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Purification and Fermentation in Vitro of Sesaminol Triglucoside from Sesame Cake by Human Intestinal Microbiota

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ABSTRACT: Sesaminol triglucoside (STG), the most abundant lignan glycoside existing in sesame cake/meal, has exhibited various biological activities. However, little information about its in vitro fermentation with intestinal microbiota is available. Therefore, the effect of STG from sesame cake on the fermentation of human fecal microbiota was evaluated. First, high-purity STG was successfully prepared from defatted sesame cake by extraction with 80% ethanol and simple purification procedures of polyamide column chromatography and Toyopearl HW-40S column chromatography. Then the influence of STG on intestinal microbiota was conducted by monitoring bacterial populations and analyzing the concentrations of short-chain fatty acids (SCFA). We found that STG could significantly induce an increase in numbers of *Lactobacillus*−*Enterococcus* group and *Bifidobacterium* in fermentation in vitro with human fecal microbiota, while it did not stimulate the bacterial growth of *Eubacterium rectale*−*Clostridium coccoides* group, *Clostridium histolyticum* group, and *Bacteroides*−*Prevotella* group. Furthermore, it was found that concentrations of formic, acetic, propionic, and butyric acids in STG culture increased significantly during the fermentation, and its total SCFA concentration was relatively higher than those of the control and glucose cultures at 6 and 12 h fermentation. Our findings provided further evidence for the importance of human intestinal bacteria in the bioactivity of STG and its metabolites in the maintenance of human health.

KEYWORDS: *Sesamum indicum L., sesaminol triglucoside, purification, fermentation in vitro, intestinal microbiota*

INTRODUCTION

Sesame (*Sesamum indicum* L.) has long been regarded as an important oilseed in the world and one of traditional health foods in Asian countries, not only because of its high oil content (about 50%) but also because of its various beneficial effects, which are mostly related to its bioactive lignans, such as sesamin, sesamolin, and sesaminol glucosides.[1−3] The major lignans in sesame oil are sesamin and sesamolin, and their biological functions, such as antihypertensive effect, antioxidant activity, and inhibitory activity for cholesterol absorption and biosynthesis, have been extensively studied.[2−5] The major lignan glycosides, hydrophilic antioxidants that exist mainly in sesame cake, are sesaminol glucosides, pinoresinol glucosides, and sesamolinol glucosides.[6−8] Although they have no direct role in antioxidative defense system against various oxidative damage within sesame seeds, they could be hydrolyzed to form sesaminol by intestinal β-glucosidase after ingestion of sesame seeds, thereby working as an antioxidant. Furthermore, it has been reported that sesaminol glucosides have a protective effect on the death of β-amyloid-induced neuronal cells and potential anticancer activity.[9,10] The defatted sesame flour that contains 1% sesaminol glucoside has been reported to decrease the susceptibility of hypercholesterolemic rabbit to oxidative stress.[11]

Sesaminol triglucoside (STG), the most abundant lignan glycoside in sesame seed, has less bioactivity in vitro. But it can be converted to bioactive phenolic compounds after oral administration.[12−15] Experiments in vitro demonstrated that STG could be metabolized by human and rat intestinal microbiota to form various phenolics with antioxidant, anti-inflammatory, and estrogenic activities.[9,15−17] It has been reported that the transformation of STG in gut involved the hydrolysis of glucoside, demethylation of a methoxy group, oxidation of dibenzylbutanediol to dibenzylbutyrolactone, and reductive cleavage of furofuran ring.[13] Further metabolism of the furan ring, demethylation, and dehydroxylation of intermediate metabolites could be carried out by intestinal microbiota in the same way as for furofuran lignans such as pinoresinol and common lignans such as lariciresinol.[14] The transformation of STG by intestinal microbiota would lead to production of abundant metabolites, which was remarkably different due to differences in the composition of intestinal microbiota.[13,14] In contrast, if STG and its metabolites were not directly absorbed, they would directly reach the gut and behave as activators or inhibitors of bacterial growth, depending on their chemical structure and concentration, thereby modifying the composition of gut microbiota. STG is a large and highly water-soluble molecule that has been considered less likely to be absorbed in the gastrointestinal tract.[18] Jan et al.[14,19] reported that only a portion of dietary STG was absorbed, metabolized, and then transported to other tissues. However, STG and its metabolites were still present in the intestines, suggesting that they might play a key role in the maintenance of intestinal microbiota composition and intestinal health.

In the colon, the human gut microbiota can be very versatile and have an enormous impact on the nutritional and health status of the host. The metabolic activity developed by the gut

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microbiota contributes to the digestion of dietary compounds, salvage of energy, supply of nutrients, and transformation of xenobiotics. Accordingly, a balanced gut microbiota composition confers benefits to the host, while microbial imbalances are associated with metabolic and immune-mediated disorders.[20] The composition of gut microbiota is influenced by endogenous and environmental factors such as diet, antibiotic intake, and xenobiotics. Of these factors, the diet is considered a major driver for changes in gut bacterial diversity that may affect its functional relationships with the host.[21] It has been reported that some species of intestinal bacteria including *Bacteroides*, *Clostridium*, *Eubacterium* species, and *Lactonifactor longoviformis* were responsible for the metabolism of dietary lignans.[22,23] In vivo, it is considered that more than one species is capable of transforming plant lignans.[24] To the best our knowledge, most studies on STG have focused on its metabolism, distribution, and elimination in rat and transformation by intestinal macrobiota.[13,14,19,25] Little information about the in vitro fermentation of STG and its effects on intestinal microbiota is available. Therefore, the main objective of the present study was to evaluate the effects of STG from sesame cake on the fermentation of human fecal microbiota. First, STG of high purity was prepared through extraction from sesame cake and simple purification procedures by using polyamide and Toyopearl HW-40S column chromatography. Then, the effects of STG on gut microbiota and production of short-chain fatty acids (SCFA) were evaluated by in vitro fermentation.

MATERIALS AND METHODS

Materials and Reagents. Sesame cake, derived from the byproduct of sesame oil, was kindly provided by Anhui Jingfeng Grain & Oils Co., Ltd. (Wuhu, China). The cake was air-dried and crushed into powders with a mill, passed through a 60-mesh sieve, and defatted thoroughly with *n*-hexane at room temperature. The resulting defatted sesame cake (DSC) was dried and stored at −18 °C in a refrigerator until use. Polyamide (80−100 mesh) for column chromatography was purchased from Qingdao Ocean Chemical Co., Ltd. (Qingdao, China). Toyopearl HW-40S resin was purchased from Tosoh Corp. (Tokyo, Japan). 1-Phenyl-3-methyl-5-pyrazolonde (PMP) was purchased from Sigma Chemical Co. (St. Louis, MO). Acetic, formic, butyric, and propionic acids were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Methanol (HPLC-grade) was purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Ethanol, *n*-hexane, and sodium hydroxide, purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), were of analytical grade.

Preparation of Sesaminol Triglucoside. STG was extracted and isolated from DSC according to the reported method with some modifications.[6] Briefly, 1.0 kg of DSC powder was extracted with 80% aqueous ethanol (v/v) in a ratio of 1:10 (w/v) at room temperature by stirring for 8 h. After centrifugation at 4000 rpm for 10 min, the solid residues were extracted twice as described above. The supernatants were combined, concentrated by rotary evaporator under reduced pressure, and lyophilized to afford the crude extract of lignan glycosides. The crude extract (200 mg) was dissolved with deionized water (20 mL), filtered through 0.45 μm microfiltration membrane, and subjected to an open column (3.0 × 60 cm) packed with polyamide. The column was washed with distilled water at a flow rate of 1.0 mL/min, and the eluate was collected (10 mL/tube) by using automatic collector of DBS-100 (Huxi Analysis Instrument Factory Co., Ltd., Shanghai, China). The fractions were detected by high-performance liquid chromatography (HPLC), and the fractions containing STG were combined, concentrated in vacuo, and loaded onto a column (3.0 × 30 cm) of Toyopearl HW-40S. Distilled water filtered through 0.45 μm microfiltration membrane was used as the eluent at a flow rate of 1.0 mL/min. The eluate was collected (10 mL/tube) by automatic collector, and the fractions containing STG were collected, evaporated, and freeze-dried, affording high-purity STG.

Analysis and Characterization of Sesaminol Triglucoside and Its Metabolites by HPLC and HPLC-ESI/MS. STG in crude extract, all fractions from column chromatography, and fermentation cultures were analyzed by an Agilent 1100 series HPLC system (Agilent, Santa Clara, CA), equipped with a model G1379A degasser, a G1311A quaternary pump, a model G1316A column thermostat, and a model G1315B diode array detector (DAD). An Agilent ChemStation Rev.A.10.02 was used for instrument control and data acquisition. Before HPLC analysis, the samples taken from fermentation culture were centrifuged at 13 000 rpm for 10 min and filtered through a 0.22 μm microfiltration membrane to remove bacterial cells and insoluble particles. The analyses were carried out on a TSKgel ODS-80TsQA column (4.6 × 250 mm, 5 μm, Tosoh, Tokyo, Japan) connected through a C_{18} guard column (Supelco). The oven temperature was set at 35 °C. The mobile phase was methanol/water (60/40 v/v), and the flow rate was 0.6 mL/min. The detector was set at 290 nm.

The structure of STG isolated from DSC was characterized by electrospray ionization tandem mass spectrometry (ESI-MS/MS) and HPLC. ESI-MS/MS analysis was performed by using TSQ Quantum Ultra triple quadrupole mass spectrometer (Thermo Scientific) in negative mode. The operation parameters of ESI ion source were as follows: drying gas, N_2; temperature, 270 °C; flow rate of drying gas, 4 L/min; pressure of nebulizer gas (N_2), 9.0 psi; capillary voltage 4 kV. The sugar composition of STG was analyzed by reverse-phase (RP) HPLC with precolumn derivatization with PMP (1-phenyl-3-methyl-5-pyrazolonde) according to the reported method.[26] In brief, STG (4.0 mg dissolved in 1.0 mL of distilled water) was hydrolyzed with 4 M trifluoroacetic acid (TFA, 100 μL) under nitrogen atmosphere at 120 °C for 2 h. After the solution was cooled to room temperature, 200 μL of methanol was added into the hydrolyzate and concentrated to dryness in vacuo, and the procedure was repeated three times to remove excess TFA. The resulting hydrolysate was dissolved with 100 μL of distilled water and derivatized with PMP as described previously.[26] The analysis of PMP derivatives was performed on an Agilent 1100 HPLC system equipped with a DAD, and the chromatography conditions were as follows: column, RP-C_{18} column (4.6 × 250 mm, 5 μm); oven temperature, 30 °C; mobile phase, a mixture of 0.1 M phosphate-buffered saline (PBS, pH 6.7) and acetonitrile in a ratio of 83:17 (v/v); flow rate, 1.0 mL/min; detector, 245 nm.

The metabolites of STG during the in vitro fermentation were analyzed by using an Agilent 1200 HPLC-DAD-ESI/MS system. The separation conditions for STG and its metabolites by HPLC were the same as mentioned above for STG analysis, and the ESI parameters were set as follows: nebulizer gas pressure, 30.0 psi; gas flow rate, 10.0 L/min; electrospray voltage of ion source, 3851 V; capillary temperature, 350 °C. Nitrogen (>99.99%) and helium (>99.99%) were used as the sheath and damping gas, respectively.

In Vitro Fermentation of Sesaminol Triglucoside. Effect of STG on human intestinal microbiota was investigated by fermentation in vitro according to the method described in the literature[27−30] with some modifications. Briefly, fecal samples were obtained from three healthy volunteers (one female and two males, age 25−30) without antibiotic treatment over the preceding 6-month period and without gastrointestinal disorders. Fecal slurries were prepared by mixing fresh fecal samples with autoclaved PBS (0.1 M, pH 7.2) to yield 10% (w/v) suspensions. STG sample was mixed with autoclaved basal nutrient growth medium to give a final concentration of 1% (w/v) in an anaerobic incubator of YQX-1 (Yuejin Medical Optical Instruments Factory, Shanghai, China). The basal nutrient medium (pH 7.0) contained (per liter) peptone 2 g, yeast extract 2 g, NaCl 0.1 g, K_2HPO_4 0.04 g, KH_2PO_4 0.04 g, $MgSO_4{\cdot}7H_2O$ 0.01 g, $CaCl_2{\cdot}6H_2O$ 0.01 g, $NaHCO_3$ 2 g, hemin 0.02 g, cysteine HCl 0.5 g, bile salts 0.5 g, resazurin 1 mg, Tween 80 2.0 mL, and vitamin K_1 10 μL. Fermentation was initiated by adding 150 μL of fecal slurry to 1350 μL of culture medium containing STG and incubated at 37 °C in the anaerobic incubator. In the present study, the culture medium without

STG was used as a control. Samples (250 μL) were taken from the inoculum at 0, 6, 12, and 24 h for enumeration of bacteria and SCFA analysis. All experiments were repeated three times.

Enumeration of Bacteria by Fluorescent in Situ Hybridization. Fluorescent in situ hybridization (FISH) technique was applied to count bacterial cells as described by Hernandez-Hernandez et al.[28] and Rycroft et al.[31] In brief, 100 μL of culture sample was added to 300 μL of filtered paraformaldehyde solution (4% w/v) and left to fix overnight at 4 °C. The fixed bacterial cells were washed twice with 400 μL of PBS (0.1 M, pH 7.2), resuspended in 600 μL of PBS/ethanol (1:1 v/v), and stored at −20 °C until used for hybridization. Hybridization was performed as described by Martín-Peláez et al.[32] using 16S rRNA-targeted oligonucleotide probes labeled with the Cy3 fluorescent dye for enumerating specific bacterial groups or with 4′,6-diamidino-2-phenylindole (DAPI) for counting total bacteria. The probes, synthesized by Shanghai Sangon Biological Engineering Technology and Service Co., Ltd. (Shanghai, China), were Bif164 specific for *Bifidobacterium*,[33] Bac303 specific for *Bacteroides* and *Prevotella* group,[34] His150 for *Clostridium histolyticum* group (clusters I and II),[35] Erec482 for *Eubacterium rectale*−*Clostridium coccoides* group,[35] and Lab158 for *Lactobacillus*−*Enterococcus* group.[36] A fluorescent Axio Imager A1 microscope (Carl Zeiss, Göttingen, Germany) was used to count the bacterial cells. At least six random fields were counted on each slide, and the counts of the bacterial numbers were expressed as log cells per milliliter ± standard deviation (SD).

Analysis of Short-Chain Fatty Acids and Lactic Acid. Samples taken from the fermentations were centrifuged at 13 000 rpm for 10 min and filtered through a 0.22 μm microfiltration membrane to remove bacterial cells and all insoluble particles. The concentration analysis of lactic, acetic, propionic, and butyric acids was performed on an Agilent 1100 series HPLC system with a Beckman Ultrasphere column (4.6 × 250 mm, 5 μm). The temperature of column oven was set at 30 °C. The mobile phase consisted of 20 mM KH_2PO_4 (pH 2.5, solvent A) and methanol (solvent B) with a gradient system: 0−16 min, 5% B; 16−30 min, 5−30% B; 30−40 min, 30% B. The flow rate was 0.8 mL/min and the detector was set at 210 nm. The injection volume was 20 μL. Concentrations of SCFA were calculated according to calibration curves of respective authentic samples, which included formic, lactic, acetic, propionic, and butyric acids. The analysis was conducted in triplicate.

Statistical Analysis. Statistical analyses were conducted with SPSS 11.5 for Windows (SPSS Inc., Chicago). Differences in the bacterial counts and SCFA concentrations between all the treatments were checked by a one-way analysis of variance (ANOVA) test. Significant difference was determined by post hoc multiple comparison tests of means at a significance level of 5%.

RESULTS AND DISCUSSION

Preparation and Characterization of Sesaminol Triglucoside. Sesame cake/meal is not only a good source of fat, carbohydrate, and protein[37,38] but also a source of lignan glycosides, especially STG.[39] The existence of fat and the high contents of carbohydrate and protein make it more difficult to extract and isolate the lignan glycosides from sesame cake. In order to extract effectively sesaminol glycosides and remove soluble sugars and proteins, aqueous solutions of methanol or ethanol have been used to extract lignans and lignan glycosides from sesame seed/meal.[6,39] For example, Suja et al.[40] reported that methanol was the most efficient solvent for extracting lignans from sesame cake. In our preliminary study, ethanol (40%, 80%, and 100%), methanol (40%, 80%, and 100%), acetone, and ethyl acetate as extraction solvents were examined to extract lignan glycosides from DSC. We found that 80% methanol provided the highest extraction yield for STG, followed by 80% ethanol (data not shown). However, no significant difference in extraction yield was observed for 80% methanol and 80% ethanol. Thus, 80% ethanol was selected as the extraction solvent since ethanol is a food-grade, reusable, and cheap solvent.

A certain amount of DSC was extracted with 80% ethanol (1:10, g/mL) by stirring at room temperature for 8 h for three times. After centrifugation, the upper layers were collected, concentrated in vacuo, and freeze-dried, resulting in an extraction yield of 13.25% based on DSC used. Then, the crude extract was fractionated by polyamide column chromatography and Toyopearl HW-40S column chromatography. As shown in Figure 1, three fractions were obtained from

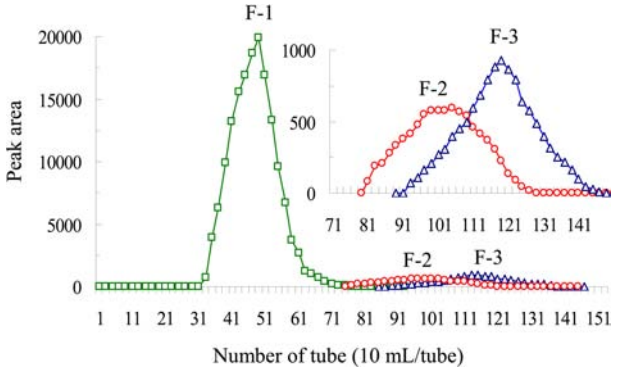

Figure 1. Elution curve of lignan glycosides with water as the eluent from polyamide column. (Inset) Zoom on the important increases of peak area vs number of tube. (□, green) Compound **1**; (△, blue) compound **2**; (○, red) compound **3**.

the crude extract by polyamide column chromatography. According to HPLC analytical results (Figure 2), the major compounds in fractions F-1 (tubes 31−71) and F-3 (tubes 91−141) were compounds **1** and **3**, respectively, while fraction F-2 (tubes 81−121) contained compounds **1**−**3**. Obviously, **1** could be isolated successfully from the mixture of compounds **1**−**3** by polyamide column chromatography, but it was difficult to separate **2** and **3** completely. For further purification, the F-1 fractions were collected, concentrated, and subjected to a column of Toyopearl HW-40S with water as the eluent. The fractions containing **1** were collected, evaporated, and freeze-dried to afford **1** with a purity of 98.86% (Figure 2). The yield of **1** was 615.3 mg/100 g of DSC, and the overall recovery for **1** was 73.16%.

The structure of **1** was characterized by ESI-MS/MS and HPLC. First, the monosaccharide composition of STG was analyzed by RP-HPLC with precolumn derivatization with PMP according to the reported method.[26] The peak with a retention time of 21.693 min (Figure 3) was identified as D-glucose by comparison with that of standard D-glucose, which indicated the monosaccharide component of **1** was glucose. Furthermore, the chemical structure of **1** was characterized by ESI-MS in negative mode (scan range m/z 30−1000), and the ESI-MS spectrum of **1** is presented in Figure 4. The fragment at m/z 855 was observed for ion $[M - H]^-$ (molecular weight 856), and the fragment at m/z 891 indicated the ion presence of $[M + 2H_2O - H]^-$. The ions at m/z 693 and 369 were attributed to the loss of one glucose residue (162 Da) and three glucose residues (486 Da) of **1**, respectively. The fragment at m/z 179 and 485 resulted from the ions of $[\text{glucose} - H]^-$ and three glucose residues, respectively, indicating that there are at least three molecules of glucose in the structure of **1**. Accordingly, **1** was identified to be STG, sesaminol 2′-*O*-*β*-

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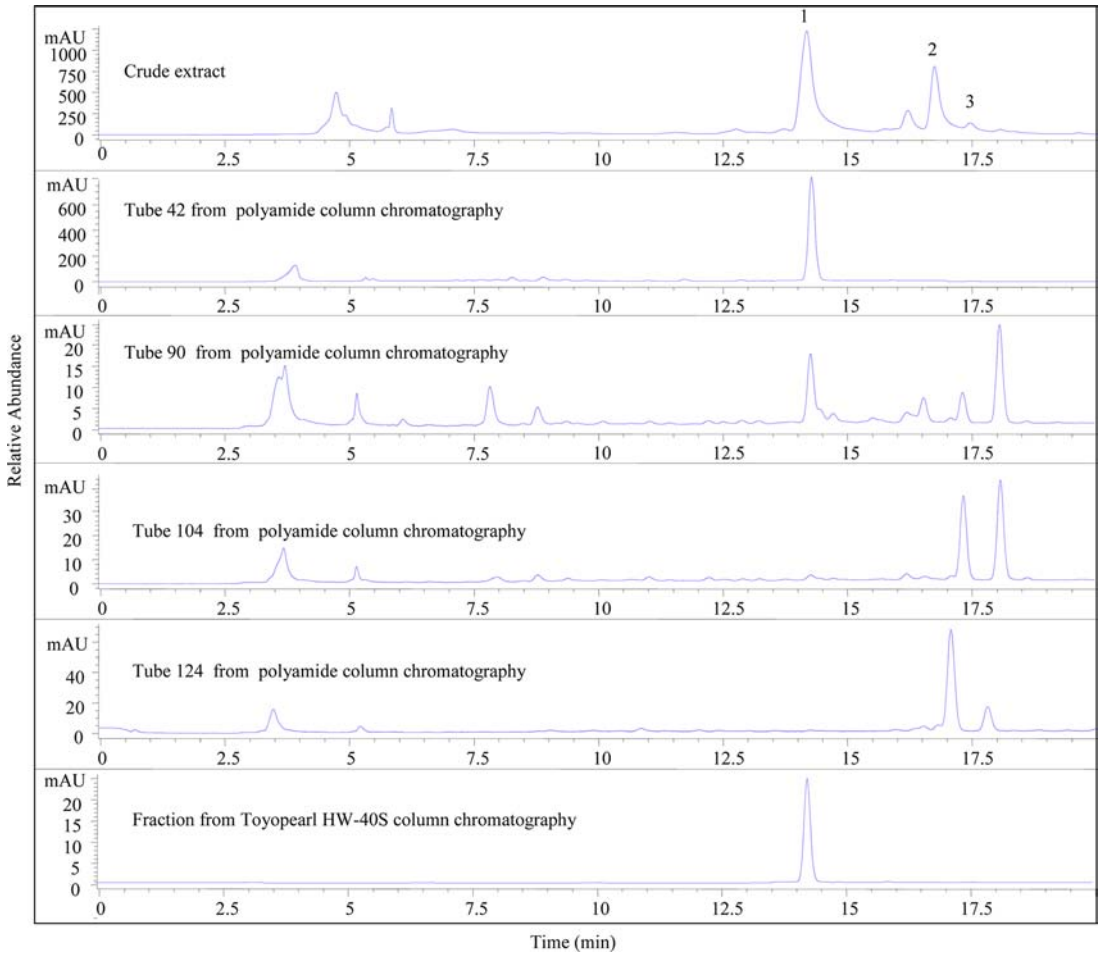

Figure 2. HPLC chromatograms of lignan glycosides from crude extract, fractions from polyamide column chromatography, and fraction from Toyopearl HW-40S column chromatography. Peak 1, STG; peaks 2 and 3, unknown.

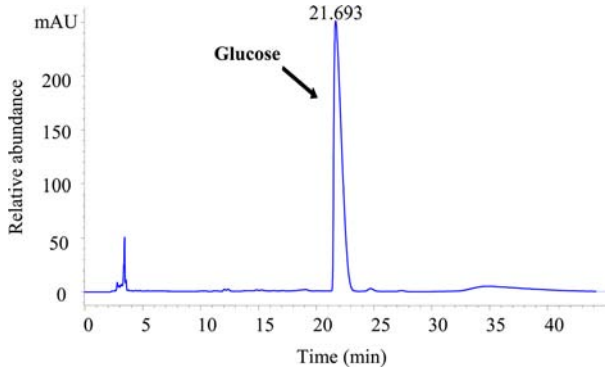

Figure 3. RP-HPLC chromatogram of PMP derivative of compound **1**.

glucopyranosyl(1→2)-*O*-[*β*-D-glucopyranosyl(1→6)]-*β*-D-glucopyranoside.[6,19]

STG is generally prepared from sesame seed or sesame cake by extraction with 80% methanol or ethanol and purification by Amberlite XAD-2 column chromatography and preparative HPLC.[6,13,14] In the present study, high-purity STG was successfully prepared from sesame cake by extraction with 80% ethanol and a simple purification procedures by using polyamide and Toyopearl HW-40S column chromatography. The results suggested that sesame cake could be a suitable source for STG production and STG could be purified from crude extract of sesame cake in large amounts by using column chromatography.

Effect of Sesaminol Triglucoside on Human Gut Microbiota. During the fermentation in vitro of STG, changes of the bacterial populations determined by FISH are shown in Table 1. In general, the total bacterial populations for all treatments significantly increased during the fermentation ($P < 0.05$), but no significant difference was found between STG and the control. At the same fermentation time, the bacterial populations in glucose culture were relatively higher than those in control and STG cultures (Table 1). The bacterial numbers detected by using probes Bac303, Bif164, Erec482, Lab158 and His150 also increased with increasing fermentation time from 0 to 24 h.

STG and glucose induced significant increases in *Bifidobacteria* numbers. However, *Bifidobacteria* number increased significantly only at the early stage of STG fermentation ($P < 0.05$). After 12 h, this situation was changed with only slight increase in both STG and control ($P > 0.05$), although *Bifidobacteria* number in STG fermentation was significantly higher than that of the control at 24 h ($P < 0.05$). STG also induced significant increase in the numbers of *Lactobacillus*–*Enterococcus* group, and the population of *Lactobacillus*–*Enterococcus* group at 24 h was the highest for STG. However, glucose significantly stimulated the growth of *Bacteroides*–*Prevotella* and *Eubacterium*–*Clostridium* groups compared to STG. During the same fermentation time, the levels of

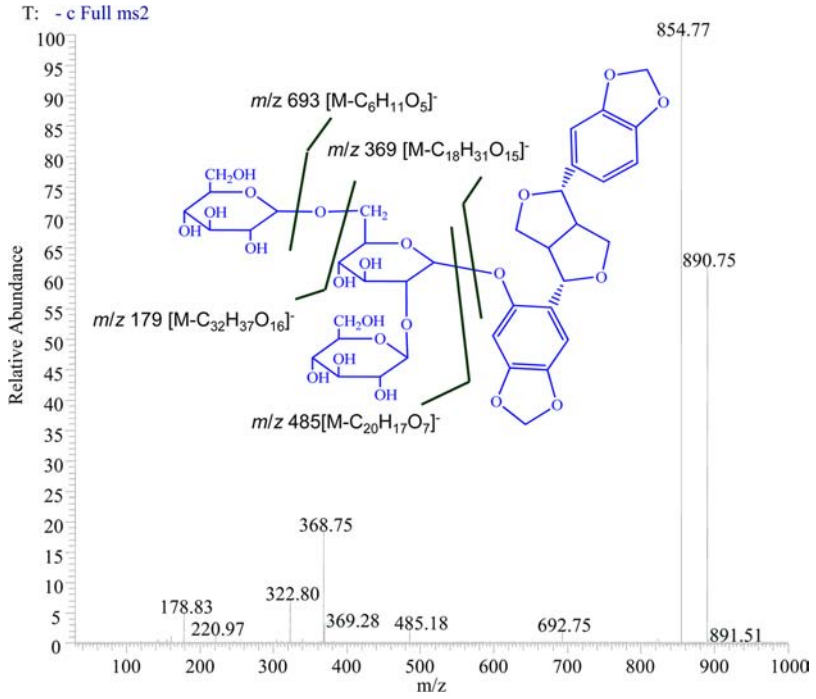

Figure 4. ESI-MS/MS spectrum of compound **1** isolated from defatted sesame cake.

Table 1. Bacterial Population in Fermentation in Vitro Measured by Fluorescence in Situ Hybridization[a]

	bacterial group (log cell/mL)					
time (h)	total cell population	Bac303	Bif164	Erec482	Lab158	His150
			Control			
0	8.67 ± 0.13 a	7.39 ± 0.10 a	7.98 ± 0.09 a	7.13 ± 0.21 a	7.78 ± 0.13 a	7.03 ± 0.11 a
6	8.85 ± 0.16 b	7.59 ± 0.13 b	8.26 ± 0.11 b	7.45 ± 0.14 b	7.87 ± 0.09 bc	7.19 ± 0.14 b
12	8.97 ± 0.13 cd	7.94 ± 0.12 c	8.33 ± 0.11 bc	7.72 ± 0.11 d	8.06 ± 0.16 d	7.51 ± 0.08 c
24	9.12 ± 0.14 e	8.11 ± 0.21 d	8.42 ± 0.12 c	7.94 ± 0.11 e	8.14 ± 0.12 e	7.68 ± 0.03 de
			STG			
6	8.88 ± 0.10 bc	7.57 ± 0.12 b	8.54 ± 0.15 d	7.44 ± 0.11 b	7.97 ± 0.11 c	7.15 ± 0.16 ab
12	9.00 ± 0.11 d	7.87 ± 0.16 c	8.82 ± 0.11 e	7.62 ± 0.12 c	8.10 ± 0.08 de	7.50 ± 0.13 c
24	9.14 ± 0.11 e	7.90 ± 0.12 c	8.89 ± 0.12 e	7.73 ± 0.11 d	8.27 ± 0.15 f	7.60 ± 0.13 cd
			Glucose			
6	10.43 ± 0.13 f	10.08 ± 0.11 e	9.81 ± 0.09 f	9.31 ± 0.07 f	7.93 ± 0.06 bc	7.26 ± 0.05 b
12	10.86 ± 0.07 g	10.14 ± 0.09 e	10.10 ± 0.10 g	9.50 ± 0.08 g	7.96 ± 0.09 c	7.51 ± 0.04 c
24	11.28 ± 0.09 h	10.29 ± 0.07 f	10.41 ± 0.10 h	9.88 ± 0.04 h	8.16 ± 0.03 e	7.75 ± 0.06 e

[a]Data in the same column with different letters are significantly different at $P < 0.05$ for each bacterial group.

Eubacterium−*Clostridium*, *Bacteroides*−*Prevotella*, and *Clostridium histolyticum* groups in STG culture were lower than those in the control and glucose cultures. Therefore, it might be considered that STG did not stimulate the growth of these three groups in human fecal microbiota, though the counts of these bacteria increased during fermentation with STG.

It has been demonstrated that the biotransformation of STG to mammalian lignans by human intestinal microbiota included four types of reaction: hydrolysis of lignan glucoside, demethylation of a methoxy group, oxidation of dibenzylbutanediol to dibenzylbutyrolactone, and reductive cleavage of furofuran rings.[14,41] In the biotransformation of lignans, *Bacteroides* and *Clostridium* species are capable of deglycosylating secoisolariciresinol diglucoside in gut and releasing the aglycons and glycosides, and *Eubacterium limosum* and *Blautia producta* can catalyze the demethylation of a methoxy group.[22−24] Therefore, we examined the changes of STG and its metabolites produced during the fermentation by HPLC and HPLC-DAD-ESI/MS. We found that only a part of STG (9.7%) remained in the medium (Figures 5 and 6) after 6 h of fermentation. During the period 6−12 h of fermentation, STG disappeared completely. The results suggested that STG should be a suitable substrate for intestinal microbiota. Meanwhile, sesaminol diglucoside (SDG), sesaminol, and sesaminol monoglucoside (SMG), identified on the basis of MS (Figure 7) and MS^2 data (Table 2) from HPLC-DAD-ESI/MS analysis,[42] were produced during the in vitro fermentation of STG. At 6 h of fermentation, the level of sesaminol was higher than that of STG, SDG, or SMG, and the maximal production of sesaminol and SDG was observed at 12 h of fermentation. After that, the levels of SDG and sesaminol gradually decreased. In contrast, a gradual increase for the level of SMG was observed. These results suggested that the first step for STG transformation in human gut should be the hydrolysis of lignan

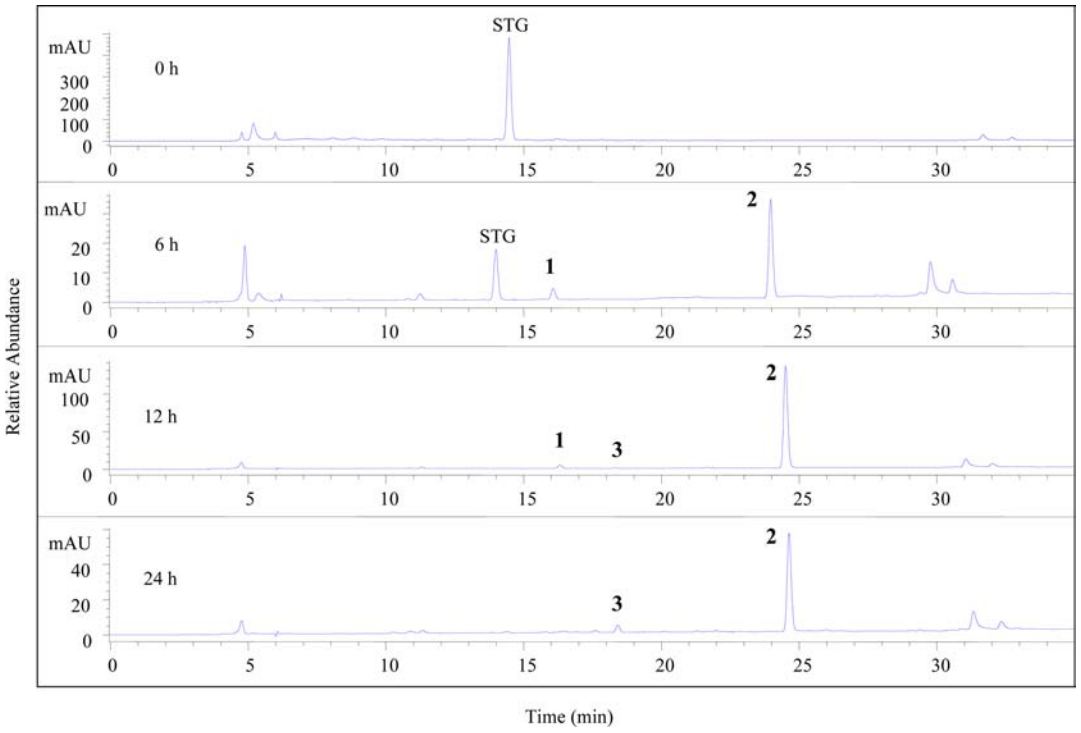

Figure 5. HPLC chromatograms showing changes of STG and its metabolites during the fermentation. Peak 1, SDG; peak 2, sesaminol; peak 3, SMG.

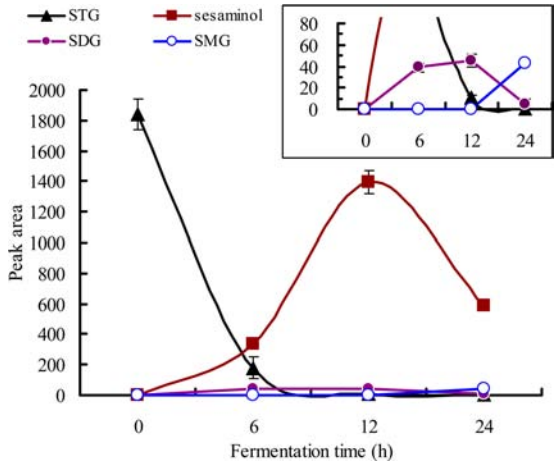

Figure 6. Changes of STG and its metabolites during fermentation. (Inset) Zoom on the 0−80 range of peak area.

glucoside, which is in good agreement with the previous reports.[13,14] Furthermore, SMG might be produced in a manner of sequential deglycosylation of SDG, and sesaminol might be further transformed into other compounds. It has been reported that intestinal microbiota play an important role in the biotransformation of dietary lignans. However, the metabolites from STG, such as sesaminol and other compounds, would reach the colon and be exposed to a substantial microbial population[18] and so have an impact on the growth and composition of intestinal microbiota. Perhaps it is the best explanation for the above variations in human gut microbiota occurring during fermentation. Furthermore, the transformation of STG by intestinal microbiota would lead to production of some amount of glycosides due to the presence of glycosyl residues in the structure of STG. Therefore, STG would provide nutrients (glucose) for the growth of intestinal microbiota.

Effect of Sesaminol Triglucoside on Short-Chain Fatty Acid Production. As shown in Table 3, SCFA production was significantly influenced by the presence of STG during fermentation. The total SCFA concentration in STG culture was relatively higher than those of the control and glucose cultures at 6 and 12 h of fermentation. The concentrations of formic, acetic, propionic, and butyric acids all increased significantly. However, the concentration of lactic acid decreased remarkably during fermentation for all treatments, particularly STG.

The molar proportions of each SCFA at different fermentation points are shown in Figure 8. Among all treatments, acetic acid was in the greatest proportion, accounting for 38.02%, 31.94%, and 39.14% for STG, control, and glucose cultures, respectively, at 24 h of fermentation. In addition, the molar proportions of acetic acid for all treatments at 6 h were the lowest during the fermentation. The high production of acetic acid might be correlated with metabolic activities of *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, and *Enterococcus*.[43,44]

During fermentation, an increased proportion of butyric acid in total SCFA for all treatments was seen (Figure 8), particularly for STG. However, butyric acid was only in a small proportion despite increasing gradually during fermentation. The production of butyric acid occurred at a later stage of fermentation, indicating that its production was correlated with the transformation of other bacterial metabolites formed during the fermentation.[29] The presence of lactic acid is an indicator of metabolic activity of the potentially beneficial bacteria from *Bifidobacterium* and *Lactobacillus*.[29,43] Accordingly, a decline of lactic acid concentration between 6 and 24 h with a

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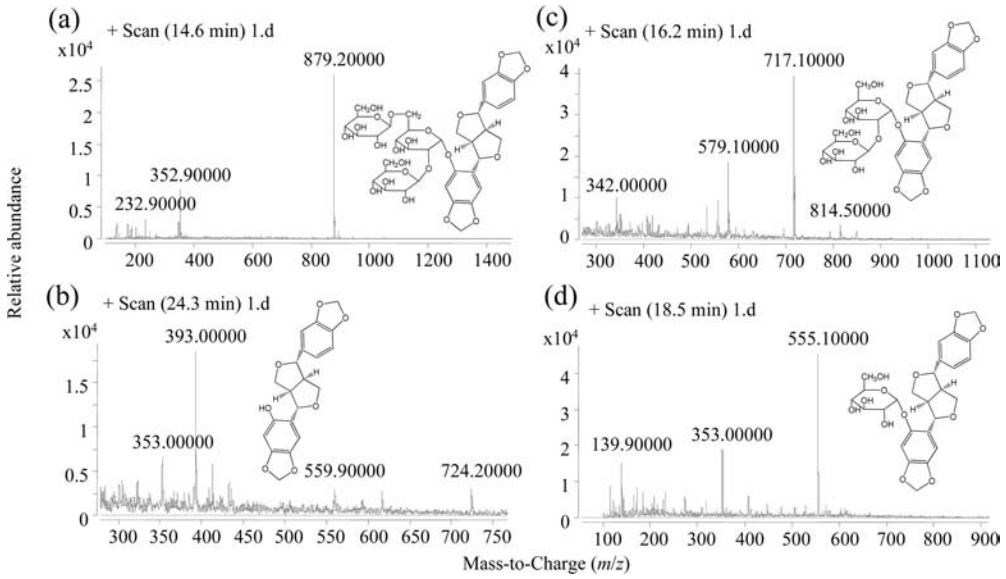

Figure 7. ESI-MS spectra of STG and its metabolites. (a) STG, m/z 879.2 $[M + Na]^+$; (b) SDG, m/z 717.1 $[M + Na]^+$; (c) sesaminol, m/z 393.0 $[M + Na]^+$; (d) SMG, m/z 555.1 $[M + Na]^+$.

Table 2. Mass Data for Sesaminol Triglucoside and Its Metabolites Obtained by HPLC-DAD-ESI/MS in Positive-Ion Mode

peak	retention time (min)	ESI/MS m/z $[M + Na]^+$	MS^2	assignment
STG	14.6	879.2	717.3, 527.1, 304.9	sesaminol triglucoside
1	16.2	717.1	555.2, 365.1, 244.9	sesaminol diglucoside
2	24.3	393.0	334.9, 322.8, 304.8	sesaminol
3	18.5	555.1	537.0, 322.9, 184.8	sesaminol monoglucoside

corresponding increase in butyric acid concentration indicated a possible stimulation of butyric acid-producing bacteria.[29]

In the control and glucose cultures, the production of lactic acid significantly increased, but their molar proportions in total SCFA were observed first to increase and reach a maximum at 6 h, and then gradually decreased after that. In STG culture, the production and molar proportion of lactic acid declined significantly ($P < 0.05$), and the molar proportion was only 9.14% at 24 h. A significant increase of lactic acid in the initial stage of fermentation was perhaps correlated with the high levels of *Lactobacillus* and *Bifidobacterium*, and thereafter, a decline in the concentration of lactic acid was observed. A similar declining trend of lactic acid in batch cultures during fermentation has been observed.[28,45] In the fermentation process, lactic acid is a key intermediate produced via the homofermentative or heterofermentative pathways. It is a precursor for the formation of acetate, propionate, and butyrate in genera such as *Megasphaera* and *Veillonella*, so that it is detected at low levels in healthy subjects.[46] During fermentation, lactic acid tends to be rapidly converted into other SCFA by bacteria in the human intestinal tract.[47−49] In the present study, we noticed a decline of lactic acid concentration between 6 and 24 h with a corresponding increase in butyric acid concentration, which might be a possible reason for the decline of lactic acid during fermentation.

As shown in Table 3, STG produced more formic acid and propionic acid than did the control and glucose at the same fermentation times. However, for all treatments, the molar

Table 3. Short-Chain Fatty Acid Levels in Batch Culture Fermentations[a]

time (h)	total SCFA (mM)	formic acid (mM)	lactic acid (mM)	acetic acid (mM)	propionic acid (mM)	butyric acid (mM)
			Control			
0	17.37 ± 0.22 a	4.52 ± 0.14 a	5.43 ± 0.05 a	7.42 ± 0.08 a	0.00 ± 0.00 a	0.00 ± 0.00 a
6	34.27 ± 0.28 b	8.50 ± 0.09 b	11.29 ± 0.05 d	11.02 ± 0.09 b	3.45 ± 0.03 b	0.00 ± 0.00 a
12	42.37 ± 0.26 c	10.21 ± 0.05 d	12.10 ± 0.03 e	14.46 ± 0.06 c	4.63 ± 0.02 c	0.99 ± 0.15 b
24	50.01 ± 0.14 d	10.30 ± 0.09 d	14.07 ± 0.04 f	15.97 ± 0.06 e	8.37 ± 0.05 e	1.29 ± 0.04 d
			STG			
6	62.35 ± 0.16 f	16.67 ± 0.06 f	15.31 ± 0.03 g	18.88 ± 0.05 f	8.03 ± 0.06 d	3.46 ± 0.05 f
12	81.76 ± 0.16 h	19.40 ± 0.05 g	10.49 ± 0.06 c	29.76 ± 0.09 g	14.63 ± 0.07 h	7.47 ± 0.04 h
24	89.85 ± 0.12 i	20.55 ± 0.04 h	8.21 ± 0.03 b	34.17 ± 0.06 i	17.45 ± 0.03 j	9.48 ± 0.04 i
			Glucose			
6	53.83 ± 0.06 e	8.75 ± 0.07 c	18.29 ± 0.04 h	15.22 ± 0.04 d	10.42 ± 0.03 f	1.15 ± 0.03 c
12	75.68 ± 0.18 g	10.28 ± 0.04 d	20.34 ± 0.06 i	30.89 ± 0.06 h	11.78 ± 0.04 g	2.40 ± 0.02 e
24	90.82 ± 0.18 j	13.06 ± 0.05 e	21.29 ± 0.03 j	35.54 ± 0.03 j	16.94 ± 0.04 i	3.98 ± 0.05 g

[a]Data in the same column with different letters are significantly different at $P < 0.05$.

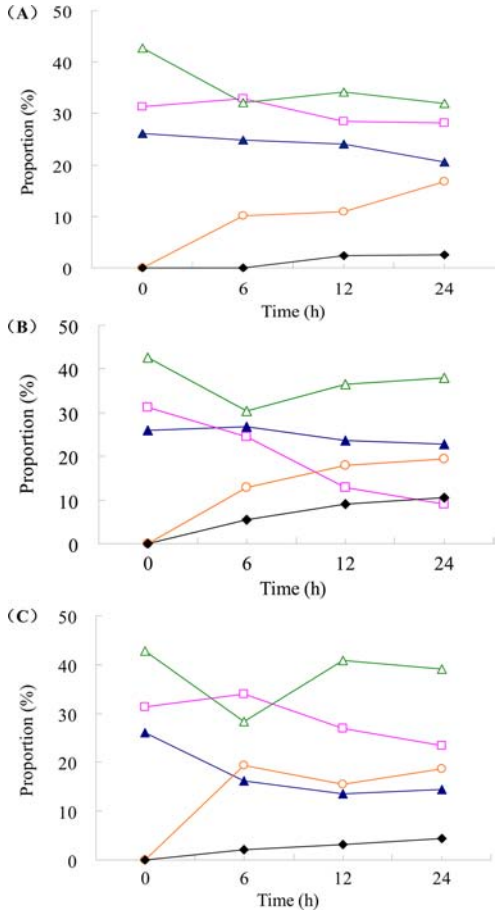

Figure 8. Molar proportion (%) of formic, lactic, acetic, propionic, and butyric acids in total SCFA at different times during fermentation with (A) control, (B) STG, and (C) glucose. (△, green) Acetic acid; (□, magenta) lactic acid; (▲, blue) formic acid; (○, orange) propionic acid; (◆, black) butyric acid.

proportion of formic acid in total SCFA decreased gradually, but the molar proportions of propionic acid increased clearly during fermentation (Figure 8). It was noticeable that acetic, lactic, and formic acids were the main SCFA at the early stage of fermentation; but at the final stage of fermentation, the main SCFA were shifted to acetic, formic, lactic, and propionic acids.

The production of SCFA is a consequence of the metabolism of the microbiota present in the fermentation medium,[31] and it has been reported that SCFA contribute to normal large bowel function and prevent pathologies through their actions in the lumen and in the colonic musculature and vasculature through their metabolism by colonocytes.[50,51] Particularly, butyrate is thought to play a role in a number of beneficial phenomena. High generation of acids upon fermentation is considered desirable, as acidifying the colonic environment can protect against carcinogenic potential and pathogenic bacteria and will be helpful for the absorption of minerals.[52] It has been reported that high concentrations of propionate and butyrate in the colon have great potential for preventing hypocholesterolemia and tumorigenesis,[50] which is consistent with the bioactivity of STG reported by Sheng et al.[10] and Kang et al.[11,53]

For the consumption of sesame seed, South Korea is the highest in the world, where about 6−7 g/day per capita is consumed, and Japan follows (2−3 g/day per capita).[2,54] It has been reported that sesame seed contains 224−1148 mg/100 g lignans, indicating that sesame seed is a source as rich in lignans as flaxseed.[7,8,55,56] The intake of STG per capita could be calculated to be about 6−22 mg/day based on the removal of 50% oil from sesame seed. Accordingly, it will play an important role in the maintenance of human gut microbiota and the production of SCFA, although it is a minor constituent of daily diets.

In conclusion, high-purity STG was successfully prepared from DSC for the first time by extraction with 80% ethanol and simple purification procedures of polyamide and Toyopearl HW-40S column chromatography. During in vitro fermentation with human fecal microbiota, STG could significantly induce an increase in numbers of *Lactobacillus*−*Enterococcus* group and *Bifidobacterium*, while it did not stimulate the growth of *Clostridium histolyticum*, *Eubacterium rectale*−*Clostridium coccoides*, and *Bacteroides*−*Prevotella* groups. Furthermore, it was found that the total SCFA concentration in STG culture was relatively higher than those of the control and glucose cultures at 6 and 12 h of fermentation, and the concentrations of formic, acetic, propionic, and butyric acids increased significantly. These findings provided further evidence for the importance of human intestinal bacteria in the bioactivity of STG and its metabolites in the maintenance of human health.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Ryu, S. N.; Ho, C. T.; Osawa, T. High performance liquid chromatographic determination of antioxidant lignan glycosides in some varieties of sesame. *J. Food Lipids* **1998**, *5*, 17−28.

(2) Namiki, M. Nutraceutical functions of sesame: a review. *Crit. Rev. Food Sci. Nutr.* **2007**, *47*, 651−673.

(3) Kamal-Eldin, A.; Moazzami, A.; Washi, S. Sesame seed lignans: potent physiological modulators and possible ingredients in functional foods & nutraceuticals. *Recent Pat. Food Nutr. Agric.* **2011**, *3*, 17−29.

(4) Matsumura, Y.; Kita, S.; Morimoto, S.; Akimoto, K.; Furuya, M.; Oka, N.; Tanaka, T. Antihypertensive effect of sesamin. I. Protection against deoxycorticosterone acetate-salt-induced hypertension and cardiovascular hypertrophy. *Biol. Pharm. Bull.* **1995**, *18*, 1016−1019.

(5) Hirose, N.; Inoue, T.; Nishihara, K.; Sugano, M.; Akimoto, K.; Shimizu, S.; Yamada, H. Inhibition of cholesterol absorption and synthesis in rats by sesamin. *J. Lipid Res.* **1991**, *32*, 629−638.

(6) Katsuzaki, H.; Kawakishi, S.; Osawa, T. Sesaminol glucosides in sesame seeds. *Phytochemistry* **1994**, *35*, 773−776.

(7) Moazzami, A.; Andersson, R. E.; Kamal-Eldin, A. HPLC analysis of sesaminol glucosides in sesame seeds. *J. Agric. Food Chem.* **2006**, *54*, 633−638.

(8) Moazzami, A.; Andersson, R. E.; Kamal-Eldin, A. Characterization and analysis of sesamolinol digulcoside in sesame seeds. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 1478−1481.

(9) Lee, S. Y.; Ha, T. Y.; Son, D. J.; Kim, S. R.; Hong, J. T. Effect of sesaminol glucosides on β-amyloid-induced PC12 cell death through antioxidant mechanisms. *Neurosci. Res.* **2005**, *52*, 330−341.

(10) Sheng, H.; Hirose, Y.; Hata, K.; Zheng, Q.; Kuno, T.; Asano, N.; Yamada, Y.; Hara, A.; Osawa, T.; Mori, H. Modifying effect of dietary sesaminol glucosides on the formation of azoxymethane-induced premalignant lesions of rat colon. *Cancer Lett.* **2007**, *246*, 63−68.

(11) Kang, M. H.; Kawai, Y.; Naito, M.; Osawa, T. Dietary defatted sesame flour decreases susceptibility to oxidative stress in hypercholesterolemic rabbits. *J. Nutr.* **1999**, *129*, 1885−1890.
(12) Miyake, Y.; Fukumoto, S.; Okada, M.; Sakaida, K.; Nakamura, Y.; Osawa, T. Antioxidative catechol lignans converted from sesamin and sesaminol triglucoside by culturing with *Aspergillus*. *J. Agric. Food Chem.* **2005**, *53*, 22−27.
(13) Jan, K. C.; Ku, K. L.; Chu, Y. H.; Hwang, L. S.; Ho, C. T. Tissue distribution and elimination of estrogenic and anti-inflammatory catechol metabolites from sesaminol triglucoside in rats. *J. Agric. Food Chem.* **2010**, *58*, 7693−7700.
(14) Jan, K. C.; Hwang, L. S.; Ho, C. T. Biotransformation of sesaminol triglucoside to mammalian lignans by intestinal microbiota. *J. Agric. Food Chem.* **2009**, *57*, 6101−6106.
(15) Katsuzaki, H.; Osawa, T.; Kawakishi, S. Chemistry and antioxidative activity of lignan glucosides in sesame seed. In *Food Phytochemicals for Cancer Prevention II*; Ho, C. T., Osawa, T., Huang, M. T., Rosen, R. T., Eds.; ACS Symposium Series *547*; American Chemical Society: Washington, DC, 1994; pp 275−280.
(16) Adlercreutz, H.; Vanderwildt, J.; Kinzel, J.; Attalla, H.; Wähälä, K.; Mäkelä, T.; Hase, T.; Fotsis, T. Lignan and isoflavonoid conjugates in human urine. *J. Steroid Biochem. Mol. Biol.* **1995**, *52*, 97−103.
(17) Mousavi, Y.; Adlercreutz, H. Enterolactone and estradiol inhibit each other's proliferative effect on MCF-7 breast cancer cells in culture. *J. Steroid Biochem. Mol. Biol.* **1992**, *41*, 615−619.
(18) Jan, K. C.; Ku, K. L.; Chu, Y. H.; Hwang, L. S.; Ho, C. T. Intestinal distribution and excretion of sesaminol and its tetrahydrofuranoid metabolites in rats. *J. Agric. Food Chem.* **2011**, *59*, 3078−3086.
(19) Jan, K. C.; Hwang, L. S.; Ho, C. T. Tissue distribution and elimination of sesaminol triglucoside and its metabolites in rat. *Mol. Nutr. Food Res.* **2009**, *53*, 815−825.
(20) Egert, M.; de Graaf, A. A.; Smidt, H.; de Vos, W. M.; Venema, K. Beyond diversity: functional microbiomics of the human colon. *Trends Microbiol.* **2006**, *14*, 86−91.
(21) Ley, R. E.; Lozupone, C. A.; Hamady, M.; Knight, R.; Gordon, J. I. Worlds within worlds: evolution of the vertebrate gut microbiota. *Nat. Rev. Microbiol.* **2008**, *6*, 776−788.
(22) Clavel, T.; Dore, J.; Blaut, M. Bioavailability of lignans in human subjects. *Nutr. Res. Rev.* **2006**, *19*, 187−196.
(23) Clavel, T.; Borrmann, D.; Braune, A.; Doré, J.; Blauta, M. Occurrence and activity of human intestinal bacteria involved in the conversion of dietary lignans. *Anaerobe* **2006**, *12*, 140−147.
(24) Woting, A.; Clavel, T.; Loh, G.; Blaut, M. Bacterial transformation of dietary lignans in gnotobiotic rats. *FEMS Microbiol. Ecol.* **2010**, *72*, 507−514.
(25) Liao, C. D.; Hung, W. L.; Lu, W. C.; Jan, K. C.; Shih, D. Y. C.; Yeh, A. I.; Ho, C. T.; Hwang, L. S. Differential tissue distribution of sesaminol triglucoside and its metabolites in rats fed with lignan glycosides from sesame meal with or without nano/submicrosizing. *J. Agric. Food Chem.* **2010**, *58*, 563−569.
(26) Dai, J.; Wu, Y.; Chen, S. W.; Zhu, S.; Yin, H. P.; Wang, M.; Tang, J. Sugar compositional determination of polysaccharides from *Dunaliella salina* by modified RP-HPLC method of precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone. *Carbohydr. Polym.* **2010**, *82*, 629−635.
(27) Zheng, Y.; Hu, J.; Murphy, P. A.; Alekel, D. L.; Franke, W. D.; Hendrich, S. Rapid gut transit time and slow fecal isoflavone disappearance phenotype are associated with greater genistein bioavailability in women. *J. Nutr.* **2003**, *133*, 3110−3116.
(28) Hernandez-Hernandez, O.; Cote, G. L.; Kolida, S.; Rastall, R. A.; Sanz, M. L. In vitro fermentation of alternansucrase raffinose-derived oligosaccharides by human gut bacteria. *J. Agric. Food Chem.* **2011**, *59*, 10901−10906.
(29) Dominika, S.; Arjan, N.; Karyn, R. P.; Henryk, K. The study on the impact of glycated pea proteins on human intestinal bacteria. *Int. J. Food Microbiol.* **2011**, *145*, 267−272.
(30) Cardelle-Cobas, A.; Olano, A.; Corzo, N.; Villamiel, M.; Collins, M.; Kolida, S.; Rastall, R. A. In vitro fermentation of lactulose-derived oligosaccharides by mixed fecal microbiota. *J. Agric. Food Chem.* **2012**, *60*, 2024−2032.
(31) Rycroft, C. E.; Jones, M. R.; Gibson, G. R.; Rastall, R. A. A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. *J. Appl. Microbiol.* **2001**, *91*, 878−887.
(32) Martín-Peláez, S.; Gibson, G. R.; Martín-Orúe, S. M.; Klinder, A.; Rastall, R. A.; La Ragione, R. M.; Woodward, M. J.; Costabile, A. In vitro fermentation of carbohydrates by porcine faecal inocula and their influence on *Salmonella typhimurium* growth in batch culture systems. *FEMS Microbiol. Ecol.* **2008**, *66*, 608−619.
(33) Langendijk, P. S.; Schut, F.; Jansen, G. J.; Raangs, G. C.; Kamphuis, G. R.; Wilkinson, M. H. F.; Welling, G. W. Quantitative fluorescence in-situ hybridization of *Bifidobacterium* spp with genus-specific 16S ribosomal-RNA-targeted probes and its application in fecal samples. *Appl. Environ. Microbiol.* **1995**, *61*, 3069−3075.
(34) Manz, W.; Amann, R.; Ludwig, W.; Vancanneyt, M.; Schleifer, K. H. Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment. *Microbiology* **1996**, *142*, 1097−1106.
(35) Franks, A. H.; Harmsen, H. J. M.; Raangs, G. C.; Jansen, G. J.; Schut, F.; Welling, G. W. Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Appl. Environ. Microbiol.* **1998**, *64*, 3336−3345.
(36) Harmsen, H. J. M.; Elfferich, P.; Schut, F.; Welling, G. W. A 16S rRNA-targeted probe for detection of lactobacilli and enterococci in faecal samples by fluorescent in situ hybridization. *Microb. Ecol. Health Dis.* **1999**, *11*, 3−12.
(37) Suja, K. P.; Abraham, J. T.; Thamizh, S. N.; Jayalekshmy, A.; Arumughan, C. Antioxidant efficacy of sesame cake extract in vegetable oil protection. *Food Chem.* **2004**, *84*, 393−400.
(38) Mohdaly, A. A. A.; Smetanska, I.; Ramadan, M. F.; Sarhan, M. A.; Mahmoud, A. Antioxidant potential of sesame (*Sesamum indicum*) cake extract in stabilization of sunflower and soybean oils. *Ind. Crop. Prod.* **2011**, *34*, 952−959.
(39) Shyu, Y. S.; Hwang, L. S. Antioxidative activity of the crude extract of lignan glycosides from unroasted Burma black sesame meal. *Food Res. Int.* **2002**, *35*, 357−365.
(40) Suja, K. P.; Jayalekshmy, A.; Arumughan, C. Antioxidant activity of sesame cake extract. *Food Chem.* **2005**, *91*, 213−219.
(41) Landete, J. M. Plant and mammalian lignans: A review of source, intake, metabolism, intestinal bacterial and health. *Food Res. Int.* **2012**, *46*, 410−424.
(42) Struijs, K.; Vinchen, J. P.; Gruppen, H. Comparison of atmospheric pressure chemical ionization and electrospray ionization mass spectrometry for the detection of lignans from sesame seeds. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 3615−3623.
(43) MacFarlane, G. T.; Allison, C. Utilisation of protein by human gut bacteria. *FEMS Microbiol. Lett.* **1986**, *38*, 19−24.
(44) Sanz, M. L.; Polemis, N.; Morales, V.; Corzo, N.; Drakoularakou, A.; Gibson, G. R.; Rastall, R. A. In vitro investigation into the potential prebiotic activity of honey oligosaccharides. *J. Agric. Food Chem.* **2005**, *53*, 2914−2921.
(45) Hughes, S. A.; Shewry, P. R.; Gibson, G. R.; McCleary, B. V.; Rastall, R. A. *In vitro* fermentation of oat and barley derived β-glucans by human faecal microbiota. *FEMS Microbiol. Ecol.* **2008**, *64*, 482−493.
(46) Muñoz-Tamayo, R.; Laroche, B.; Walter, É.; Doré, J.; Duncan, S.; Flint, H. J.; Leclerc, M. Kinetic modelling of lactate utilization and butyrate production by key human colonic bacterial species. *FEMS Microbiol. Ecol.* **2011**, *76*, 615−624.
(47) Bourriaud, C.; Robins, R. J.; Martin, L.; Kozlowski, F.; Tenaileau, E.; Cherbut, C.; Michel, C. Lactate is mainly fermented to butyrate by human intestinal microfloras but inter-individual variation is evident. *J. Appl. Microbiol.* **2005**, *99*, 201−212.
(48) Duncan, S. H.; Louis, P.; Flint, H. J. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Appl. Environ. Microbiol.* **2004**, *70*, 5810−5817.

(49) Kedia, G.; Vázquez, J. A.; Charalampopoulos, D.; Pandiella, S. S. In vitro fermentation of oat bran obtained by debranning with a mixed culture of human fecal bacteria. *Curr. Microbiol.* **2009**, *58*, 338−342.
(50) Kim, H. J.; White, P. J. In vitro fermentation of oat flours from typical and high β-glucan oat lines. *J. Agric. Food Chem.* **2009**, *57*, 7529−7536.
(51) Topping, D. L.; Clifton, P. M. Short chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol. Rev.* **2001**, *81*, 1031−1064.
(52) Cummings, J. H. Short chain fatty acids in the human colon. *Gut* **1981**, *22*, 763−779.
(53) Kang, M. H.; Naito, M.; Sakai, K. Mode of action of sesame lignans in protecting low-density lipoprotein against oxidative damage in vitro. *Life Sci.* **2000**, *66*, 161−171.
(54) Namiki, M. The chemistry and physiological functions of sesame. *Rood Rev. Int.* **1995**, *11*, 281−329.
(55) Moazzami, A.; Kamal-Eldin, A. Sesame seed is a rich source of dietary lignans. *J. Am. Oil. Chem. Soc.* **2006**, *83*, 719−723.
(56) Moazzami, A.; Haese, S. L.; Kamal-Eldin, A. Lignan contents in sesame seeds and products. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 1022−1027.