Digestibility of squash polysaccharide under simulated salivary, gastric and intestinal conditions and its impact on short-chain fatty acid production in type-2 diabetic rats

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ABSTRACT

In vitro digestive conditions were simulated to investigate the digestibility of polysaccharides prepared from squash (SPS). A small amount of free glucose monosaccharide was released after salivary and intestinal digestion due to the breakdown of α-(1→4)-glucose linkages and may form SPS or a starch impurity. At the same time, there was no obvious change in molecular weight distribution and reducing sugar content throughout this digestion period, demonstrating that the main structure of SPS was relatively stable under the simulated digestive conditions. Thus, most SPS can be transported intact to the large intestine. In addition, SPS alleviated type 2 diabetes (T2D) in rats. Moreover, the content of short-chain fatty acids (SCFAs) in the colon significantly increased after treatment with SPS. The present research provides insight into the non-digestibility of SPS, and suggests its utility to alleviate T2D by promoting the production of SCFA in the colon.

1. Introduction

Squash, belonging to the family Cucurbitaceae, is a commercially important crop widely grown throughout the world. Research indicates that squash polysaccharides (SPS) have beneficial effects on several lifestyle-associated diseases, including obesity, type 2 diabetes (T2D) and hyperlipidemia (Habtemariam, 2019; Yang, Zhao, & Lv, 2007; Zhang et al., 2013). Because of these various biological functions, interest in the consumption of SPS, as part of a healthy diet, has increased in recent years. Our previous research has investigated the separation, structure and functions of polysaccharides extracted from squash and pumpkin (Song, Li, Hu, & Li, 2011; Song, Ni, Hu, & Li, 2015; Zhao et al., 2017).

Recent studies reported that many dietary polysaccharides show resistance to enzymatic and chemical digestion (Carnachan, Bootten, Mishra, Monro, & Sims, 2012; Wang, Xu, Liu, & Li, 2015; Yi et al., 2019). These undigestible polysaccharides can then be transported intact to the colon after which they can exhibit probiotic properties by influencing the composition of gut microbiota (Kaoutari, El Armougom, Gordon, Raoul, & Henrissat, 2013). As a result, the production of short-chain fatty acids (SCFAs) can be increased in the colon resulting in health-promoting effects and the improvement of some chronic diseases, such as T2D (Fernández et al., 2016; Nie, Lin, & Luo, 2017; Priyadarshini, Wicksteed, Schiltz, Gilchrist, & Layden, 2016). Previous reports demonstrated polysaccharides extracted from pumpkin and squash showed anti-diabetic effects on T2D (Habtemariam, 2019), but the mechanisms behind these effects still remain unclear. We hypothesized that the SCFA production pathway might be important in the mechanism of SPS. Digestibility and changes in polysaccharide structure can play a major role in how a polysaccharide exerts its bioactivities (Edwards et al., 2014). Unfortunately, there is limited information on the salivary, stomach and small intestinal digestibility of SPS and the structure of the SPS reaching the colon and its impact on SCFAs.
In this study, salivary, gastric and small intestinal conditions were simulated to investigate the digestibility of SPS. We then studied the effects of SPS on T2D in rats. SCFAs in the colon were analyzed after SPS treatment and the results contribute to our understanding of the structural changes of SPS in the digestive tract and suggest a possible mechanism by which SPS might impact T2D.

2. Materials and methods

2.1. Materials and reagents

Fresh squash (Cucurbita moschata) Duch fruits were purchased from a local commercial market in Beijing, China. 3-methyl-1-phenyl-2-pyrrolizin-5-one (PMP), pepsin 3000 units/g, trypsin 300 units/mg, pancreatic, bile salt, and monosaccharide standards of HPLC-grade including fucose Fuc, arabinose Ara, rhamnose Rha, mannose Man, xylose Xyl, galactose Gal, galacturonic acid GalA and glucose Glc were purchased from Sigma Chemical Co. St. Louis, MO, USA. a-amylase from Aspergillus oryzae 40,000 u/g was purchased from Solarbio Science & Technology Co., Ltd. Beijing, China. All other used chemicals and solvents used were analytical grade and purchased from Fisher Scientific Springfield, NJ, USA.

2.2. Extraction of SPS

SPS was isolated from squash according to our previously reported method (Liang, Liu, Yu, Song, & Li, 2019) with some modifications. Briefly, crushed squash fruit (100 g) was added into distilled water (1:3, v/w), then it was mixed with 15 mg of a-amylase. The mixture was kept at 90 °C for 3 h and then centrifuged at 8000 rpm for 20 min. The supernatant was collected and concentrated for deproteinization by using Sevag reagent (Navarini et al., 1999). The extract was mixed with 95 % ethanol (1:3, v/v) and maintained at 4 °C to obtain the precipitate of polysaccharide. After 12 h, centrifugation (8000 rpm, 20 min) was performed to obtain the final precipitate. The precipitate was redissolved in water and dialyzed against distilled water using 3000 Da molecular weight cut-off (MWCO) membrane. Finally, SPS was concentrated and lyophilized before further analysis.

2.3. Determination of saliva digestion

According to the reported methods (NAVAZESH, 1993; Stokes & Davies, 2007), simulated saliva digestion was carried out. The fresh saliva samples were provided by four healthy non-smoking volunteers before drinking or eating in the morning. The age of volunteers ranges from 20 to 30, and they had not been treated with antibiotics over the previous 3 months. Each volunteer was asked to rinse the mouth with distilled water for 45 s and discard the saliva of the first 30 s. Then, the saliva was collected for 5 min. All the collected saliva samples were mixed and centrifuged (4000 rpm, 10 min) to remove impurities. The supernatant was collected, affording the simulated saliva fluid (SSF).

SPS was dissolved in distilled water (4.0 g/mL). Test tube A, B and C contained the mixtures of 4.0 mL SPS solution with 4.0 mL SSF, 4.0 mL SSF with 4.0 mL distilled water and 4.0 mL SPS solution with 4.0 mL distilled water, respectively. All the tubes were kept in the incubator shaker at 37 °C to mimic the conditions in the human mouth. Samples were collected during simulated salivary digestion (0 h, 0.5 h and 1 h), then they were boiled for 5 min to inactivate enzymes.

2.4. Determination of simulated gastric digestion

According to the reported methods (Hur, Lim, Decker, & McClements, 2011; Minekus et al., 2014; Zhao et al., 2018), simulated gastric digestion was established. The simulated gastric medium was composed of NaCl (3.1 g/L), KCl (1.1 g/L), NaHCO₃ (0.6 g/L) and CaCl₂ (0.15 g/L) and was adjusted to pH 2.5 by HCl solution (0.1 M). Then, 150 g of the gastric medium was mixed with 1 mL of CH₃COONa (1.0 M, pH 5) and 35.0 mg of pepsin. The mixture was adjusted to pH 2.0, affording the simulated gastric fluid (SGF).

SPS was dissolved in distilled water (8.0 mg/mL). For simulated gastric digestion, three mixtures of 8.0 mL SPS solution with 8.0 mL SGF, 8.0 mL SGF with 8.0 mL distilled water and 8.0 mL SPS solution with 8.0 mL distilled water were put into tubes A, B and C, respectively. All the tubes were kept in the incubator shaker at 37 °C. Samples were collected during simulated gastric digestion (0 h, 0.5, 1, 2, 4 and 6 h), then they were boiled for 5 min to inactivate enzymes.

2.5. Determination of simulated small intestinal digestion

Simulated small intestinal digestion was performed according to the reported methods (Minekus et al., 2014; Yi et al., 2019). The simulated small intestinal medium was composed of NaCl (5.4 g/L), KCl (0.65 g/L) and CaCl₂·2H₂O (0.33 g/L), and was adjusted to pH 7 by NaOH solution (0.1 M). Next, 13 mg trypsin, 100 g pancreatin solution (7 %, w/w) and 200 g bile salt (4 %, w/w) were mixed with 100 g of the small intestinal medium. The mixture was adjusted to pH 7.5, affording the simulated small intestinal fluid (SIF).

The pH of the gastric digested (after 6 h) SPS solution was increased to 7.0 by using NaHCO₃ solution (1 M) to investigate simulated small intestinal digestion. The mixtures of 3.0 mL of SIF with 10.0 mL of gastric digested SPS solution, 3.0 mL of SIF with 10.0 mL of distilled water, and 3.0 mL of distilled water with 10.0 mL of digested SPS solution were put into tubes labeled A, B and C, respectively, and these tubes were maintained in the incubator shaker at 37 °C. Samples were collected during simulated small intestinal digestion (0, 0.5, 1, 2, 4 and 6 h), then they were boiled for 5 min to inactivate enzymes.

2.6. Determination of mw distribution

High-performance gel permeation chromatography (HPGPC), as described by our previously reported method (Yu et al., 2017), was applied to determine the Mw distribution of samples before and after digestion. The HPGPC system was equipped with a refractive index detector, TSK G4000 SWXL column (30 cm × 7.8 mm) and G3000 SWXL column (30 cm × 7.8 mm), operating at 30 °C. 20 μL of sample solution was injected in each run, taking 0.1 M ammonium acetate with 0.02 % (w/v) sodium azide as the mobile phase at a flow rate of 0.6 mL/min. The separation was calibrated with a series of dextran standards using linear regression.

2.7. Determination of free monosaccharide

High-performance liquid chromatography (HPLC) with pre-column derivatization (Chen et al., 2012; Zhao et al., 2017), was applied to determine the free monosaccharide of samples before and after digestion. Briefly, 100 μL of samples or standards were mixed with 100 μL of NaOH solution (0.6 M), and 100 μL of the mixture was labeled by 120 μL of 1-phenyl-3-methyl-5-pyrazolone (PMP) methanol solution (methanol solution), kept at 70 °C for 1 h. After cooling down, the mixture was neutralized with 100 μL HCl solution (0.3 M). Chloroform was then added to remove the excess PMP. The organic phase was discarded after vortex and standing for a few minutes. Finally, the aqueous layer was filtered through a 0.22 μm nylon membrane for further analysis. The HPLC system was equipped with UV detector and Agilent XDB-C18 column (4.6 mm × 250 mm), operating at 35 °C. A 10 μL aliquot of PMP-labeled samples was injected in each run using a mixture of 0.1 mol/L KH₂PO₄ and acetoniitrile (83:17, v/v) as mobile phase. The flow rate was 1.0 mL/min and the detection wavelength was 245 nm.
2.8. Determinations of reducing sugar

A 3, 5-dinitrosalicylic acid (DNS) method (Miller, 1959) was applied with minor modification to determine the content of reducing sugar in samples before and after digestion. Briefly, 1 mL of the sample and 2 mL of DNS reagent were mixed and heated to 100 °C for 5 min and after the reaction cooled to room temperature 12 mL of distilled water was added to the mixture. The detection wavelength was 245 nm and reducing sugar content was calculated based on a glucose standard curve.

2.9. Animal experiments

Our previously reported animal protocol (Liang, Liu, Yu, Zhang et al., 2019) was applied with slight modification. Adult male Wistar rats, weighing 180 – 220 g, were supplied by Beijing Laboratory Animal Research Center (Beijing, China). All the investigations on rats were approved of the Ethics Committee of Beijing Laboratory Animal Research Center (SYXK:2015-0046; Beijing, China). All rats were sheltered in an SPF barrier environment under the regulated environmental conditions (constant temperature: 21 – 23 °C, relative humidity: 40 – 45 %, 12 h light/12 h dark cycle, ad libitum access to food and water). After acclimatization for one week, the rats were fed on a high-fat diet (HFD), containing 10 % lard, 20 % sucrose, 2.5 % cholesterol, 1 % sodium cholate and 66.5 % pulverized standard rat pellet. The rats were then injected with a low-dose of streptozocin (STZ) (30 mg/kg body weight) that after 4 weeks resulted in moderate destruction of β-cells. These rats, having the fasting blood glucose level ≥ 11.1 mmol/L and the presence of polyuria and polydipsia, were considered as successfully induced with T2D. T2D rat models were randomly allocated to three experimental groups with 10 rats in each based on the following design of an experiment: (1) control group (T2D group), intragastrically administered with saline; (2) metformin (MT) treated group (MT group), intragastrically administered with 200 mg/kg body weight metformin once daily; and (3) SPS treated group (SPS group), intragastrically administrated with 1000 mg/kg body weight of SPS once daily. All the rats were fed on HFD over the entire period of the experiment. After treatment of 4 weeks, blood samples were then collected from three groups (T2D, n = 8, MT, n = 9, SPS, n = 9). Blood glucose (Glu), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) and other blood biochemical parameters were measured as previously described (Zhang, Xu, Yu, Wu, & Sui, 2017). Insulin (Ins) was determined using an ELISA kit according to the manufacturer’s instructions. The colon content also collected and frozen rapidly in liquid nitrogen for subsequent analyses.

2.10. Determination of SCFAs in colon content

Based on a reported method (Liu, Wang, Yu, & Li, 2018; Xia, Wang, Yu, Liang, & Kuang, 2019), gas chromatography-mass spectrometer (GC-MS) was applied to determine SCFAs. The colon content samples were suspended in phosphoric acid solution (0.5 %, 1 mL). After centrifugation at 10,000 rpm/min for 10 min supernatant 800 μL was collected and 800 μL of ethyl acetate was added to this supernatant. After vortexing and centrifugation, a 600 μL aliquot of the organic ethyl acetate phase was added to 500 μM 4-methylpentanoic acid in ethyl acetate for further analysis.

GC-MS analysis was carried out on an Agilent 7890A/5975C system, equipped with a DB-WAX MS silica capillary column (30 m × 0.25 mm × 0.25 μm). The initial oven temperature (90 °C) was maintained for 1 min before being raised to 120 °C at a rate of 10 °C/min. The oven temperature was then increased to 150 °C at a rate of 5 °C/min and then increased to 250 °C at 25 °C/min. The temperature of the injection port was 250 °C. A 1 μL aliquot of the prepared sample was injected in each run, using helium as the carrier gas at a flow rate of 1.0 mL/min with a split ratio of 10:1.

2.11. Statistical analysis

Statistical analysis was performed by SPSS for Windows, Version 16.0 (SPSS, Chicago, IL). The data were shown as mean ± standard deviation (SD) and the analysis of one-way analysis of variance followed by Duncan’s multiple range test was used for evaluation of the significance. The difference was considered to be statistically significant if P < 0.05.

3. Results and discussion

The carbohydrate content of SPS was 92.05 ± 2.85 % and its Mw ranged from 12.28 kDa to 270.35 kDa. SPS was composed of GalA, Rha, Gal, Ara and Glc in a molar ratio of 35.93 ± 0.82 %, 22.49 ± 0.56 %, 21.68 ± 1.10 %, 12.05 ± 0.79 % and 7.85 ± 0.87 %, respectively.

3.1. Change in Mw of SPS after simulated digestion

The change of molecular weight distribution was calculated during its passage through the simulated digestive conditions to investigate the digestibility of SPS.

In the oral cavity, food masticated into small particles and lubricated by salivary secretion (Mathur & Mathur, 2006; Ziebarth, Spiegelhalder, & Bartsch, 1997). The pH in human saliva is in the range of 6.5–7.2 (Ziebarth et al., 1997). The constituents of human saliva are complex, and the key enzyme present is α-amylase, of which the activity was determined to be approximately 18–208 U/mL. This α-amylase catalyzes the hydrolysis of α-(1→4) linkages in polysaccharides. Fig. 1 shows the results of Mw changes obtained by HPGPC determination. There were two peaks (peak I and peak II) in the HPGPC chromatograms of the initial SPS solution (Fig. 1A). Besides, peak III and peak IV were detected within 40 min in the HPGPC chromatogram of SGF. Following in vitro salivary digestion, there was no obvious change in the retention time and response value at different digestion times, showing that the salivary amylase does not hydrolyze SPS. The results are consistent with previous reports that non-starch polysaccharides are generally resistant to the salivary digestion (Chen et al., 2016). Moreover, oral digestion time in vivo is generally quite short and saliva amylase is inactivated rapidly by gastric acid (Minekus et al., 2014; Rosenblum, Irwin, & Alpers, 1988). Therefore, we could conclude that saliva has no significant effect on SPS.

In the gastric phase of digestion, the emptying of daily meals usually takes about 4 h. Thus, the Mw distributions of SPS were investigated during the simulated gastric process (0, 0.5, 1, 2, 4, 6 h, respectively), along with initial SPS solution and blank SGF (Fig. 1B). Peak V corresponding to pepsin in SGF appears at 31.7 min in the HPGPC chromatogram. After simulated gastric digestion, the retention times and response value of the two peaks are unaltered in all of the samples. This suggests that SPS had no degradative impact on two major components in SPS after a 6 h digestion. This result is consistent with previous research in which similar polysaccharides showed resistance to digestion in SGF at pH 2 (Wang et al., 2015). Although, these are reports that the glycosidic bonds could be broken when carbohydrates were incubated in SGF (Hu, Nie, Min, & Xie, 2013). The decreased response value of peak V is related to the self-degradation of pepsin over time.

After 6 h of gastric digestion, SPS was transferred to the small intestinal phase of digestion. The peak, associated with components of SIF, appearing late in the HPGPC chromatogram had no influence on the determination of the Mw change of SPS (Fig. 1C). SPS treated by SIF for different times showed no significant changes in Mw distribution. The pH was increased to neutral in SIF, leading to further inactivation of pepsin (Minekus et al., 2014). Because of pepsin decomposition, peak V gradually vanished and new peaks (peak VI and peak VII) appeared in the chromatogram of 0 h and 0.5 and finally disappear after 6 h of digestion.

In summary, SPS showed resistance to digestion under the simulated...
conditions and no changes in molecular weight. Thus, SPS should reach the colon to be used by gut microbiota.

3.2. Reducing sugar content of SPS after simulated digestion

The factors that influence the digestibility of SPS are numerous, including pH, glycohydrolases present, temperature, and digestion media (Carnachan et al., 2012; Chen et al., 2016). However, Mw change is not the sole standard for measuring polysaccharide digestibility. A slight change in Mw, caused by a small amount of polysaccharide degradation may be undetectable by HPGPC (Savary, Hotchkiss, & Cameron, 2002). The breakage glycosidic linkages result in an increase of reducing ends (Harrington & Zimm, 1965). Thus, the DNS method was next applied to study the changes of reducing sugars.

During the simulated digestion process, the amount of reducing sugars was determined at different time points (Table 1). At 0 h of salivary digestion, the reducing sugar content was 0.13 ± 0.01 mg/mL. After salivary digestion, there was an observable but insignificant increase (0.14 ± 0.01 mg/mL) of reducing sugar content. The reducing sugar content was nearly constant over the 6 h of gastric phase digestion. At 6 h of processing time, there was no significant increase compared to the 0 h time point. For simulated digestion in SIF no significant increase of the reducing ends was observed during the digestion process. Overall, the result of reducing sugar content indicated that the SPS showed resistance to be broken down in the simulated digestive tract. This is also consistent with the results of previous research (L. Zhao et al., 2018). The slight variability in reducing sugar content is

Table 1

<table>
<thead>
<tr>
<th>Samples</th>
<th>Reducing sugars (Glucose equivalent mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSF</td>
<td></td>
</tr>
<tr>
<td>0 h</td>
<td>0.13 ± 0.01 a</td>
</tr>
<tr>
<td>0.5 h</td>
<td>0.15 ± 0.02 a</td>
</tr>
<tr>
<td>1 h</td>
<td>0.14 ± 0.01 a</td>
</tr>
<tr>
<td>SGF</td>
<td></td>
</tr>
<tr>
<td>0 h</td>
<td>0.22 ± 0.02 ab</td>
</tr>
<tr>
<td>0.5 h</td>
<td>0.19 ± 0.03 ab</td>
</tr>
<tr>
<td>1 h</td>
<td>0.17 ± 0.05 a</td>
</tr>
<tr>
<td>2 h</td>
<td>0.16 ± 0.04 a</td>
</tr>
<tr>
<td>4 h</td>
<td>0.23 ± 0.01 ab</td>
</tr>
<tr>
<td>6 h</td>
<td>0.24 ± 0.03 b</td>
</tr>
<tr>
<td>SIF</td>
<td></td>
</tr>
<tr>
<td>0 h</td>
<td>1.11 ± 0.10 a</td>
</tr>
<tr>
<td>0.5 h</td>
<td>1.19 ± 0.06 a</td>
</tr>
<tr>
<td>1 h</td>
<td>1.28 ± 0.08 a</td>
</tr>
<tr>
<td>2 h</td>
<td>1.24 ± 0.05 a</td>
</tr>
<tr>
<td>4 h</td>
<td>1.14 ± 0.07 a</td>
</tr>
<tr>
<td>6 h</td>
<td>1.16 ± 0.02 a</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.
Values in the same group with different letters (a–b) are significantly different, p < 0.05.
consistent with the breakage of a few glycosidic linkages (Chen et al., 2016).

3.3. Determination of free monosaccharide of SPS after simulated digestion

SPS is composed of five monosaccharides, including GalA, Rha, Gal, Ara and Glc. The release of these monosaccharides during the digestive process was also determined by HPLC-ELSD to further understand the digestibility of SPS. Nine standard monosaccharides could be well separated as shown in Fig. 2. Free monosaccharides released under simulated digestive conditions were calculated, along with initial SPS solution and blank simulated digestive fluids as control. The initial SPS solution contained no free monosaccharides, but there were some unknown substances in SSF (peak a and b), SGF (peak c) and SIF (peak d) (Fig. 2A1, B1 and C1).

A small peak (peak A) was obtained during in vitro salivary digestion and increased gradually over time (Fig. 2A). Peak B appeared in chromatograms after the simulated small intestinal digestion. Based on the retention times of peak A and B, the free monosaccharide released after salivary and intestinal digestion was glucose. No difference between the chromatograms of SPS at different time points was found after gastric digestion. The known characteristics of α-amylase and pancreatin suggest that the released free glucose monosaccharide may come from a starch impurity or less likely from the breakdown of an α-(1→4)-linkage in SPS (Hu et al., 2013).

Based on the lack of change in Mw distribution and reducing sugar content and the small increase in free glucose monosaccharide we conclude the structure of SPS is not affected by simulated digestion. In summary, the structure of SPS is relatively stable under simulated digestions and should reach the colon where the gut microbiota can use it as a carbon source.

3.4. SPS alleviates T2D in rats

After 4 weeks of HFD feeding and one intraperitoneal injection of STZ, T2D was induced in rats. Over the next 4 weeks, all rats were continuously fed an HFD, while SPS and MT were given through intragastric administration.

SPS supplementation significantly (P < 0.05) reduced the level of glucose, LDL and TG as compared with the T2D group (Table 2).
The effects of SPS and MT treatment on serum biochemical parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T2D</th>
<th>MT</th>
<th>SPS</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu (mmol/L)</td>
<td>29.8 ± 4.7 a</td>
<td>19.5 ± 7.4 b</td>
<td>20.9 ± 6.9 b</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.8 ± 0.6 a</td>
<td>1.5 ± 1.1 ab</td>
<td>0.7 ± 0.3 b</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>5.7 ± 1.5 a</td>
<td>3.1 ± 1.0 b</td>
<td>3.6 ± 1.9 ab</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.2 ± 0.6 a</td>
<td>1.9 ± 0.8 a</td>
<td>0.8 ± 0.3 b</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.3 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.4</td>
<td>0.35</td>
</tr>
<tr>
<td>Ins (μIU/mL)</td>
<td>45.2 ± 3.6</td>
<td>48.1 ± 7.7</td>
<td>47.3 ± 6.4</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.

Notably, the level of LDL in SPS group was also significantly decreased (P < 0.05) when compared with MT group. The results were in line with previous studies (Poovitha, Siva Sai, & Parani, 2017), indicating that SPS had hypolipidemic effect on T2D in rats. In this study, TC of SPS group was lower than that of T2D group, but the difference was not significant (P = 0.09). Additionally, no significant difference was found in HDL (P = 0.35) and Ins (P = 0.54) among the three groups.

T2D, characterized by hyperglycemia and dyslipidemia, is a chronic metabolic disorder (Kumar, Arora, Ram, Bhandari, & Vyas, 2015). High levels of TC, TG and LDL in serum are more likely to cause macrovascular complications in patients with T2D (Jones et al., 1989). Because of the adverse effects of drugs, there is a trend to use functional food components having anti-diabetic activities as adjuvants for T2D prevention and treatment. In present study, SPS shows the potential for alleviating T2D as well as a hypolipidemic effect on T2D in rats, suggesting that SPS may be a good dietary supplement for T2D patients.

3.5. SCFA concentration in the colon content

SCFAs, derived from gut microbiota fermentation of indigestible carbohydrates, not only impact gut health but may also pass into the circulatory system exhibiting beneficial effects on human health (Canfora, Jocken, & Blaak, 2015). It has been reported that polysaccharides improve the symptoms of T2D and obesity by affecting lipids and energy metabolism (Anderson & Bryant, 1986; Byrne, Chambers, Morrison, & Frost, 2015). Thus, SPS may also serve as a carbon source for gut microbiota and promote health beneficial compounds such as SCFAs in T2D rats.

The colon contents were collected after the animal experiment. GC–MS was then applied to determine the concentration of SCFAs (acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid and caproic acid) (Fig. 3). As compared to the T2D group, butyric acid, isobutyric acid, isovaleric acid and total SCFAs were significantly increased (P < 0.05) in SPS group and MT group, respectively. In addition, there was an obvious increase (P < 0.05) of valeric acid in SPS group. These results are consistent with previous studies on other indigestible polysaccharides (Henningsson, Björck, & Nyman, 2001). Additionally, two SCFA receptors (FFA2 and FFA3) were found to be metabolic sensors in β cells. Moreover, longer SCFAs (4–6 carbons) and SCFAs with branched alkyl groups (isovalerate and isobutyrate) show the ability to activate FFA3 better than FFA2 (Priyadarshini et al., 2016). Thus, indigestible SPS promotes the production of butyric acid, isobutyric acid and isovaleric acid in the colon, which has the potency for FFA3. Moreover, the hypolipidemic and alleviation effect of SPS on T2D may associate with the activation of FFA3 by SCFAs. Further evidence of FFA3 changes in pancreatic β cells is still needed.

4. Conclusion

Under the simulated digestive conditions, although a small amount of free monosaccharide released after salivary and intestinal digestion, there was no obvious change in Mw distribution and reducing sugar content throughout the whole digestion period. Taking into account all of the above results, SPS showed resistance to enzymatic and chemical digestion and the main structure of SPS is relatively stable. Thus, most SPS should be transported into the large intestine without being broken down. The biochemical parameters of serum in T2D rats showed the regulation of Glu, LDL and TG after treated with SPS, which demonstrates alleviation in T2D. SCFAs associated with health-promoting effects, especially butyric acid, isobutyric acid and isovaleric, were also found significantly increased after treated with SPS. Therefore, the present results provide insight into the undigestible properties of SPS, and reveal that the T2D alleviating effects of SPS might be associated with SCFAs production.

Declaration of Competing Interest

The authors have declared no conflict of interest.