty acids in fecal samples (*n*=6)

Name	Precision (%)		Stability (%)		Extraction recovery (%)		Matrix effect (%)	
	Intra-day	Inter-day	−80°C	Room temperature	Mean	RSD	Mean	RSD
Acetic acid								
Low	2.52	6.58	8.35	7.39	89.34	4.37	87.77	2.73
Middle	4.68	7.83	9.74	6.62	96.25	2.14	99.08	1.94
High	1.29	4.77	5.22	6.11	105.31	1.95	76.41	3.28
Propionic acid								
Low	2.24	7.65	5.32	8.75	94.54	3.91	99.49	0.97
Middle	2.99	7.54	5.69	5.37	86.80	2.60	89.81	1.46
High	3.64	8.96	7.18	9.21	86.53	2.89	82.95	1.99
Isobutyric acid								
Low	1.73	5.78	3.63	6.15	107.02	0.88	89.53	5.21
Middle	0.71	3.62	6.14	9.06	86.18	1.32	112.37	2.45
High	2.65	5.84	7.33	5.99	85.22	2.97	92.39	3.55
Butyric acid								
Low	2.33	6.69	7.55	9.84	95.37	5.16	79.25	4.37
Middle	1.59	4.27	5.91	11.71	85.58	2.72	86.07	5.13
High	1.24	3.99	7.22	8.43	85.40	3.65	111.30	4.92
Isovaleric acid								
Low	0.94	3.47	4.58	8.33	92.84	1.38	92.28	6.46
Middle	2.32	6.56	7.61	10.87	85.52	4.57	105.33	7.52
High	2.01	7.11	8.93	7.39	86.87	3.44	87.74	3.58
Valeric acid								
Low	3.47	9.25	9.10	5.77	100.25	7.73	88.22	3.36
Middle	1.62	5.61	3.74	4.82	89.17	2.14	98.71	7.43
High	2.21	5.19	4.43	7.93	90.18	3.32	103.21	5.77
Hexanoic acid								
Low	4.53	13.41	9.66	13.40	85.62	9.97	79.59	7.81
Middle	2.35	7.74	4.27	9.64	97.93	5.31	88.25	5.46
High	2.66	5.38	7.57	10.29	89.76	3.56	82.83	6.29

SCFAs: Short-chain fatty acids; RSD: Relative standard deviation

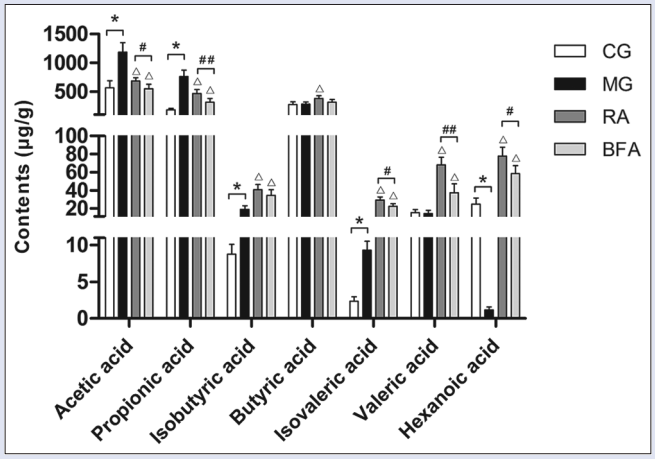

Figure 3: Column chart of seven short-chain fatty acid contents in feces of rats among four groups (n = 10). *P < 0.01 when compared with the control group; $^{\triangle}P$ < 0.01 when compared with the model group; $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01 when compared with the raw atractylodis rhizoma group

are two important gastrointestinal hormones, which play an important role in preserving normal gastrointestinal purpose.[19,20] AMS and TRY are two enzymes related to digestive function *in vivo*.[21] In this study, we initiate that SDS rats had an irregular expression in MTL, GAS, AMS, and TRY. Furthermore, we also detected that SDS had some abnormal indications such as depressed mood and loose stools. This was steady with the results of previous study,[22] and also meets the standards of SDS diagnosis.[23] All of these displayed the success of our establishment of SDS model. After administering raw and BFA, the expression of MTL, GAS, AMS, and TRY and the behaviors of SDS rats recovered to a certain extent, which recommended that the raw and BFA had a good therapeutic effect on SDS. Moreover, BFA had significant rewards over raw AR in regulating MTL, GAS, AMS, and TRY, which exposed BFA had advantages over raw AR in refining gastrointestinal function.

SCFAs contribute in the normal operation of gastrointestinal function, which have been found related to many ailments.[24] In order to study the effect of raw and BFA on the metabolism of SCFAs in SDS rats, a quantitative method was recognized in the study, which was proved to be simple and precise with short time, and had high sensitivity and a suitable recovery. Compared with the healthy rats, the acetic acid, propionic acid, isobutyric acid, and isovaleric acid contents in the feces of SDS rats augmented significantly, while the content of hexanoic acid diminished significantly. This unnecessary accumulation or inadequate secretion showed that the metabolism of SCFAs was seriously disturbed when suffering from SDS. After the mode of administration of raw and BFA, the acetic acid, propionic acid, and hexanoic acid contents in SDS rats inclined to recover to the normal levels to a certain extent, and BFA had benefits over raw AR. SCFAs are produced by the metabolism of gut flora, therefore, their content levels imitate the structure of gut flora to some extent.[25] In our earlier study, we found that the abundance of *Ruminococcus* and *Bacteroides*, measured to produce acetic acid mostly, significantly augmented in SDS rats compared with the healthy rats, and significantly lessened after the mode of administration of raw and BFA, and BFA had advantages over raw AR.[8] These two studies results were reliable to a certain extent, and the findings of this study also confirmed the previous study.

Fascinatingly, we found that raw and BFA had some accumulation effects in isobutyric acid, butyric acid, isovaleric acid, valeric acid, and hexanoic acid. We risked that this accumulation effect of AR may be due to its own toxicity. In this study, we employed the powder solution of medicinal materials to give medicine because of the doubt of dominant material basis of AR. It had been showed that the volatile oil of AR had some toxic and side effects such as stomach irritation, reduced alertness, locomotion, and reduced response to touch and balance.[26] This may be one of the reasons for the accumulation of SCFAs. The content of volatile oil in AR can be condensed by stir frying with bran,[27] which is in accordance with the result that the accumulation effect of BFA was less than that of raw AR in this study. Of course, it leftovers to be further considered whether there are other substances of toxic and side effects in AR. In the future, the correlation analysis between chemical components and SCFAs can be performed, which is also conducive to our considerate of the specific mechanism of SCFAs metabolism in SDS rats after the mode of AR administration.

There are still some absences in this study. In this study, we only preliminarily analyzed the SCFA metabolism in feces of SDS rats after the administering of raw and BFA. SCFA metabolism in other intestinal sections or tissues persisted unclear. Research has shown that the effects of SCFAs and gut flora are reciprocal. On the one hand, SCFAs are formed by the metabolism of gut flora. On the other hand, SCFAs can promote or inhibit gut flora and therefore move the structure of intestinal microecology.[28] There are huge changes in the number and types of micro-organisms dispersed in the intestine due to the differences of internal environment in diverse intestinal segments.[29] For instance, Meng *et al.*[30] found that the accumulation of SCFAs was detected in colon contents while the inadequate secretion of SCFAs was pragmatic in cecum contents when analyzing the metabolism of SCFAs in enteritis mice. In this study, we only examined the metabolism of SCFAs in feces of SDS rats. The metabolism of SCFAs in other intestinal segments and the potential mechanism of SCFA metabolism in SDS rats endured to be further considered.

CONCLUSION

In this study, a quantitative method was recognized to regulate seven SCFAs in rat feces by GC-MS, which was modest and precise with short time, and had high sensitivity and a satisfactory retrieval. We also established that raw and BFA can recover the acetic acid, propionic acid, and hexanoic acid metabolism in SDS rats, and BFA was more powerful than raw AR.

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Conflicts of interest

There are no conflicts of interest.

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