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Alleviating effects of tumorous stem mustard fermented by starter cultures on ulcerative colitis through modulating gut microbiota and inhibiting the TLR4/MyD88/NF- κ B pathway

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ABSTRACT: Tumorous stem mustard (TSM) is one kind of important cruciferous vegetables for directly consumption and processing in China. Inoculation of lactic acid bacteria (LAB) starters can enhance the maturing of fermented vegetables and improve the sensory quality. However, it is unknown whether homo- and hetero-fermentative LAB starters exert distinct effects on the functional activities of fermented vegetables. Fermented with homo-fermentative *Lactiplantibacillus plantarum* S7-12 and heterofermentative *Lentilactobacillus parafarraginis* L-7 enhanced the ripening of fermented tumorous stem mustard (FTSM), improved the glucosinolate contents and showed higher antioxidant activities than spontaneous fermentation TSM. Apart from the differences in the production of organic acids, S7-12 starter retained higher content of total phenolics than L-7. Furthermore, FTSM treatment alleviated the DSS-induced colitis symptoms including body weight loss, DAI values and shortened colonic length. Both S7-12 and L-7 FTSM better ameliorated colonic pathological damage and exhibited good antioxidative activity *in vivo* than SF FTSM, but there was no significant difference between them. Immunohistochemical results further confirmed that FTSM maintained the colon mucosal barrier function by restoring TJ proteins. Moreover, FTSM supplementation could reduce the levels of serum and colonic proinflammatory cytokines IL-6, TNF- α and IL-1 β and enhance the expression of anti-inflammatory cytokine IL-10 by inhibiting the TLR4/MyD88/NF- κ B signaling pathway. FTSM treatment reversed the decline in the microbial diversity induced by DSS and reduced the pro-inflammatory gram-negative bacteria *Enterobacteriaceae*. In conclusion, S7-12 and L-7-inoculated FTSM relieved the DSS-induced colitis through regulating the composition of intestinal microbiota and inhibiting the TLR4/MyD88/NF- κ B signaling pathway.

Keyword: fermented tumorous stem mustard, inoculated fermentation, lactic acid bacteria, colitis, gut microbiota

1. Introduction

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Tumorous stem mustard (TSM) (*Brassica juncea* var. *tumida*), characterized by its swollen succulent stem, is a critical cruciferous vegetable in Asia, especially in China [1]. Its stem is rich in vitamins, dietary fiber, amino acids and mineral substances as well as phytochemicals such as glucosinolates (GLSs) [1,2]. TSM are mainly consumed as fresh state or processing into *Zhacai*, a famous salted pickle in China.

The industrial scale pickling process of *Zhacai* involves repeated marinating in salt solution (12%-14%, w/w) and pressing to remove the brine, followed by fermentation for several months. High concentration of salt contributes to the distinctive flavor and crispy texture through inhibiting the growth of autochthonous microorganisms on the surface of the raw TSM, inactivating TSM-derived enzymes and reducing water activity [3]. However, this traditional salted pickling not only leads to products causing health concerns, but also a large amount of high salinity wastewater.

Low salt fermentation of TSM could be considered an alternative process from the perspective of nutritional values and health benefits. It has been observed that microbial community is correlated with the maturing, sensory quality and the metabolism of phytochemicals of fermented vegetables [3]. Inoculation of starter cultures could enhance the ripening of fermented vegetables, reduce rapidly the pH value and influence the microbial flora of fermented vegetable, thus altering the metabolite composition. Homofermentative and heterofermentative lactic acid bacteria (LAB) starters play different roles in the fermentation process. Heterofermentative LAB starters only dominate during the early stage of the fermentation and produce acetic acid, mannitol and carbon dioxide, while homofermentative LAB produce rapidly lactic acid [4]. Differences in the contents of amino acids and volatiles compounds were observed between Paocai fermented with homofermentative *Lactiplantibacillus pentosus* and *L. plantarum* and heterofermentative *Leuconostoc mesenteroides* and *L. brevis* [5]. Heterofermentative starters affected the composition of volatile aroma compounds in semi-hard cheese [6]. However, the effect of homo- and heterofermentative LAB on the metabolism of bioactive substances like GLSs and the functional properties of fermented foods are still unknown to our knowledge.

Ulcerative colitis (UC) is one of chronic inflammatory bowel disease (IBD), which has been a global health burden worldwide. It is suggested that UC is attributed to genetic, microbial and environmental factors [7]. Apart from medicals treatment, dietary intervention is also a potential intervention strategy for UC. Our study aimed to investigate the effects of homo- and heterofermentative LAB on the phytochemicals and antioxidant activities of TSM. Health benefits of two forms of fermented tumorous stem mustard (FTSM) were further evaluated in DSS-induced UC mice model. The study can provide a reference for the starter fermentation of cruciferous vegetables on the UC intervention.

2. Materials and methods

2.1 Reagents and kits

Chemicals including 3,5-dinitrosalicylic acid, gallic acid, FeSO₄, salicylic acid, sodium acetate, tripyridyltriazine (TPTZ) and FeCl₃ were obtained from Kelong Chemical Reagent Factory (Chengdu, China).

Sinigrin, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and dextran sulfate sodium (DSS, MW 40000) were purchased from Sigma (Shanghai, China).

Kits for measuring the levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), catalase (CAT), malondialdehyde (MDA) and myeloperoxidase (MPO) were purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). Elisa kits of IL-10, IL-6, TNF- α and IL-1 β were obtained from Shanghai Enzyme Biotechnology Co., Ltd. (Shanghai, China).

2.2 Preparation of fermented tumorous stem mustard (FTSM)

L. plantarum S7-12 and *Lentilactobacillus parafarraginis* L-7 were separated from traditional Paocai, identified and stocked in the lab. Both strains were successively transferred to MRS broth and cultured at 30°C for 20 h after activation, and then centrifuged at 4000 $\times g$, 4°C for 10 min. After washing twice with saline solution (0.85%), the cell pellets were then resuspended in saline solution.

Tumorous stem mustard (TSM) was purchased from a local supermarket, (Chongqing, China). After removing the bruised, insect-damaged and aged parts of TSM, the mustard was cut into 4-millimeter shreds and blanched for 1 min to avoid the potential environmental microbial contamination. The sliced mustard was pressed into glass fermentation jar after sterilizing by boiling water and covered with enough saline (2.5%, w/v). The bacterial suspension of S7-12 and L-7 (10^8 CFU/mL) were inoculated into the corresponding jar respectively. TSM with only saline (2.5%, w/v) was considered as spontaneous FTSM. All fermentation jars then were incubated at room temperature (around 25°C) for 7 days. Each FTSM group was performed in biological triplicate.

2.3 Physicochemical analyses

2.3.1 pH

pH values of FTSM brines were measured using a pH Meter at an ambient temperature around 25°C.

2.3.2 Total acid content (TAC)

10 g of crushed FTSM samples were mixed with 100 mL Mill-Q water. After ultrasonic treatment for 30 min and filtered through filter paper, total acid content (TAC) of FTSM was determined based on the description in Chinese national standard (GB 12456-2021).

2.3.3 Organic acids

The content of organic acids was determined using a Shimadzu HPLC LC-20A system (Shimadzu, Japan) equipped with Shim-pack GIST column (C18, 5 μm , 4.6 mm $\times$ 150 mm) and a UV detector (λ = 210 nm) at 30°C with a flow rate of 0.3 mL/min. The mobile phase is consisting of 0.1% phosphoric acid solution (A) and methanol (B) (A:B = 97.5:2.5). And the injection volume is 20 μL .

2.3.4 Others

The content of dietary fiber and total phenolic compounds (TPC) were determined as the description of our previous study [8].

Total GLSs content was analyzed using the spectrophotometric method [9]. TSM extract (0.1 mL) was mixed with 0.1% sodium carboxymethylcellulose (0.2 mL) and 4 mmol/L (0.2 mL) PdCl₂. The volume of mixture was supplemented with distilled water to 1 mL, vortexed and kept at 25°C for 3 h. The absorbance was measured at 450 nm with sinigrin as a standard.

2.4 Determination of antioxidant activity

The antioxidant activities of FTSM were determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) [8] and hydroxyl radical [10] scavenging abilities and FRAP assay [11]. Trolox was used as standard for FRAP assay.

2.5 Animal experiments

Fifty 8-week-old SPF male C57BL/6 mice were obtained from Jiangsu Huachuang Xinnuo Pharmaceutical Technology Co. (Taizhou, China) and adopted in a constant SPF environment (12 h light-12 h dark cycle, 23°C ± 1°C, 55%-65% relative humidity) for one week, then randomly divided into five groups: normal (NM), DSS, spontaneous fermentation of TSM (SF), fermented TSM by *L. plantarum* S7-12 (S7-12) and fermented TSM by *Lent. parafarraginis* (L-7). Sample size of animal experiments is determined based on a preliminary study (data not published) and calculation of software G Power (<http://www.gpower.hhu.de/>) [11].

FTSM samples of SF, S7-12 and L-7 were freeze-dried using a SCIENTZ-20F lyophilization equipment (Scientz Biotechnology, Ningbo, China) and then ground into fine powder for making 5% (w/w) FTSM mouse chow with Ain-93G diet [12]. Mice of the NM group and DSS group were fed Ain-93G diet (Jiangsu Xietong Pharmaceutical Bio-engineering Co., Nanjing, China), whereas those of intervention group were fed Ain-93G diet supplemented with SF, S7-12 or L-7 FTSM powder from the beginning, respectively. All chews and water were offered ad libitum. From 14th day, mice of DSS and intervention group drank water containing 3% (w/v) DSS for 7 days, while sterile water for NM group. The mice were monitored for weight changes per day during the DSS treatment. Mouse feces were collected at the end. After the mice were sacrificed, blood was collected and spleen, kidney, colon and liver samples were harvested, washed and weighed after removing the water. The colon length was also measured. There were 10 samples per group for animal experiments except where otherwise specified.

All protocols for the animal experiment were approved by the Institutional Animal Care and Use Committee of Southwest University (reference number IACUC-20231101-06) and complied with EU guidelines (Directive 2010/63/EU).

2.6 Assessment of disease activity index (DAI)

DAI scores were obtained as the total scores of weight loss, altered stool consistency and signs of fecal blood (**Supplementary Table 1**).

2.7 Histopathological evaluation

0.5 cm of distal colonic tissue was collected and washed with PBS solution (pH 7.4) twice and then fixed in a 4% paraformaldehyde overnight. The colon tissues were then embedded in paraffin and cut at 5 µm

thickness slices. Finally, the tissue sections were stained with hematoxylin and eosin (H&E) and examined under an optical microscope (100x and 400x). The histological scores were assessed as the criteria shown in **Supplementary Table 2**.

2.8 Determination of serum antioxidant levels

The levels of serum glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and malondialdehyde (MDA) were measured using the kits from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China) according to the manufacturer's guidelines.

2.9 Determination of myeloperoxidase (MPO) and inflammatory cytokine levels

After the colon tissue was homogenized in PBS (1:9, w/v) using a tissue homogenizer, the homogenates were centrifuged at 1800 g for 10 min at 4°C. The supernatant was collected and used for the measurement of MPO activity and inflammatory cytokines. Colon MPO activity was determined using the corresponding kits. Serum and colonic IL-6, IL-10, IL-1 β and TNF- α (Shanghai Enzyme Biotechnology, Shanghai, China) were determined using the corresponding Elisa kits following the instructions from the manufacturer.

2.10 Immunohistochemical (IHC) assay

The expressions of tight junction (TJ) proteins including occludin, ZO-1 and claudin-1 and colonic TLR4、MyD88、NF- κ B p65 proteins were examined using IHC assay. Firstly, the deparaffinized tissues were subjected to antigen repair, and then placed in a 3% H₂O₂ in a dark for 25 min. A blocking step was performed using serum for 30 min, and then the primary antibody was added to the tissue section and kept at 4°C overnight. The secondary antibody was added and placed at room temperature for 50 min after decolorization. DAB color developing solution was added for color development, and then the cell nuclei were counterstained with hematoxylin. After dehydration, the sections were sealed with sealing glue and placed under a white light microscope for microscopic examination, and the positive area of the protein was analyzed using Image Pro Plus 6.0.

2.11 16S rRNA sequencing

Total microbial genomic DNA was extracted from the collected mouse feces using the FastPure Stool DNA Isolation Kit (MJYH, Shanghai, China). The concentration and quality of DNA were determined by 1.0% agarose gel electrophoresis and a NanoDrop2000 spectrophotometer (Thermo Scientific, United States). The V3-V4 region of 16S rRNA were amplified using primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR products were extracted, purified, eluted, and detected, and then sequenced on an Illumina Nextseq2000 platform (Illumina, San Diego, USA) by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). After demultiplexing, the resulting sequences were quality filtered with fastp (v0.19.6) and merged with FLASH (v1.2.7), and then de-noised using DADA2 in the Qiime2 (version 2020.2). And the data were further analyzed using the Majorbio Cloud Platform (<https://cloud.majorbio.com>). Alpha diversity indices including Ace, Shannon and coverage were calculated using the package Mothur v1.30.1. Beta diversity were analyzed by principal coordinate analysis (PCoA)

based on Bray Curtis metrics. Statistical significance of microbial community was analyzed by permutational multivariate analysis of variance (PERMANOVA) test using the package Vegan v2.5-3.

2.12 Statistical analysis

Data were expressed as mean $\pm$ SEM of at least triplicate experiments. Differences of physicochemical and biochemical indexes were evaluated via one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test in SPSS 20 (SPSS Inc., Chicago, IL, USA). Differences at $P < 0.05$ were considered statistically significant.

3. Results and discussion

3.1 pH values and TAC of different FTSM

As one of most popular traditional fermented vegetables in China, Paocai is immersed in brines and anaerobically fermented in closed jar [8]. Starter culture can improve the acid-producing rate and enhance the mature of fermented vegetables compared to traditional spontaneous fermentation. pH value is not only associated with the safety and sensory quality, but also a critical parameter indicating the maturity of fermented vegetables [13]. It is generally acknowledged that when the pH of fermented vegetable reaches between 3.0 and 4.0, indicating that the Paocai has matured.

For homofermentative S7-12 group, the pH decreased and TAC increased rapidly during 3 days, while the pH and TAC altered more slowly in L-7 group (**Fig. 1A** and **1B**), because the heterofermentative LAB produced carbon dioxide, mannitol in addition to organic acids. As a result, the S7-12 group obtained a lower pH and a higher TAC than other two groups at the later stages of fermentation. After the sliced mustard was blanched for 1 min, the LAB count of SF group on day 0 still reached to 10^2 CFU/mL (**Supplementary Fig. 1**), suggesting that blanching did not completely remove the endogenous microorganisms from the mustard, although it was far lower than those in starter groups. Therefore, LAB grew slowly and yielded low acid production during the initial fermentation stage in SF group, which required longer time for dominating the fermentation process [14]. One study of inoculated sauerkraut with single LAB strains also demonstrated that the pH decreased more rapidly in homofermentative *L. plantarum*-inoculated sauerkraut along with higher acid production than that inoculated with heterofermentative *Leuc. Mesenteroides* [15]. *Leuc. Mesenteroides* sauerkraut had slight lower pH value and higher TAC than spontaneous fermentation during 7 days. Similar trends of pH and TAC were also observed in Paocai inoculated with homo- fermentative *L. pentosus* and *L. plantarum* and hetero- fermentative *Leuc. mesenteroides* and *L. brevis* [5]. These showed that inoculation of LAB starter cultures, especially homofermentative strains, is beneficial to accelerate the fermentation process of TSM and shorten the maturation period.

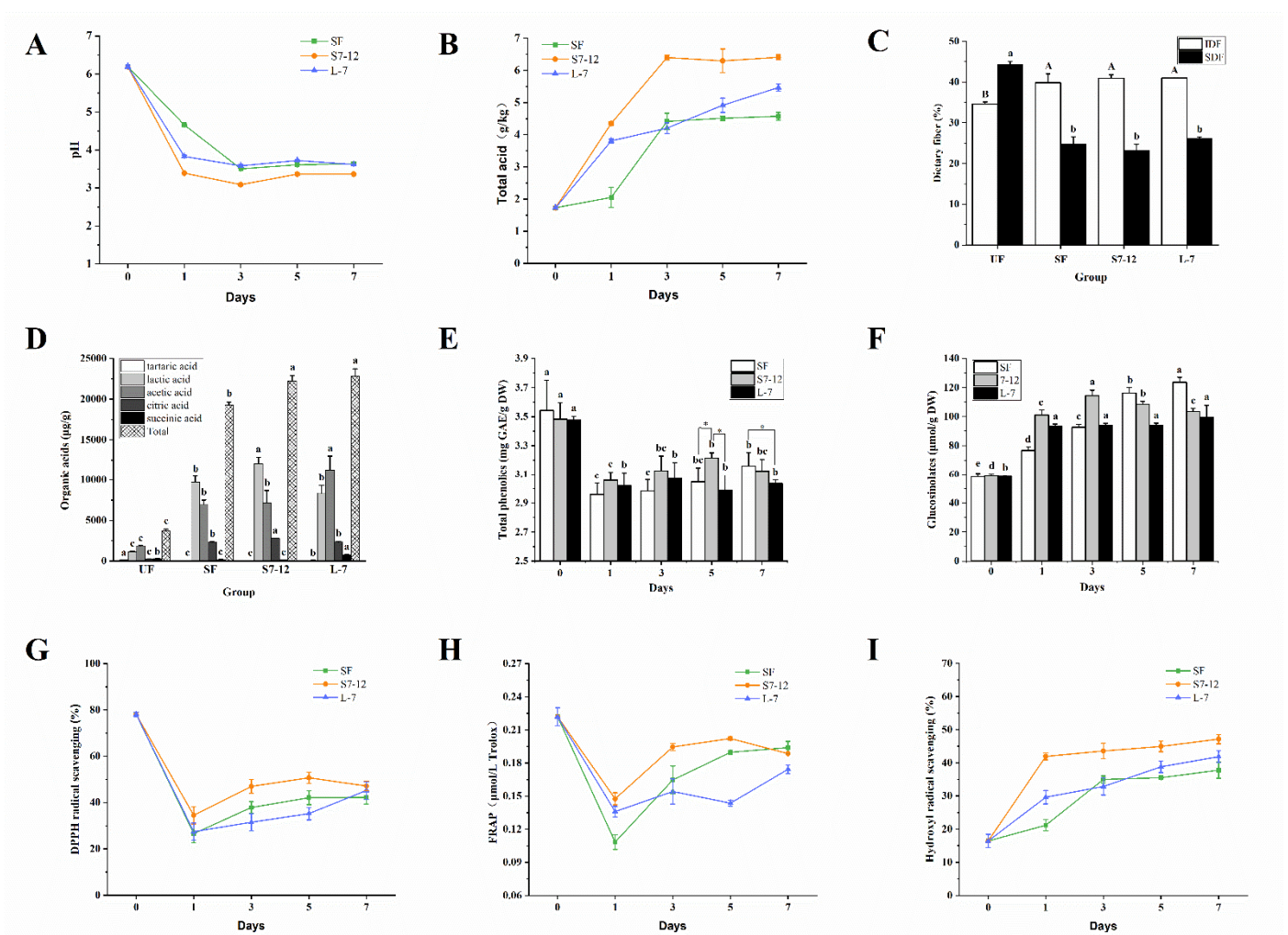


Fig. 1 Effects of S7-12 and L-7 starter cultures on the physicochemical indices (A, pH; B, total acid; C, dietary fiber; D, organic acids; E, total phenol; F, glucosinolates) and antioxidant activities (G, DPPH· radical scavenging ability; H, FRAP assay; I, OH scavenging ability) of FTSM. Statistically significant results in different groups are marked by different letters.

3.2 Dietary fiber of different FTSM

Dietary fiber including soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) is resistant to digestion by human alimentary enzymes. SDF is fermented and utilized by gut microorganisms to produce beneficial metabolites in the large intestine, effectively protecting the intestinal barrier and intestinal microbiota homeostasis. IDF can effectively increase fecal bulk and promote intestinal peristalsis through particle formation and water-holding capacity, ameliorating constipation, lowering blood glucose and cholesterol levels, and improving gastrointestinal disorders [16]. In this study, the SDF content of FTSM decreased from 44.35% to 23.25% (S7-12) after 7 days of fermentation, while the IDF content increased from 34.60% to 40.97% (L-7), no significant difference among three FTSM groups. The altering trend of SDF and IDF was different from the findings of Kaczmarek et al [17], but the total dietary fiber content decreased after fermentation in all cases, which may be due to the different microbial communities and the pectinase-producing species leading to the decrease in SDF content (**Fig. 1C**).

3.3 Organic acids of different FTSM

Organic acids are the main flavor substances of fermented vegetables. The composition and content of organic acids are correlated to the microbial composition, growth conditions and raw materials. Tartaric acid, lactic acid, acetic acid, citric acid and succinic acid were detected in raw TSM (**Fig. 1D**). The total content of organic acids in FTSM increased rapidly as fermentation progressed, finally exhibiting higher levels in starter-inoculation SF than SF. In particular, the tartaric acid content remarkably decreased and was significantly higher in L-7 group than other FTSM after 7 days fermentation. The concentration of succinic acid reduced in SF and S7-12-inoculation FTSM, while increased in L-7 group after 7 days of fermentation. S7-12 starter fermentation induced higher production of lactic acid ($P < 0.05$) because homofermentative LAB almost only produce lactic acid. L-7 FTSM had significantly higher content of acetic acid than other groups ($P < 0.05$). Lactic acid is characterized as “tart” and “acid”, exerting a mild acidic note in aqueous solution [18]. Acetic acid could contribute to a distinct flavor of FTSM inoculated with hetero- fermentative starter because it not only has a sharp, spicy and sour taste but also forms aromatic substances such as esters with alcohols and ketones [19]. Tartaric acid tastes slightly astringent and possesses a strong sour taste, while citric acid has a mild and refreshing sour taste, which can impart fermented vegetables a good sour taste. Succinic acid has a weaker sour taste and is similar to the umami taste of shellfish. Overall, heterofermentative L-7 FTSM had the highest contents of total organic acids, tartaric acid and tartaric acid, whereas homofermentative S7-12 had higher levels of lactic acid and citric acid. Organic acids mainly contribute to the sour profile of fermented vegetables, and the overall flavor still needs to be evaluated via sensory evaluation in future.

3.4 TPC and GLS contents of different FTSM

The TPC in three types of FTSM decreased sharply and then showed a slight fluctuating up and down until the day 7, which was consistent with the results of previous study [20] (**Fig. 1E**). At the early stage of fermentation, phenolic compounds could leach into the brines, leading to a remarkable decline of TPC in TSM matrix. The TPC then slightly increased, which could be attributed to the LAB-degradation of the plant cell wall and microbial production of acids, promoting the release of phenolic compounds and the inhibition of polyphenol oxidase activity [21]. The loss of TPC ranged from 9.7% to 13.3% during the fermentation process of TSM, indicating that fermentation is a relatively good method for retaining the phenolics of TSM, and the homofermentative S7-12 strain were more effective in retaining the total phenolics than L-7 strain. The loss of total phenols is mainly attributed to polyphenol oxidases, in addition to including the utilization by microorganisms [22], precipitation or oxidation, binding, adsorption, and polymerization of phenolic compounds with proteins, microorganisms, and other substances [20].

GLSs are one group of important health-promoting compounds unique to *Brassica* vegetables. Spontaneous fermentation increased gradually with the GLS content of TSM, and it reached the highest on the 7th day (123.63 $\mu\text{mol/g DW}$), which was 2.1 times that of TSM (**Fig. 1F**). The GLS contents peaked on the day 3 for S7-12 group and on the day 7 for L-7 group, 114.36 and 99.50 $\mu\text{mol/g DW}$, respectively. There was no significant difference for GLS metabolism between S7-12 and L-7 groups. Similar results of total GLS content were observed in the study of broccoli juice fermentation by *Lactobacillus* spp by Ye et al [23], which

may be attributed to the fact that bacterial enzymes are capable of degrading the cell wall structure and enhancing the release of bound phytochemicals, including GLSs.

3.5 Antioxidant activities of FTSM

The DPPH scavenging ability and FRAP values of FTSM (**Fig. 1G and 1H**) exhibited a decrease followed by a gradual increase, being consistent with the trend of TPC and similar with the antioxidant ability of a fermented leaf mustard [24]. Pearson correlation analysis showed a significant positive association between the DPPH scavenging and FRAP value and TPC ($P < 0.01$) (**Table 1**), which is in agreement with the results of a previous study [25]. But the $\bullet$ OH scavenging capacity increased from 16.4% to 37.8%-47.2% after fermentation, and was positively correlated with the GLS content ($P < 0.01$) (**Fig. 1I**). Therefore, the decrease of DPPH scavenging and FRAP values could be attributed to the decrease of TPC and the $\bullet$ OH scavenging is mainly associated with GLSs. Besides, S7-12 FTSM displayed higher antioxidant capacity, which could be explained by S7-12 fermentation deconjugating bound phenolic compounds [10]. Similarly, lactic starter fermentation showed higher antioxidant activity than spontaneous fermentation of Chinese cabbage, white cabbage, and red cabbage [26].

Table 1 Correlation between total phenols and glucosinolates with antioxidant capacity

Items	DPPH radical scavenging (%)	Hydroxyl radical scavenging (%)	FRAP (μ mol/L Trolox)
Total phenolics	.961**	-.611*	.835**
Glucosinolates	-.619*	.876**	-.212

Note: ** indicates $P < 0.01$; * indicates $P < 0.05$

3.6 Alleviating effects of different FTSM on DSS-induced colitis symptoms

It is generally considered that treatment of vegetables and probiotics have an alleviating impact on the DSS-induced colitis symptoms [7, 27]. DSS-induced colitis is characterized by body weight loss, shorten colon length, fecal bleeding and disrupted intestinal morphology. In our study, the body weight of mice in the DSS group declined remarkably compared to the NM group (**Fig. 2A**). The body weight of mice of three FTSM intervened mice initially increased, and then decreased from the day 5 of DSS administration. It showed that FTSM had a significant mitigating effect ($P < 0.05$) on the decrease in body weight of mice with colitis.

The DAI scores of DSS-treated mice increased sharply, while SF, S7-12, and L-7 FTSM partly reduced the DAI scores (**Fig. 2B**). The colon length of mice in the model group was significantly shorter than that of the NM group ($P < 0.05$) (**Fig. 2C and 2D**). FTSM feeding extended the colon length to different degrees, and there is no significant difference between FTSM intervention and NM group. Besides, the organ indexes of the spleen, liver and kidney in the model group increased significantly ($P < 0.05$) compared to the NM group (**Table 2**), which revealed that DSS-induced colitis caused the extra-intestinal organ swelling. The damaged intestinal barrier cannot prevent the migration of microorganisms and microbial products to the liver, leading to the liver injury [28]. Intervention of SF, S7-12 and L-7 FTSM reversed these organ indexes to different extent, indicating that FTSM treatment could prevent the extra-intestinal organ inflammatory. L-7 FTSM significantly reduced the liver index to the level of NM mice ($P < 0.05$).

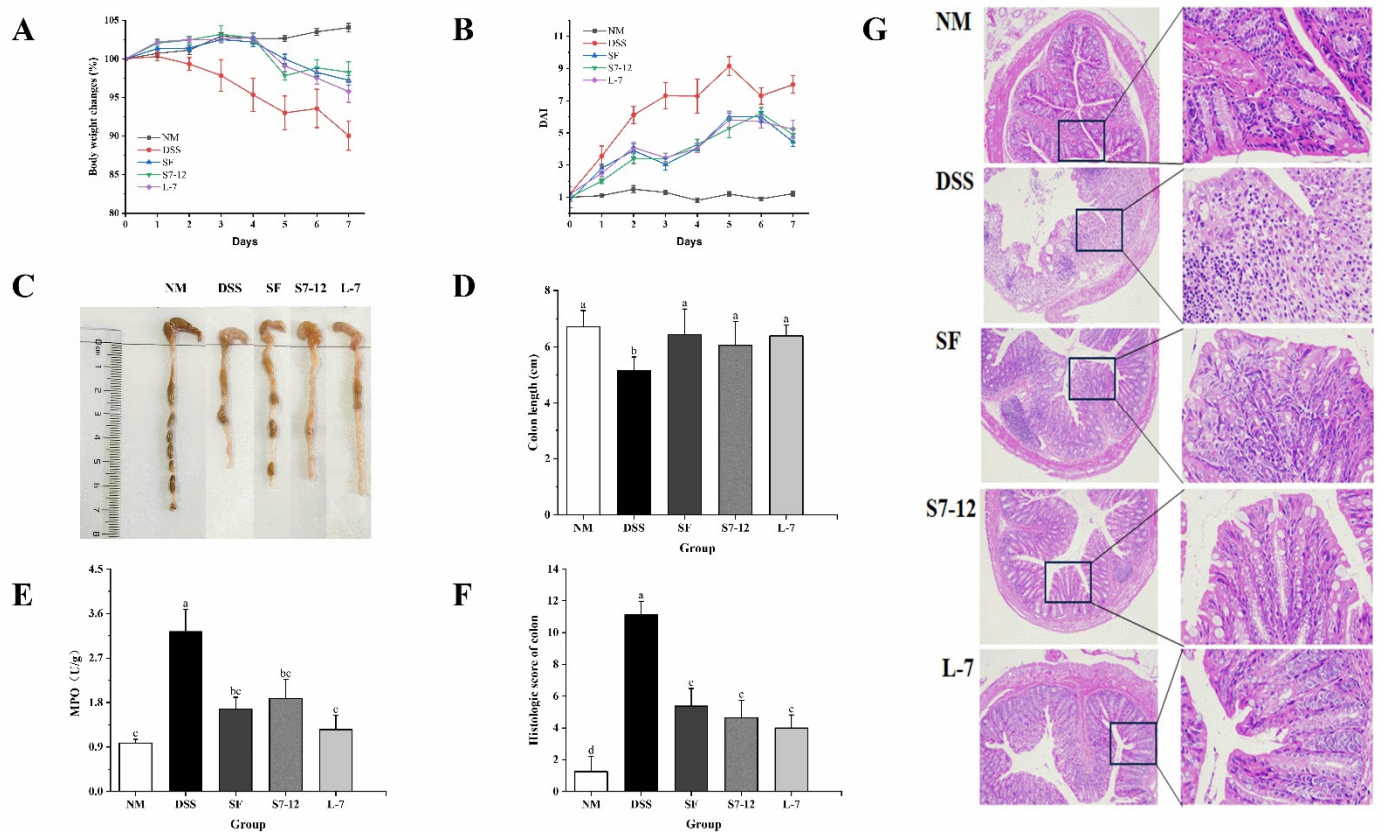


Fig. 2 Effects of different FTSM on body weight (A), DAI (B), colon length (C, D), MPO (E) and colon histologic score and morphology (F, G).

Table 2 Effects of different FTSM on organ indexes in mice

Group	Spleen index (mg/g)	Liver index (mg/g)	Kidney index (mg/g)
NM	2.33 ± 0.31 ^c	34.89 ± 2.23 ^b	11.08 ± 0.37 ^b
DSS	3.83 ± 1.06 ^a	40.78 ± 3.75 ^a	12.27 ± 1.11 ^a
SF	3.02 ± 0.21 ^b	36.43 ± 1.09 ^b	11.15 ± 0.54 ^b
S7-12	2.88 ± 0.15 ^{bc}	36.90 ± 3.59 ^b	11.38 ± 0.86 ^b
L-7	2.76 ± 0.26 ^{bc}	35.47 ± 1.78 ^b	11.03 ± 0.33 ^b

Note: different letters in the same column indices significant difference ($P < 0.05$)

As a pro-inflammatory and pro-oxidative enzyme that secreted by macrophages and granulocytes, MPO level was sharply increased in model group and FTSM significantly reduced the MPO level ($P < 0.05$) (Fig. 2E). L-7 group has a similar level of MPO to the control, suggesting that L-7 FTSM could relieve neutrophil infiltration in colon tissue. It was demonstrated that homo- or hetero- fermentative FTSM could prevent DSS-induced weight loss, increase of DAI and organ indexes, no significant from spontaneous fermentation FTSM.

The protective effects of FTSM on chemically-induced colitis could attribute to dietary fibers, phenolic compounds and glucosinolates [29]. Higher content of IDF had a positive effect on ulcerative colitis [30]. TSM possessed strong antioxidant and anti-tumor activities *in vitro* due to polyphenols, sinigrin hydrate and other important antioxidants [1]. For FTSM, although lactic acid, acetic acid and citric acid lack direct antioxidant activity, these provide a stabilizing low pH environment that maintains the antioxidant activity of polyphenols. Moreover, our reviewed that GLSs attenuated inflammatory bowel disease through multiple mechanism

including regulating gut microbiota, exerting antioxidant and anti-inflammatory activities, maintaining intestinal barrier and regulating epigenetic mechanisms [7].

3.7 FTSM could reduce the microscopic colonic damage

As shown in **Fig. 2F** and **2G**, the colon in the NM group had the intact mucosal lamina propria, submucosa, muscularis propria, and plasma layer, with no obvious inflammatory damage [27]. Conversely, the mice of the DSS group showed degeneration and necrosis of the colonic mucosa, accompanied by ulceration and submucosal edema. It was also observed that degeneration and necrosis of the mucosal epithelial cells, the deformation or disappearance of crypts, loss of goblet cells and infiltration of inflammatory cells. SF, S7-12 and L-7 treatment alleviated intestinal inflammation to different degrees, including attenuating the damage of mucosal structure, restoring the crypt structure, reduced infiltration of inflammatory cells, and the increasing number of goblet cells. Meanwhile, the colonic histologic scores of mice in each treatment group (**Fig. 2F**) were significantly lower than those of the model group ($P < 0.05$), and the colonic pathological features of S7-12 and L-7 mice were closer to those of the normal group, suggesting that feeding different FTSM could ameliorate the colonic mucosal pathological damage and inflammatory response induced by DSS. Similar results were observed in treatment with fermented wallace melon juice on DSS-induced mice [31].

3.8 FTSM improved the serum antioxidative activity

Elevation of oxidative stress markers including MDA are observed in individuals with IBD, which further contributes to the development of intestinal inflammation through causing degradation of intestinal mucosal layer and bacterial invasion [32]. DSS-induced a significant increase in the serum MDA content and decrease in the activities of SOD and GSH-PX ($P < 0.05$). Administration of FTSM reversed the decrease in level of SOD induced by DSS and there is no significant difference among three groups (**Fig. 3A**). S7-12 and L-7 treatment induced a lower MDA content and a higher GSH-PX level than SF group, suggesting starter-FTSM exerted good antioxidative activity *in vivo* (**Fig. 3B and 3C**).

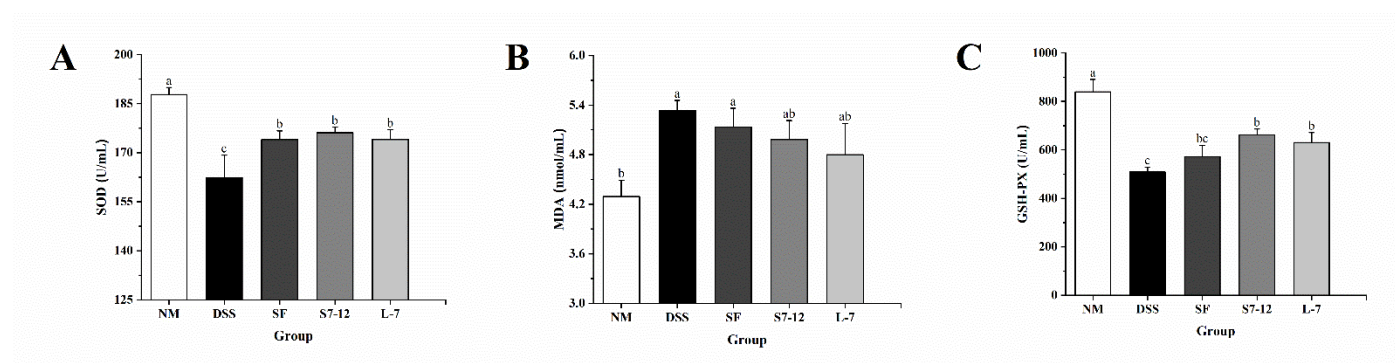


Fig. 3 Effects of different FTSM on serum oxidative stress of mice (A, SOD; B, MDA; C, GSH-PX activity)

3.9 Regulation of FTSM on inflammatory cytokine expression

Inflammatory cytokines have a critical role in the pathogenesis of IBD. It is generally found that an imbalance between proinflammatory and anti-inflammatory cytokines in patients and mouse models of IBD [33]. Regulation of cytokine has been considered as a therapeutic target for chronic intestinal inflammation.

Similar changes of inflammatory cytokines were also observed both in serum and colon of model mice in the study (**Table 3**). FTSM supplementation could reduce the levels of serum and colonic proinflammatory cytokines IL-6, TNF- α and IL-1 β and enhance the expression of anti-inflammatory cytokine IL-10 compared to the model group. Moreover, S7-12 and L-7 have a more potent impact in reversing the changes of cytokines than SF FTSM. Although treatment with homofermentative S7-12 FTSM lead to slight lower levels of serum and colonic TNF- α and higher of IL-10 than heterofermentative L-7 FTSM, but the differences were not significant.

Table 3 Effects of different FTSM on inflammatory factor levels in serum and colon of mice

	Cytokines (pg/mL)	NM	DSS	SF	S7-12	L-7
Serum	IL-6	32.17 $\pm$ 1.53 ^b	36.70 $\pm$ 2.12 ^a	34.96 $\pm$ 1.26 ^{ab}	32.42 $\pm$ 0.54 ^b	32.17 $\pm$ 1.44 ^b
	TNF- α	344.69 $\pm$ 21.21 ^b	422.62 $\pm$ 10.08 ^a	376.93 $\pm$ 13.85 ^{ab}	364.84 $\pm$ 13.81 ^b	369.64 $\pm$ 19.22 ^b
	IL-1 β	82.92 $\pm$ 2.75 ^b	92.93 $\pm$ 2.98 ^a	85.19 $\pm$ 0.70 ^{ab}	83.17 $\pm$ 4.04 ^b	84.52 $\pm$ 1.79 ^b
	IL-10	34.60 $\pm$ 1.00 ^a	28.61 $\pm$ 2.00 ^c	29.69 $\pm$ 1.32 ^{bc}	33.92 $\pm$ 1.39 ^{ab}	32.90 $\pm$ 1.16 ^{abc}
Colon	IL-6	29.89 $\pm$ 3.58 ^d	59.37 $\pm$ 3.00 ^a	50.85 $\pm$ 1.14 ^b	46.29 $\pm$ 1.08 ^{bc}	42.58 $\pm$ 1.46 ^c
	TNF- α	255.28 $\pm$ 8.93 ^c	370.77 $\pm$ 12.97 ^a	301.95 $\pm$ 7.72 ^b	285.94 $\pm$ 6.77 ^b	292.26 $\pm$ 6.96 ^b
	IL-1 β	62.23 $\pm$ 2.68 ^d	85.73 $\pm$ 3.80 ^a	79.52 $\pm$ 3.45 ^{ab}	71.81 $\pm$ 2.18 ^{bc}	68.65 $\pm$ 1.68 ^{cd}
	IL-10	38.62 $\pm$ 2.08 ^a	25.48 $\pm$ 1.99 ^c	32.46 $\pm$ 1.59 ^b	36.14 $\pm$ 1.49 ^{ab}	34.88 $\pm$ 1.31 ^{ab}

Note: different letters in the same line indices significant difference ($P < 0.05$)

3.10 FTSM restored intestinal barrier function

The balance between these inflammatory cytokines exerts a fundamental role for mucosal healing in experimental colitis and in IBD patients. It has been demonstrated that some pro-inflammatory cytokines like TNF alters TJ activity and induces apoptosis of intestinal epithelial cells (IECs), which contributes to intestinal barrier damage [34]. TJ proteins regulate epithelial barrier function and control intestinal permeability, and prevent harmful substances from entering other parts of the body from the intestine and play multiple roles in intestinal homeostasis. Compared to the NM group, the expressions of TJ proteins such as claudin-1, occludin and ZO-1 in the model group significantly reduced ($P < 0.05$), indicating that colon TJs and intestinal integrity were destroyed (**Fig. 4**). Three FTSM can restore the levels of TJ proteins in colon tissue, and the expression of colonic TJ proteins was significantly increased in S7-12 and L-7 group ($P < 0.05$). while only occludin was significantly increased in SF ($P < 0.05$). The trend of TJ proteins is consistent with inflammatory cytokines among three FTSM group. It showed that feeding FTSM, especially LAB starter FTSM, can alleviate the reduction of TJ proteins and is beneficial to maintaining the colon mucosal barrier function.

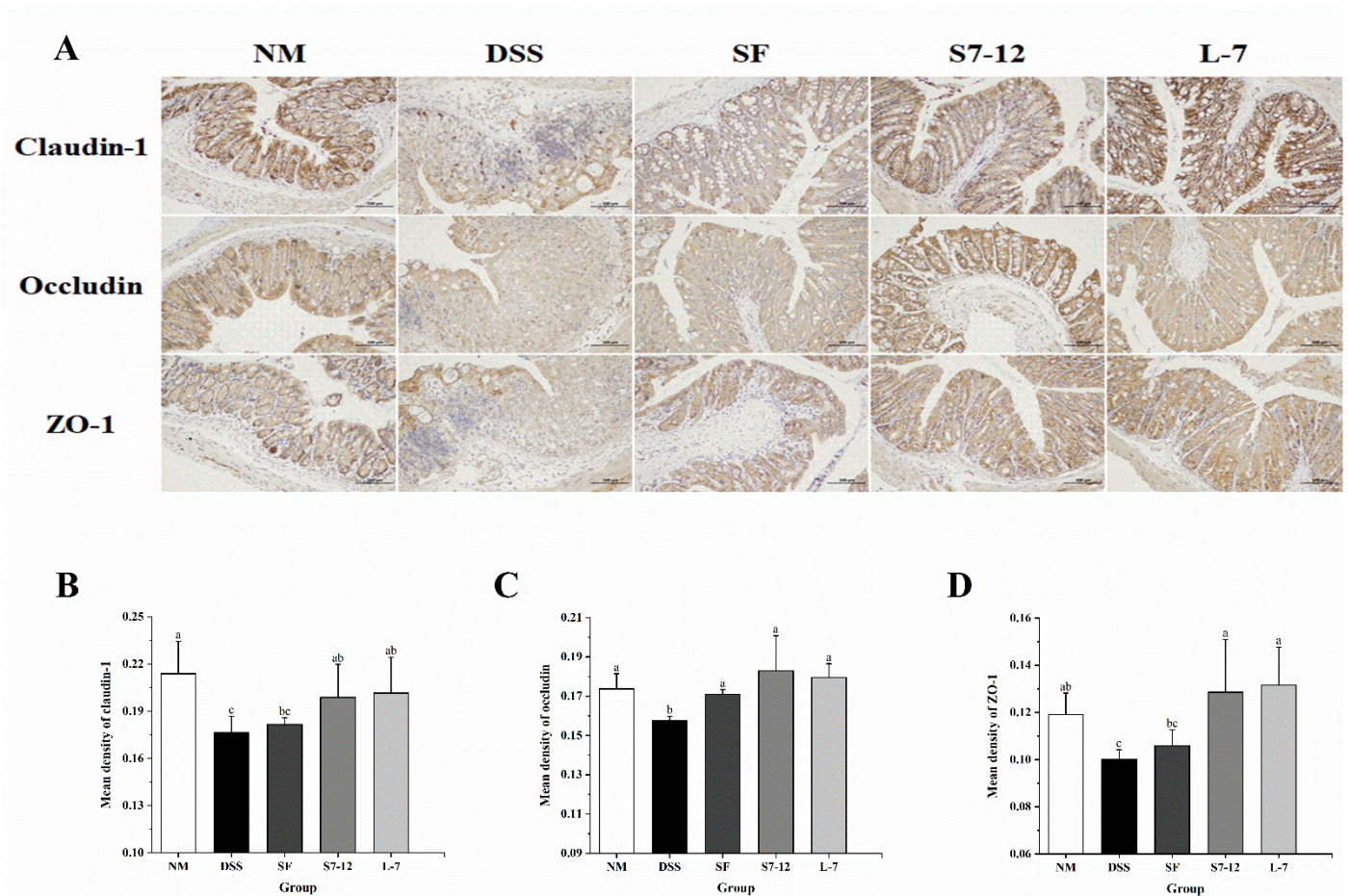


Fig. 4 Effects of different FTSM on the expression of TJ proteins: (A, immunohistochemical staining of TJ proteins; B, claudin-1; C, occludin; D, ZO-1) (n = 5 per group)

3.11 Anti-inflammatory mechanism of FTSM through TLR4/MyD88/NF- κ B pathways

TLR4/NF- κ B signaling pathway plays a critical role in the regulation of a variety of inflammatory cytokines. Toll-like receptor 4 (TLR4) is a crucial transmembrane signal transduction receptor that is activated by inflammation stimulating and leads to the activation of the adaptor protein, myeloid differentiation 88 (MyD88), which then increases the transcriptional activity of NF- κ B and transfers it to the cell nucleus, turning on the gene expression of a variety of pro-inflammatory cytokines [35]. Immunohistochemical analysis showed that DSS administration led to a significant increase in the expression of TLR4, MyD88 and NF- κ B p65 protein in the colon tissue ($P < 0.05$), indicating that DSS induction activated the TLR4/MyD88/NF- κ B signaling pathway (**Fig. 5**). Feeding SF, S7-12 and L-7 FTSM inhibited the TLR4, MyD88 and NF- κ B p65 expression to a certain extent, and SF and L-7 had a significant inhibitory effect on these three proteins, and S7-12 had a significant inhibitory effect on TLR4 and NF- κ B p65 protein ($P < 0.05$). These results showed that feeding different FTSM can exert anti-inflammatory activity by inhibiting the TLR4/MyD88/NF- κ B signaling pathway.

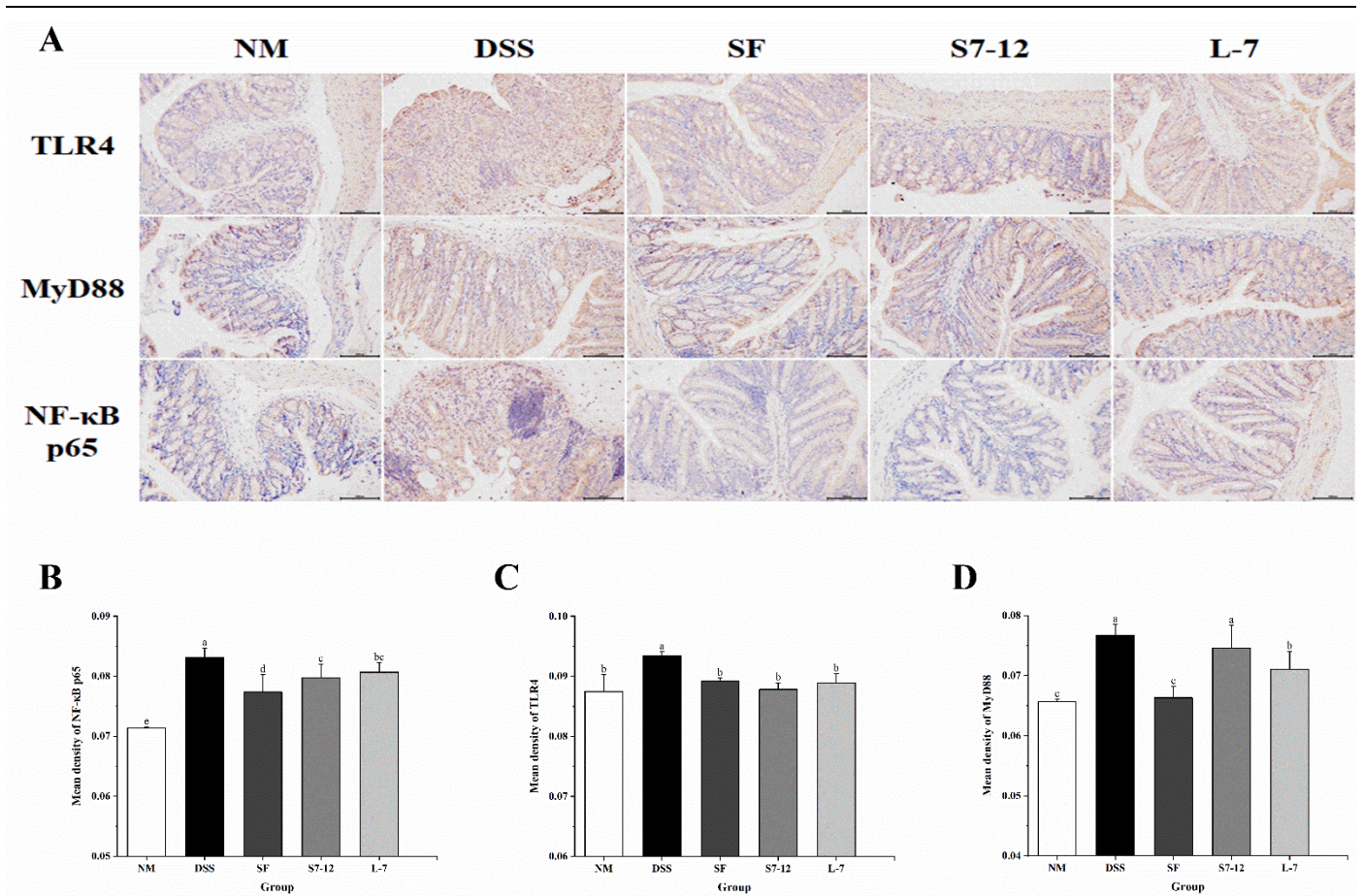


Fig. 5 Effects of different FTSM on TLR4, MyD88, NF-κB p65 proteins expression in colon of mice (A, immunohistochemical staining of proteins; B, NF-κB p65 protein; C, TLR4 protein; D, MyD88 protein) (n = 5 per group)

3.12 FTSM altered the composition of intestinal microbiota in DSS-treated mice

Dysbiosis of intestinal flora is correlated with colitis of experimental model and patients [7]. Results of 16S rRNA sequencing showed that FTSM, especially SF and L-7 group restored the richness and diversity of the intestinal microbiota to some extent compared to the DSS group (Table 4). Rarefaction curve indicated that the sequencing depth was sufficient to reflect the microbial diversity in samples (Fig. 6A). Apart from 72 shared ASVs, 650, 674, 461, and 451 unique ASVs were in the NM, SF, S7-12, and L-7, respectively, whereas the DSS had only 219 ASVs, suggesting that FTSM treatment improved the microbial diversity (Fig. 6B). β -diversity index showed that there was remarkable difference between DSS and NM group, and FTSM induced the changes of intestinal microbiota towards NM group (Fig. 6C).

Table 4 Effects of different FTSM on alpha diversity index of intestinal microbiota in mice

Group	Ace	Shannon	Coverage
NM	405.15 ± 33.04 ^a	4.04 ± 0.44 ^a	1.00 ± 0.00 ^a
DSS	132.89 ± 51.38 ^c	2.63 ± 0.38 ^{bc}	1.00 ± 0.00 ^a
SF	329.71 ± 113.08 ^{ab}	3.57 ± 0.31 ^{ab}	1.00 ± 0.00 ^a
S7-12	172.50 ± 93.26 ^c	1.99 ± 1.15 ^c	1.00 ± 0.00 ^a
L-7	215.28 ± 63.32 ^{bc}	2.84 ± 0.22 ^{bc}	1.00 ± 0.00 ^a

Note: different letters in the same column indices significant difference ($P < 0.05$)

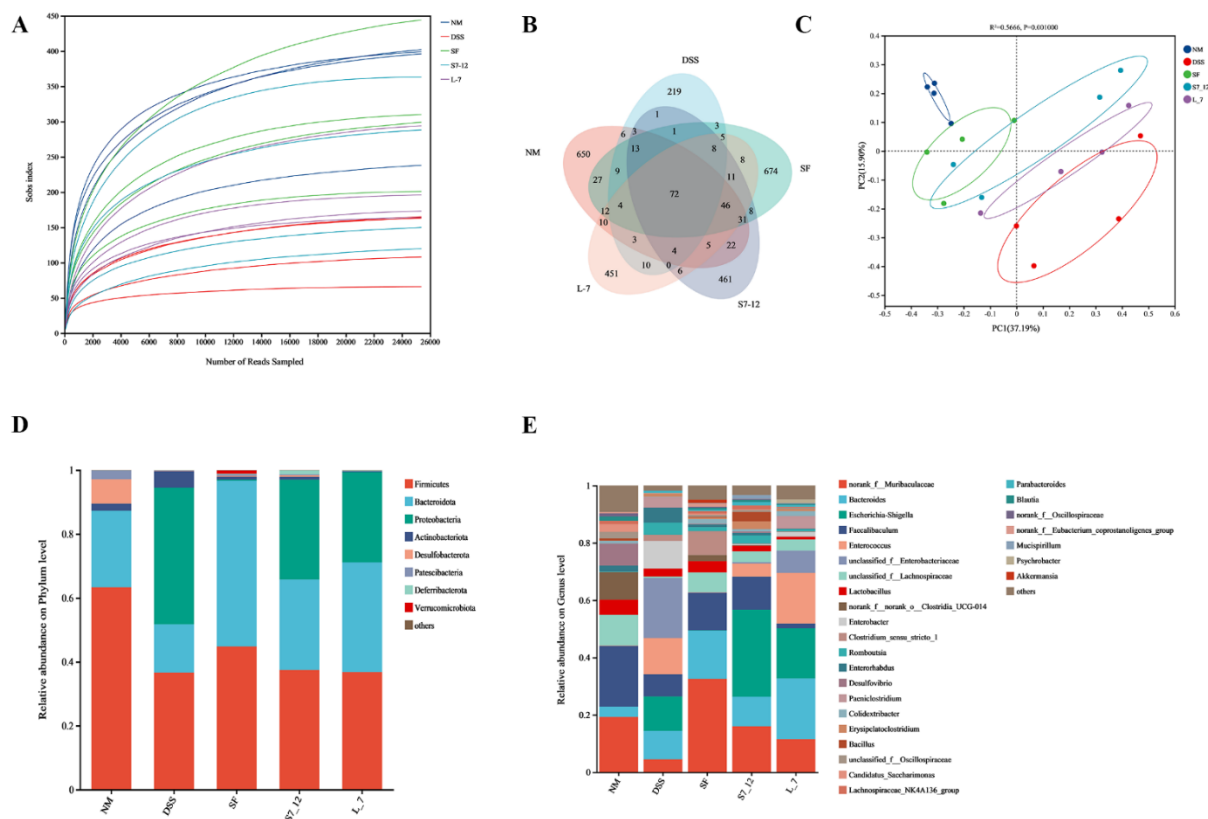


Fig.6 Effects of FTSM on intestinal microbiota composition in mice (A, rarefaction curves of Sobs; B, venn diagram of ASV level; C, PCoA analysis; D, the composition of gut microbiota at phylum levels; E, the composition of gut microbiota at genus levels)

Firmicutes, *Bacteroidota*, *Actinobacterota*, *Desulfobacterota* and *Patescibacteria* are the five dominant phyla in the normal group at the phylum level (**Fig. 6D**). However, the relative abundance of *Proteobacteria*, and *Actinobacterota* sharply increased in the model group, while the proportion of *Firmicutes*, *Bacteroidota* and *Desulfobacterota* decreased. Shi et al. reported that the proportion of *Firmicutes* and *Bacteroidota* were reduced in DSS-induced chronic colitis of mice [36]. The abundance of pro-inflammatory bacteria *Proteobacteria* increased in the colitis model of mice of previous studies [28]. FTSM treatment reduced the proportion of *Proteobacteria*, and improved the relative abundance of *Bacteroidota*.

At the genus level, the relative abundance of *norank_f_Muribaculaceae*, *Faecalibaculum*, *unclassified_f_Lachnospiraceae*, and *Lactobacillus* decreased, whereas the proportion of *Bacteroides*, *Escherichia-Shigella*, *Enterococcus*, *unclassified_f_Enterobacteriaceae* and *Enterobacter* increased in the DSS group compared to the control group (**Fig. 6E**). It is well known that *Lactobacillus*, as carbohydrate-utilizing and lactate/acetate-producing bacteria, are often regarded as probiotics to treat intestinal inflammation, while *Bacteroides* increased inflammation in UC patients [37, 38]. The abundance of *Muribaculaceae* also decreased in the DSS-induced mice of previous studies, and were negatively correlated with inflammatory markers in diet-induced insulin resistant mice, and positively correlated with the propionate content [39]. *Faecalibaculum* is a short chain fatty acid (SCFA) producer and showed anti-inflammatory effects [40]. *Lachnospiraceae* is one of butyrate-producing *Firmicutes* families of the normal microbiome and responsible for degrading carbohydrates into SCFAs, whose decrease is associated with earlier onset and enhanced colitis severity in rodent [41].

SF and S7-12 FTSM improved the relative abundance of *norank_f_Muribaculaceae*, *Faecalibaculum* and *unclassified_f_Lachnospiraceae* and decreased *Enterococcus*, *unclassified_f_Enterobacteriaceae* and *Enterobacter*. L-7 increased the relative abundance of *norank_f_Muribaculaceae* and decreased the proportion of *unclassified_f_Enterobacteriaceae* and *Enterobacter*. The expansion of *Enterobacteriaceae* that contain microbe-associated molecular patterns such as lipopolysaccharide (LPS) enhanced the inflammatory response through interacting with toll-like receptor (TLR) 4 and 9 [42]. Wilcoxon rank-sum test further revealed the significant differences on genus between NM vs DSS and DSS vs SF, S7-12 and L-7 (Fig. 7A-7D). More specifically, administration of S7-12 FTSM significantly reduced the abundance of *Enterobacter* and *Paeniclostridium*, whereas L-7 mainly decreased *norank_f_Erysipelotrichaceae* ($P < 0.05$).

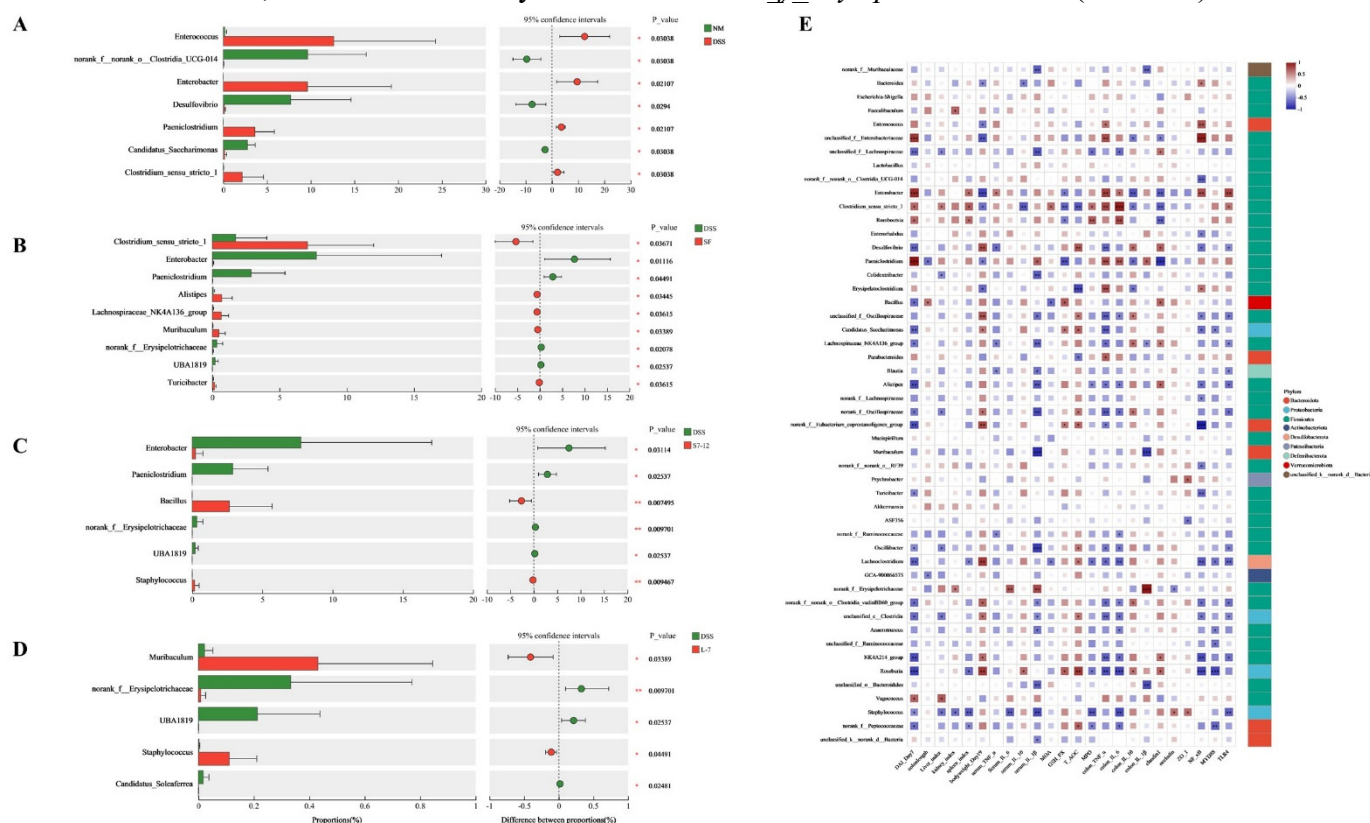


Fig. 7 Differences in intestinal microbiota on genus between NM and DSS (A), DSS and SF (B), DSS and S7-12 (C), DSS and L-7 (D) and correlation analysis between colitis-related parameters and intestinal microorganisms (E) ($n = 5$ per group)

The correlation analysis between colitis-related parameters and intestinal microorganisms further exhibited the roles of gram-negative bacteria such as *Enterobacteriaceae* in the development of colitis (Fig. 7E). The relative abundance of *Enterobacter*, *Enterococcus*, *unclassified_f_Enterobacteriaceae*, *Enterobacter*, *Clostridium_sensu_stricto_1*, and *Paeniclostridium* was positively correlated with the levels of inflammatory cytokines such as colonic TNF- α , IL-6 and DAI, and negatively associated with the activities of GSH-PX and T-AOC. Moreover, the genera *Enterobacteriaceae*, *unclassified_f_Enterobacteriaceae* and *Enterobacter* also showed significant positive correlation with the expression of NF- κ B, TLR4 and MyD88. These findings suggest that FTSM regulated the composition of intestinal microbiota by reducing the proportion of pro-inflammatory bacteria, thus inhibiting the relative expression of TLR4/MyD88/NF- κ B, and alleviating inflammatory response [43]. Further studies are warranted to elucidate the causal correlations

between TLR4 pathway and intestinal microbial alteration using fecal microbiota transplantation (FMT) or antibiotic-depletion models ^[44].

4. Conclusion

Both starter homo-fermentative *L. plantarum* S7-12 and hetero-fermentative *Lent. parafarraginis* L-7 enhanced the ripening of FTSM, characterized by a higher TAC and lower levels of pH values. S7-12 fermentation produced higher content of lactic acid, while L-7 group has the highest content of acetic acid. S7-12 fermentation retained the total phenolic content and both starter fermentation increased the GLS contents, and showed higher antioxidant activities than SF-FTSM.

Furthermore, FTSM intervention alleviated the colitis symptoms including loss of body weight, DAI values, colonic length and MPO activity. S7-12 and L-7 FTSM also ameliorated the colonic pathological damage and exhibited good antioxidative activity *in vivo* than SF FTSM. Immunohistochemical analysis further confirmed that FTSM maintained the colon mucosal barrier function by restoring TJ proteins. Moreover, FTSM supplementation could reduce the levels of serum and colonic proinflammatory cytokines IL-6, TNF- α and IL-1 β and enhance the expression of anti-inflammatory cytokine IL-10 by inhibiting the TLR4/MyD88/NF- κ B signaling pathway. FTSM treatment reversed the decline in the microbial diversity induced by DSS and reduced the pro-inflammatory gram-negative bacteria *Enterobacteriaceae*. Both homo-fermentative S7-12 and hetero-fermentative L-7 inoculated FTSM showed the stronger alleviating effects on the colitis of mice than SF, but there was no significant difference between them. In conclusion, starter culture FTSM relieved the DSS-induced colitis through regulating the composition of intestinal microbiota and inhibiting the TLR4/MyD88/NF- κ B signaling pathway.

Conflicts of Interest

The authors declare that there is no competing interests.

References

- [1] Li, W., et al., Chemical composition, antioxidant activity and antitumor activity of tumorous stem mustard leaf and stem extracts, *CyTA - Journal of Food*, 2019. 17(1): p. 272-279. <https://doi.org/10.1080/19476337.2019.1577303>.
- [2] Zhang, C., J. Zhang, and D. Liu, Biochemical changes and microbial community dynamics during spontaneous fermentation of Zhacai, a traditional pickled mustard tuber from China, *International Journal of Food Microbiology*, 2021. 347. <https://doi.org/10.1016/j.ijfoodmicro.2021.109199>.
- [3] Yang, J., et al., Metagenomic analysis of microbial community succession during the pickling process of Zhacai (preserved mustard tuber) and its correlation with Zhacai biochemical indices, *Journal of the Science of Food and Agriculture*, 2021. 101(4): p. 1646-1658. <https://doi.org/10.1002/jsfa.10785>.
- [4] Moon, S.H., C.R. Kim, and H.C. Chang, Heterofermentative lactic acid bacteria as a starter culture to control kimchi fermentation, *LWT*, 2018. 88: p. 181-188. <https://doi.org/10.1016/j.lwt.2017.10.009>.
- [5] Liu, Z., et al., Effect of homo- and hetero-fermentative lactic acid bacteria on physicochemical properties, amino acid, and volatile flavor compounds during paocai fermentation by pure culture, *Journal of Food Processing and Preservation*, 2022. 46(12): p. e17052. <https://doi.org/10.1111/jfpp.17052>.
- [6] Pedersen, T.B., F.K. Vogensen, and Y. Ardö, Effect of heterofermentative lactic acid bacteria of DL-starters in initial ripening of semi-hard cheese, *International Dairy Journal*, 2016. 57: p. 72-79. <https://doi.org/10.1016/j.idairyj.2016.02.041>.

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- [7] Zhao, J., et al., The Effects of Interventions with Glucosinolates and Their Metabolites in Cruciferous Vegetables on Inflammatory Bowel Disease: A Review, *Foods*, 2024. 13(21): p. 3507. <https://doi.org/10.3390/foods13213507>.
- [8] Zhao, J., et al., Effects of brine- and NaCl- fermented carrots on lipid metabolism and gut microbiota in high-fat diet-fed mice, *Journal of Functional Foods*, 2023. 105: p. 105564. <https://doi.org/10.1016/j.jff.2023.105564>.
- [9] Thies, W.J.F., Seifen, Anstrichmittel, Complex-formation between glucosinolates and tetrachloropalladate (II) and its utilization in plant breeding, 1982. 84(9): p. 338-342. <https://doi.org/10.1002/lipi.19820840903>
- [10] Shang, Z., et al., Analysis of phenolic compounds in pickled chayote and their effects on antioxidant activities and cell protection, *Food Research International*, 2022. 157: p. 111325. <https://doi.org/10.1016/j.foodres.2022.111325>.
- [11] Charan, J. and N.D. Kantharia, How to calculate sample size in animal studies?, *J Pharmacol Pharmacother*, 2013. 4(4): p. 303-6. <https://doi.org/10.4103/0976-500x.119726>.
- [12] Zhang, T., et al., A steamed broccoli sprout diet preparation that reduces colitis via the gut microbiota, *Journal of Nutritional Biochemistry*, 2023. 112. <https://doi.org/10.1016/j.jnutbio.2022.109215>.
- [13] Zhang, F., et al., Microbial composition of spoiled industrial-scale Sichuan paocai and characteristics of the microorganisms responsible for paocai spoilage, 2018. 275: p. 32-38. <https://doi.org/10.1016/j.ijfoodmicro.2018.04.002>
- [14] Xu, J., et al., A review on fermented vegetables: Microbial community and potential upgrading strategy via inoculated fermentation, *Comprehensive Reviews in Food Science and Food Safety*, 2024. 23(3): p. e13362. <https://doi.org/10.1111/1541-4337.13362>.
- [15] Yang, X., et al., Comparison of northeast sauerkraut fermentation between single lactic acid bacteria strains and traditional fermentation, *Food Research International*, 2020. 137. <https://doi.org/10.1016/j.foodres.2020.109553>.
- [16] Wu, L., et al., Modified dietary fiber from soybean dregs by fermentation alleviated constipation in mice, *Food Chemistry: X*, 2023. 19: p. 100810. <https://doi.org/10.1016/j.fochx.2023.100810>.
- [17] Kaczmarek, K.T., et al., Effect of germination and fermentation on carbohydrate composition of Australian sweet lupin and soybean seeds and flours, *Journal of Agricultural and Food Chemistry*, 2017. 65(46): p. 10064-10073. <https://doi.org/10.1021/acs.jafc.7b02986>.
- [18] Peyer, L.C., E. Zannini, and E.K. Arendt, Lactic acid bacteria as sensory biomodulators for fermented cereal-based beverages, *Trends in Food Science & Technology*, 2016. 54: p. 17-25. <https://doi.org/10.1016/j.tifs.2016.05.009>.
- [19] Wang, L., et al., The dynamic changes and correlations between biochemical properties, flavor and microbial community during fermentation of asparagus by-products, *Food Chemistry*, 2025. 463: p. 141173. <https://doi.org/10.1016/j.foodchem.2024.141173>.
- [20] Chen, R., et al., Comparative evaluation of the antioxidant capacities, organic acids, and volatiles of papaya juices fermented by *Lactobacillus acidophilus* and *Lactobacillus plantarum*, *Journal of food quality*, 2018. 2018(1): p. 9490435. <https://doi.org/10.1155/2018/9490435>.
- [21] Bhat, R., et al., *Lactobacillus plantarum* mediated fermentation of *Psidium guajava* L. fruit extract, *Journal of bioscience and bioengineering*, 2015. 119(4): p. 430-432. <https://doi.org/10.1016/j.jbiosc.2014.09.007>.
- [22] Hervert-Hernández, D., et al., Stimulatory role of grape pomace polyphenols on *Lactobacillus acidophilus* growth, *International Journal of Food Microbiology*, 2009. 136(1): p. 119-122. <https://doi.org/10.1016/j.ijfoodmicro.2009.09.016>.
- [23] Ye, J.-H., et al., Fermentation-based biotransformation of glucosinolates, phenolics and sugars in retorted broccoli puree by lactic acid bacteria, *Food Chemistry*, 2019. 286: p. 616-623. <https://doi.org/10.1016/j.foodchem.2019.02.030>.
- [24] Di, H., et al., Correlations between flavor and glucosinolates and changes in quality-related physiochemical characteristics of Guizhou suancai during the fermentation process, *Food Chemistry*, 2023. 405. <https://doi.org/10.1016/j.foodchem.2022.134965>.
- [25] Liu, M.C., et al., Changes in volatile compounds of pickled mustard tuber (*Brassica juncea* var. tsatsai) during the pickling process, *International journal of food science and technology*, 2009. 44(11): p. 2278-2286. <https://doi.org/10.1111/j.1365-2621.2009.02070.x>
- [26] Parada, R.B., et al., Improved antioxidant capacity of three Brassica vegetables by two-step controlled fermentation using isolated autochthonous strains of the genus *Leuconostoc* spp. and *Lactiplantibacillus* spp, *Food Chemistry: Molecular Sciences*, 2023. 6: p. 100163. <https://doi.org/10.1016/j.fochms.2023.100163>.

- [27] Yan, S., et al., A ropy exopolysaccharide producing strain *Bifidobacterium longum* subsp. *longum* YS108R alleviates DSS-induced colitis by maintenance of the mucosal barrier and gut microbiota modulation, *Food & Function*, 2019. 10(3): p. 1595-1608. <https://doi.org/10.1039/c9fo00014c>.
- [28] Xia, P., et al., Konjac oligosaccharides attenuate DSS-induced ulcerative colitis in mice: mechanistic insights, *Food & Function*, 2022. 13(10): p. 5626-5639. <https://doi.org/10.1039/D1FO04004A>.
- [29] Li, W., et al., Salted and Unsalted Zhacai (*Brassica juncea* var. *tumida*) Alleviated High-Fat Diet-Induced Dyslipidemia by Regulating Gut Microbiota: A Multiomics Study, *Molecular Nutrition & Food Research*, 2020. 64(24). <https://doi.org/10.1002/mnfr.202000798>.
- [30] Wang, Y., et al., Long-term soy dietary fiber diet modifies the intestinal internal environment: Focus on chronic ulcerative colitis, *Food Research International*, 2025. 209. <https://doi.org/10.1016/j.foodres.2025.116193>.
- [31] Zhang, J., et al., Wallace melon juice fermented with *Lactobacillus* alleviates dextran sulfate sodium-induced ulcerative colitis in mice through modulating gut microbiota and the metabolism, *Journal of Food Science*, 2024. 89(4): p. 2450-2464. <https://doi.org/10.1111/1750-3841.16973>.
- [32] Muro, P., et al., The emerging role of oxidative stress in inflammatory bowel disease, *Frontiers in Endocrinology*, 2024. 15. <https://doi.org/10.3389/fendo.2024.1390351>.
- [33] Neurath, M.F., Cytokines in inflammatory bowel disease, *Nature Reviews Immunology*, 2014. 14(5): p. 329-342. <https://doi.org/10.1038/nri3661>.
- [34] Su, L., et al., TNFR2 Activates MLCK-Dependent Tight Junction Dysregulation to Cause Apoptosis-Mediated Barrier Loss and Experimental Colitis, *Gastroenterology*, 2013. 145(2): p. 407-415. <https://doi.org/10.1053/j.gastro.2013.04.011>.
- [35] Wang, Y.-Y., et al., GABA regulates the proliferation and apoptosis of MAC-T cells through the LPS-induced TLR4 signaling pathway, *Research in Veterinary Science*, 2018. 118: p. 395-402. <https://doi.org/10.1016/j.rvsc.2018.04.004>.
- [36] Shi, D.-C., et al., Potential of *Dendrobium officinale* oligosaccharides to alleviate chronic colitis by modulating inflammation and gut microbiota, *Food & Medicine Homology*, 2024. <https://doi.org/10.26599/FMH.2025.9420077>.
- [37] Zhang, X., et al., Microbiome–Metabolomics Analysis Reveals the Mechanism of *Holothuria leucospilota* Polysaccharides (HLP) in Ulcerative Colitis, *Molecular Nutrition & Food Research*, 2023. 67(10): p. 2200633. <https://doi.org/10.1002/mnfr.202200633>.
- [38] Wang, J., et al., Core Gut Bacteria Analysis of Healthy Mice, *Frontiers in Microbiology*, 2019. Volume 10 – 2019. <https://doi.org/10.3389/fmicb.2019.00887>.
- [39] Zhao, R. and G.X. Shen, Impact of anthocyanin component and metabolite of Saskatoon berry on gut microbiome and relationship with fecal short chain fatty acids in diet-induced insulin resistant mice, *The Journal of Nutritional Biochemistry*, 2023. 111: p. 109201. <https://doi.org/10.1016/j.jnutbio.2022.109201>.
- [40] Mao, G., et al., Depolymerized RG-I-enriched pectin from citrus segment membranes modulates gut microbiota, increases SCFA production, and promotes the growth of *Bifidobacterium* spp., *Lactobacillus* spp. and *Faecalibaculum* spp, *Food & Function*, 2019. 10(12): p. 7828-7843. <https://doi.org/10.1039/C9FO01534E>.
- [41] Harrison, C.A., et al., Microbial dysbiosis associated with impaired intestinal Na⁺/H⁺ exchange accelerates and exacerbates colitis in ex-germ free mice, *Mucosal Immunology*, 2018. 11(5): p. 1329-1341. <https://doi.org/10.1038/s41385-018-0035-2>.
- [42] Baldelli, V., et al., The Role of Enterobacteriaceae in Gut Microbiota Dysbiosis in Inflammatory Bowel Diseases, *Microorganisms* 2021. 9(4): p. 697. <https://doi.org/10.3390/microorganisms9040697>.
- [43] Wang, N., et al., Fermented *Spirulina platensis* alleviated DSS-induced ulcerative colitis by regulating gut microbiota and MyD88/TLR4 signaling pathway, *Asian Journal of Agriculture and Biology*, 2024. 2024(1). <https://doi.org/10.35495/ajab.2023.232>.
- [44] Wen, X., et al., Fecal microbiota transplantation alleviates experimental colitis through the Toll-like receptor 4 signaling pathway, *World Journal of Gastroenterology*, 2023. 29(30): p. 4657-4670. <https://doi.org/10.3748/wjg.v29.i30.4657>.