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·Digestion and Gut-Modulating Functions of Six Dietary Oils: A Multi-Omics-Based Investigation into Oxidative Stability, Gas Production Profiles, and Microbiota Shifts

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Abstract

Dietary fats are crucial energy sources, carriers of fat-soluble vitamins, and providers of essential fatty acids; however, global dietary transitions have increasingly associated lipid metabolism dysregulation with chronic diseases. Using an *in vitro* continuous digestion model that includes small and large intestinal phases, we evaluated six dietary oils, namely, coconut, olive, camellia, fish, arachidonic acid (ARA), and peanut oils, for their oxidative stability, antioxidant activity, colonic fermentation, and gut microbiota modulation. Oxidative stability followed a hierarchy determined by unsaturated fatty acids: camellia ≈ peanut > coconut > ARA > olive > fish oil, with polyunsaturated oils showing the most pronounced decline in antioxidant properties. Based on colonic gas profiles, the six oils were classified into three categories: a high-activity gas-producing group (fish oil, ARA, and peanut oil) with elevated H_2S (0.78 ppm) and VOC (0.48 ppm); a high hydrogen-producing group (coconut oil, 0.42 ppm H_2); and a mild fermentation group (olive and camellia oil) with the lowest gas output. These patterns were correlated with shifts in gut microbiota, particularly the proliferation of Desulfobacterota and Proteobacteria in high gas-producing groups ($p < 0.05$). Metabolomics revealed seven distinct metabolite subclasses, with organosulfur compounds enriched in high gas-producing oils and organic acids in coconut oil. Collectively, these findings provide a framework for rational oil selection and targeted development of functional lipid products.

Highlights:

1.Classified six dietary oils into three distinct colonic fermentation fates based on gas metabolism profiles.

2.Revealed strong gas-producing oils (fish oil, ARA, peanut oil) enriched

Desulfobacterota and Proteobacteria.

3.Identified coconut oil as a unique high hydrogen producer linked to specific SCFA-generating genera.

4.Demonstrated olive oil and camellia oil exhibited mild fermentation with minimal gut microbiota disturbance.

5.Established multi-omics correlations between oil structure, oxidative stability, and microbial met.

1. Introduction

Rapid global economic development has resulted in significant changes to human diets and lifestyles. Chronic diseases such as obesity and hyperlipidemia, characterized by lipid metabolism disorders, have surged dramatically, posing severe challenges to public health in modern society [1]. Dietary lipids, as essential nutrients, are closely associated with human health based on their intake levels and types [2]. In recent years, the growing attention to precision nutrition and functional foods has highlighted the dynamic digestive behavior of lipids in the gut, their antioxidant properties, and their regulatory effects on the gut microbiome, establishing them as leading research topics in food science and lipid nutrition [3]. Among dietary lipids, fats and oils from different sources and structures exhibit significant variations in digestion rates, oxidative stability, and bioavailability. These differences not only influence energy intake and postprandial metabolic responses but also directly modulate gut microbiota composition and metabolic functions by altering the fermentation of undigested lipids in the colon [4].

Coconut oil, as a representative of medium-chain fatty acids (MCFAs), has been demonstrated to undergo rapid hydrolysis and direct absorption via the portal vein, possessing unique energy metabolism pathways [5]. Olive oil, rich in oleic acid, has been shown to help maintain lipid equilibrium and exhibit excellent oxidative stability [6]. Camellia oil is also renowned for its high oleic acid content and is considered to possess nutritional properties similar to olive oil [7]. Fish oil, rich in omega-3 PUFAs such as EPA and DHA, has been extensively reported for its anti-inflammatory and neurodevelopmental properties [8]. Arachidonic acid (ARA), a crucial long-chain polyunsaturated fatty acid, is recognized for its key role in infant growth and development [9]. Furthermore, peanut oil, a common dietary fat, has been

74 studied for its fatty acid composition and digestion characteristics [10].

75 However, the fermentation outcomes of dietary lipids are not solely determined
76 by oil composition; individual variations in gut microbiota composition also play a
77 critical role in affecting metabolic responses to the same dietary substrate [11].
78 Moreover, the production of microbiota-derived metabolites such as short-chain fatty
79 acids is subject to complex homeostatic mechanisms that are influenced by substrate
80 availability and host factors [12]. These factors highlight the need for a systematic,
81 multi-omics evaluation of lipid fermentation in structurally diverse oils.

82 However, current research predominantly focuses on the absorption and primary
83 metabolism of lipids in the upper digestive tract. Although a previous study [13]
84 employed an integrated *in vitro*–in vivo model and revealed that the structured lipid
85 SBL delays butyric acid release during gastric digestion, prolongs glycerol
86 monolaurate release in the small intestine, and promotes beneficial bacteria such as
87 *Bifidobacterium* and Clostridiales within 24 h, their study still primarily focused on
88 release kinetics and microbial composition, neglecting the gaseous metabolites
89 produced during colonic fermentation. Regarding the gaseous metabolites (such as
90 hydrogen, hydrogen sulfide, and methane) produced during the fermentation of
91 unabsorbed lipids in the colon, systematic monitoring and in-depth analysis of their
92 production patterns, microbial sources, and potential effects on the host's intestinal
93 environment remain lacking.

94 The underlying rationale for expecting differential gas metabolism among oils is
95 their unique fatty acid compositions. Oils rich in polyunsaturated fatty acids (PUFAs),
96 such as fish oil and ARA, contain a high number of double bonds that are susceptible
97 to lipid peroxidation during gastrointestinal digestion [14]. The resulting oxidative

products, such as lipid hydroperoxides and aldehydes, may serve as alternative electron acceptors or substrates for specific gut microbial taxa, particularly sulfate-reducing bacteria such as *Desulfovibrio*, thereby promoting hydrogen sulfide (H_2S) production [15]. By contrast, medium-chain triglycerides, predominantly found in coconut oil, are rapidly β-oxidized by gut microbes, generating reducing equivalents that facilitate hydrogen (H_2) production [16]. Monounsaturated fatty acid-rich oils, such as olive and camellia oils, are expected to induce mild fermentation responses due to their enhanced oxidative stability and reduced availability as microbial substrates [17]. Therefore, we hypothesize that dietary oils with different degrees of unsaturation will exhibit differentiated gas metabolism profiles during colonic fermentation. Specifically, PUFA-rich oils enhance H_2S and volatile organic compound (VOC) synthesis, MCT-rich oils promote H_2 generation, and MUFA-rich oils result in minimal gas production, which are closely associated with specific shifts in gut microbiota and metabolite signatures. Regarding the six structurally distinct oils mentioned above, critical research gaps remain unaddressed: the dynamic changes in oxidative stability, the progressive development of antioxidant capacity, and the underlying mechanisms linking these lipids to microbial gas metabolism throughout the digestive chain (duodenum–jejunum–ileum–colon). Comprehensive comparative data are notably lacking.

This study employed a standardized *in vitro* intestinal digestion model, innovatively integrating continuous monitoring data from small and large intestinal stages to evaluate the digestive characteristics of the following six lipid raw materials: coconut oil, olive oil, camellia oil, fish oil, ARA, and peanut oil. This study aimed to compare differences in intestinal digestion among lipids of varying structures and sources, revealing their digestive patterns and influencing factors. The findings will

provide scientific references for rational dietary combinations, functional oil product development, and nutritional intervention strategies for specific populations, carrying significant implications for the progression of lipid nutrition research and advancement of the food industry.

2. Materials and Methods

2.1 Materials and Reagents

Coconut oil was obtained from Shenzhen Huiyi Food Co., Ltd. (Shenzhen, China), and was mainly composed of lauric acid (C12:0, ~45–53%), myristic acid (C14:0, ~17–19%), and palmitic acid (C16:0, ~8–11%). Olive oil was sourced from Jiangxi Senhai Natural Plant Oil Co., Ltd (Jiangxi, China), and predominantly contained oleic acid (C18:1, ~70–77%), palmitic acid (C16:0, ~8–15%), and linoleic acid (C18:2, ~10–14%). Camellia oil was acquired from Jiangxi Tiancheng Oil Co., Ltd. (Jiangxi, China), and contained the highest oleic acid content (~67–83% C18:1), with total unsaturated fatty acids accounting for approximately 90% of its composition. Fish oil was obtained from JD Health International Inc. (Beijing, China), and was rich in eicosapentaenoic acid (EPA, C20:5, ~13–50%) and docosahexaenoic acid (DHA, C22:6, ~9–28%). Arachidonic acid (ARA) oil was acquired from Hefei Bomei Biotechnology Co.,Ltd. (Hefei, China), and contained approximately 40% arachidonic acid (C20:4n-6), while Peanut oil was sourced from Shandong Luhua Group Co., Ltd. (Shandong, China), and was characterized by oleic acid (~41–67%) and linoleic acid (~14–35%) as its major constituents. The peroxide values and acid values of all oils were within the acceptable limits specified by the Codex Alimentarius standards for edible oils (Codex Stan 210-1999), confirming the freshness and quality of the oil samples prior to digestion experiments.

Potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, and sodium sulfate were purchased from Sinopharm Chemical Reagent Co., Ltd. Lipase was obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). Mucin was purchased from Shanghai McLean Biochemical Co., Ltd. All other experimental reagents used were of analytical grade.

2.2 *In Vitro* Small Intestinal Digestion Simulation of Oils

Remove the artificial gastric fluid from the -40° C freezer and activate it in a 37° C water bath for later use. Prepare duodenal fluid as follows: Dissolve 1.7 g potassium dihydrogen phosphate, 2.5 g lipase, and 0.1 g bile salts [18] in 250 mL distilled water. Adjust pH to 6.0 using 0.2 mol/L NaOH and 0.2 mol/L HCl. The pH of jejunal and ileal fluids was adjusted to 6.5 and 7.0, respectively, with the remaining preparation steps identical to duodenal fluid. Digestion proceeded at 37° C with a peristaltic frequency of 5 – 8 cycles per min for 1 h. Samples were collected after 1 h for chemical and structural analysis. In this experiment, six samples—coconut oil, olive oil, camellia oil, fish oil, peanut oil, and ARA—were sequentially digested with gastric fluid and small intestinal fluids (duodenal, jejunal, and ileal fluids). After all digestion steps were completed, the samples were frozen for storage.

2.3 *In Vitro* Simulated Dynamic Digestion of Lipids in the Large Intestine

Fresh feces were collected from three healthy female donors (age 20 years, mean body mass index 22.04 kg/m^2, who had not consumed probiotics or prebiotics

for at least two months prior to donation and had no intestinal diseases) into sterile stool collection containers. Samples were diluted with 10% sterile PBS buffer and filtered through four layers of sterile gauze to remove impurities, yielding stool homogenates. Before the experiment, prepare a basic colon medium. Dissolve 11.2 g of BHI, 0.28 g of mucin, 0.14 g of bile salts, and 0.84 g of sodium sulfate in 280 mL of distilled water. Adjust the pH of the medium to 6.5, 7.0, and 7.5 using 0.5 mol/L NaOH and HCl, respectively, to simulate the ascending colon, transverse colon, and descending colon segments. 10 mL of the sample was added to prepared coliform culture medium. This medium, along with 1 mol/L HCl solution, 1 mol/L NaOH solution, and the coliform reactor, was sterilized at 121° C for 20 mins. Following sterilization, the entire culture medium was transferred into the reactor. Nitrogen gas was circulated three times to remove oxygen. Activate the water bath to maintain the reactor at 37° C. After peristaltic agitation for 1 min to stabilize the system, introduce 10 mL of fecal homogenate through the sampling port. Adjust the pH to approximately 6.8 using HCl and NaOH solutions. Collect 10 mL liquid samples at 0, 12, and 24 hs. Collect and analyze gas samples at 12 and 24 hs. Centrifuge the liquid samples at 4000 r/min for 10 mins to obtain supernatant and solid precipitate. Store the separated components at -25° C until analysis.

2.4 Analytical Methods

2.4.1 Lipid Peroxidation Assay

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) content

using a commercial assay kit (TBA method, catalog No. A003-2-2, Nanjing Jiancheng Bioengineering Institute, China). According to the manufacturer's instructions. Blank, standard, assay, and control tubes were prepared simultaneously. Into both the blank and standard tubes, introduce 0.1 mL of absolute ethanol. For the assay and control tubes, add 0.1 mL of the sample to be tested. Then, 4 mL of the appropriate working solution is dispensed into each tube. After vortexing to ensure thorough mixing, pierce the tube caps and incubate the tubes in a water bath set at 95°C for 40 minutes. Upon removal, promptly chill the tubes in an ice-water bath. Once the reaction is complete, record the absorbance of the supernatant at 532 nm. The MDA concentration is calculated and normalized to the sample mass, with the final value reported as nmol per mg, using the formula given below.

$$MDA\ content(nmol/mL) = (A_{1}\text{-}A_{2})/(A_{3}\text{-}A_{0}) \times 10/n \quad (1)$$

A_0: Blank tube absorbance value; A_1: Test tube absorbance value; A_2: Control 210 tube absorbance value; A_3: Standard tube absorbance value, 10:Standard 211 concentration (nmol/mL); n: Sample concentration (mg/mL).

2.4.2 DPPH Radical Scavenging Activity (Antioxidant Capacity) Analysis

Referencing the methodology described in [19] , the DPPH radical scavenging capacity was determined. Digested samples were mixed with DPPH solution and reacted in the dark for 30 min. Absorbance was measured at 517 nm. Subsequently,

samples were centrifuged at 4000 r/min for 5 min. Zero the instrument using an 80% methanol solution. Transfer 800 μL of supernatant to a cuvette and measure the absorbance at 517 nm. A control tube (400 μL sample + 600 μL 80% methanol) and a blank tube (400 μL 80% methanol + 600 μL working solution) were processed and measured simultaneously. The formula for calculating the DPPH radical scavenging rate is shown in Equation (2):

$$DPPH\ Radical\ Scavenging\ Rate（\%）= [1 - （A_{1}\text{-}A_{2}）/A_{0}] \times 100\% \quad (2)$$

A_0: Blank tube absorbance value; A_1: Test tube absorbance value; A_2: Control tube absorbance value.

2.4.3 Analysis of Gas Components and Concentrations

The gas detection system from BGR was employed to collect and analyze intestinal gas production. Real-time monitoring of CO_2, H_2, H_2S and VOC concentrations in the colon reactor was achieved using an electrochemical gas detection device. The formula for calculating intestinal gas content is shown in Equation (3):

$$Gas\ content(ppm) = \frac{V1}{V2} \times n \quad (3)$$

V_1: Volume of the gas detection device; V_2: Gas volume sampled during fermentation in the bionic colon reactor; n: Data displayed by the gas detection

device.

2.4.4 16S rRNA Amplicon Sequencing

Total genomic DNA was extracted from bacterial pellets using a soil/fecal genomic DNA extraction kit following the manufacturer's instructions. The V3–V4 hypervariable regions of the 16S rRNA gene were amplified using barcoded primers 341F (CCTACGGGNGGCWGCAG) and 806R (GGACTACHVGGGTATCTAAT). The PCR reaction mixture (50 μL) contained 10 μL of 5× Q5® reaction buffer, 10 μL of 5× Q5® High GC Enhancer, 1.5 μL of dNTPs (2.5 mM), 1.5 μL of forward and reverse primers (10 μM each), 0.2 μL of Q5® High-Fidelity DNA Polymerase, and 50 ng of template DNA. The amplification protocol was as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min. The purified amplicons were then subjected to library construction and paired-end sequencing on the Illumina platform.

Raw sequencing data were processed using FASTP for quality filtering, FLASH for paired-end read assembly, and the UPARSE pipeline in USEARCH for operational taxonomic unit (OTU) clustering at 97% similarity. Chimeric sequences were identified and removed using the UCHIME algorithm. In this study, an average of approximately 115,000 raw reads per sample were generated from 27 samples. After quality control and chimera removal, an average of 113,000 high-quality effective tags per sample were retained, with an average effective ratio of 98.0%, indicating

good sequencing quality and sufficient depth for downstream analyses.

Taxonomic assignment was performed using the RDP Naïve Bayesian classifier against the UniDB database with a confidence threshold of 0.8–1.0. Alpha and beta diversity indices were calculated using the QIIME2 pipeline to evaluate differences in microbial community structure and composition across treatment groups. All bioinformatics analyses were conducted on the Omicsmart real-time interactive online data analysis platform (https://www.omicsmart.com).

2.4.5 Non-targeted Metabolomics

Following colonic fermentation, fermentation supernatant was collected from each group and stored at −80°C until analysis. For sample preparation, 50 μL of thawed supernatant was mixed with 150 μL of ice-cold extraction solvent (20% acetonitrile in methanol, v/v) containing internal standards. The mixture was vortexed for 3 min and centrifuged at 12,000 rpm for 10 min at 4°C. The supernatant (150 μL) was transferred to a new tube and kept at −20°C for 30 min, followed by another centrifugation at 12,000 rpm for 3 min at 4°C. Finally, 120 μL of supernatant was transferred to an autosampler vial for LC-MS/MS analysis.

Chromatographic separation was performed on a Waters ACQUITY Premier HSS T3 column (1.8 μm, 2.1 mm × 100 mm) using a Vanquish UHPLC system. The column temperature was maintained at 40°C with a flow rate of 0.4 mL/min and an injection volume of 3 μL. The mobile phases consisted of (A) 0.1% formic acid in

water and (B) 0.1% formic acid in acetonitrile. The gradient elution program was as follows: 0–2 min, 5% B; 2–19 min, 5–95% B; 19–24 min, 95% B; 24.1 min, 5% B; and re-equilibration at 5% B until 30 min.

Mass spectrometry detection was performed on a Q Exactive HF-X mass spectrometer (Thermo Scientific) equipped with an electrospray ionization (ESI) source operating in both positive and negative ion modes. Full scan MS data were acquired over the mass range of m/z 70–1000 at a resolution of 60,000 (MS1), followed by data-dependent MS/MS acquisitions at a resolution of 15,000 (MS2) with stepped collision energies of 30, 40, and 50 eV.

Raw data were converted to mzML format using ProteoWizard and processed using the XCMS program for peak detection, alignment, and retention time correction. Features with >50% missing values across samples were filtered out. Remaining missing values were imputed using KNN imputation for features with <50% missing values, or replaced with 1/5 of the minimum value for features with >50% missing values. Peak areas were normalized using the SVR method. Metabolite identification was performed by matching against an in-house database, public databases (HMDB, MassBank), and prediction libraries. Metabolites with a confidence score $\geqslant$0.5 and QC relative standard deviation (RSD) <30% were retained, and positive/negative ion mode data were merged by keeping the entry with the highest identification level and lowest CV value.

QC samples were prepared by pooling equal aliquots of all samples and injected every 10 samples to monitor system stability and repeatability. Principal component analysis (PCA) was performed using R software (prcomp function) with unit variance scaling. Orthogonal partial least squares discriminant analysis (OPLS-DA) was conducted using the MetaboAnalystR package in R, with log2 transformation and mean-centering prior to modeling, and validated by 200 permutation tests. Differential metabolites between groups were screened based on VIP >1 and ANOVA $p < 0.05$ (for multi-group comparisons). KEGG pathway annotation and enrichment analyses were performed using the KEGG database [20].

2.5 Statistical Analysis

All samples were measured in triplicate. Data are presented as mean ± standard deviation. Origin 2024 software was used for graphing. One-way ANOVA and Tukey's post hoc test were performed using SPSS 27.0 software. $p < 0.05$ indicates statistical significance; $p < 0.01$ indicates significant difference; $p < 0.001$ indicates extreme significance.

3. Results and Discussion

[21] This study found that the gastric digestion phase can be regarded as a homogenizing prelude, where no significant differences in hydrolysis were observed among the six lipids. True digestion commences in the small intestine—under the influence of pancreatic enzymes and bile salts, the diversity in digestive kinetics and

oxidative stability among lipids is fully revealed.

3.1 Small Intestine Spatiotemporal Digestion

MDA, a key end-product of lipid peroxidation, directly reflects the extent of lipid oxidation by free radical attack [22] This study measured MDA levels in the digestive fluids of the duodenum, jejunum, and ileum for six lipid raw materials. Most oils begin to be digested in the duodenum, where the addition of pancreatic enzymes and bile salts creates a pro-oxidative environment [23].As shown in **Figure 1 A**, the MDA levels of olive oil (48.29 nmol/mL) and fish oil (42.44 nmol/mL) were significantly higher than other oils, indicating intense oxidation during early digestion. The high MDA value of fish oil is attributed to its abundance of long-chain omega-3 polyunsaturated fatty acids. [24] reported that emulsions with higher DHA proportions are more susceptible to oxidation during digestion, likely due to DHA's higher unsaturation and unique molecular conformation ARA (23.17 nmol/mL) and coconut oil (11.71 nmol/mL) exhibited moderate oxidation. Camellia oil and peanut oil showed zero MDA values, indicating exceptional oxidative stability during early digestion and suggesting their components effectively resist oxidative stress in this phase [13].

In the duodenum, oxidative stability ranked as follows: Camellia oil $\approx$ Peanut oil > Coconut oil > ARA > Fish oil > Olive oil. Significant differences existed in the initial oxidative risk among these oils.

This study employed the DPPH radical scavenging assay to evaluate antioxidant capacity changes during digestion. Higher DPPH radical scavenging rates indicate stronger antioxidant activity in lipids [25] . As shown in **Figure 1 B**, ARA exhibited exceptionally high radical scavenging efficiency (99.15%) in the duodenum, significantly outperforming other lipids. Its outstanding initial antioxidant activity likely stems from the ability of its highly unsaturated molecular structure to rapidly donate hydrogen atoms during early digestion [26] . This mechanism has been elucidated in studies on the radical scavenging kinetics of polyunsaturated fatty acids[27] .Olive oil (77.08%) also demonstrated notable activity at this stage, with its antioxidant efficacy potentially linked to its rich phenolic content. Population intervention studies further confirm that olive oil rich in hydroxytyrosol enhances antioxidant capacity in elderly subjects [28] .

The jejunum serves as the primary site for digestion and absorption. Sustained enzymatic action and a neutral environment may alter oxidative processes [29] . As shown in **Figure 1 A**, the MDA value for olive oil remained elevated (35.25 nmol/mL), indicating ongoing oxidation. The MDA values for ARA (14.63 nmol/mL) and fish oil (14.39 nmol/mL) showed reduced MDA values compared to the duodenum, potentially indicating that their easily oxidized components were largely depleted in the anterior segments. Coconut oil exhibited a significant decrease in MDA to 3.66 nmol/mL, demonstrating enhanced stability. Camellia oil and peanut oil maintained MDA values of 0, exhibiting consistent stability throughout. Camellia oil

and peanut oil demonstrated absolute stability across intestinal segments [30].

Upon entering the jejunum, antioxidant activity of all oils underwent significant changes. The radical scavenging rates of camellia oil and peanut oil increased sharply, indicating that their fat-soluble antioxidant components (such as vitamin E) were extensively released at this stage under the action of pancreatic lipase and bile salts. This "time-dependent release" phenomenon aligns with the mechanism observed in studies on [31] , where bile salts significantly enhance the dissolution and activity of hydrophobic antioxidant components in vegetable oils by forming micelles. Coconut oil, camellia oil, and peanut oil maintained high antioxidant activity even in the terminal ileum, demonstrating greater persistence of their antioxidant systems throughout the extended digestive process. This persistence may relate to the physical encapsulation of active components within the lipid matrix and the slow digestion rate. Similar sustained antioxidant effects were observed in the study by [32] , attributed to the microstructural characteristics of lipids.

The ileum represents the final stage of digestion, characterized by decreased pH and potential changes in substance concentration that may influence oxidative endpoints. As shown in **Figure 1 A**, fish oil MDA values rebounded to the highest level (28.05 nmol/mL), followed by olive oil (22.68 nmol/mL), indicating ongoing oxidative reactions during late digestion. Camellia oil and peanut oil MDA values remained at 0, while coconut oil showed extremely low levels (0.24 nmol/mL), demonstrating perfect terminal stability. ARA: MDA values decreased to 5.61

nmol/mL, indicating a moderation in oxidation.

The oxidative stability of oils during digestion is a complex outcome of their inherent chemical structure interacting with the dynamic digestive environment [33]. This finding provides critical evidence for accurately assessing the nutritional retention rate, bioavailability, and gastrointestinal health effects of dietary oils.

By the ileum segment, coconut oil, camellia oil, and peanut oil maintained high activity, while fish oil and ARA activity declined. This indicates that the antioxidant systems of coconut oil, camellia oil, and peanut oil exhibit good digestive tolerance [34].The decline in fish oil and ARA activity aligns with the "lipid peroxidation chain reaction" theory, wherein highly unsaturated fatty acids preferentially consume their own and environmental antioxidant components during digestion to counter oxidation, leading to apparent antioxidant capacity depletion [35] .

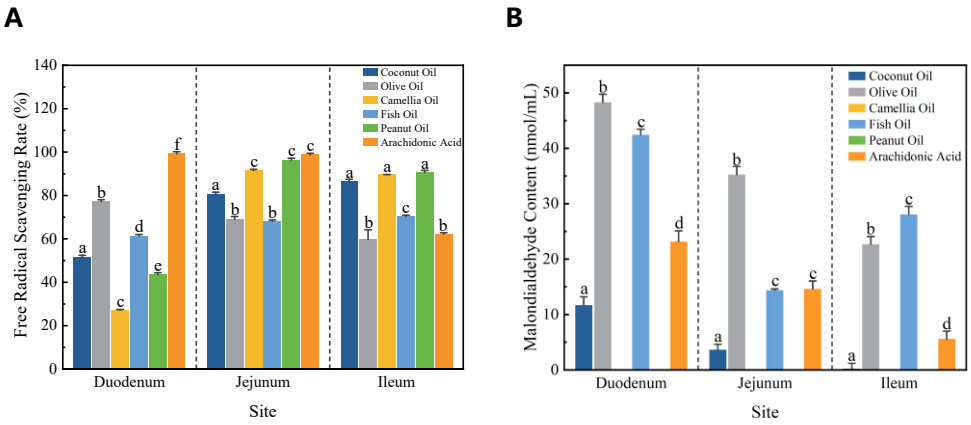


Figure 1.Characteristics of six dietary oils during small intestinal digestion. (A) Malondialdehyde (MDA) content in duodenum, jejunum, and ileum; (B) DPPH radical scavenging rate in duodenum, jejunum, and ileum. Note: Results are expressed as mean ± standard deviation (n=3). Different

lowercase letters indicate significant differences among oil groups within the same intestinal segment ($p < 0.05$).

3.2 Analysis of Lipid Oxidation Stability in the Colon

As shown in **Figure 2 A**, fish oil exhibited a MDA content as high as 0.428 nmol/mL at 0 h, significantly exceeding the initial levels of all other oils. This indicates that its abundant omega-3 polyunsaturated fatty acids underwent substantial oxidation at the onset of digestion. This phenomenon is typical in the oxidation kinetics of oils rich in unsaturated bonds. Similar rapid oxidation trends of highly unsaturated fish oils in the environment were also observed in studies on [36] . The continuous increase in MDA levels of ARA during digestion further confirms the oxidative sensitivity of highly unsaturated fatty acids in the gastrointestinal environment [37].

In contrast, camellia oil and peanut oil demonstrated excellent oxidative stability throughout the digestion process, with MDA values of only 0.0085 nmol/mL and 0.0128 nmol/mL, respectively, after 24 h. This confirms the strong antioxidant capacity of plant oils rich in oleic acid. [38] A systematic comparison of oxidative stability among different vegetable oils further supports this conclusion. Additionally, the MDA values of coconut oil and olive oil fluctuated minimally over time, remaining stable within ranges of 0.033 nmol/mL and 0.028 nmol/mL, respectively. This indicates that medium-chain fatty acids and monounsaturated fatty acids are structurally resistant to degradation in the gastrointestinal environment [39].The minimal

MDA value changes over time in coconut oil and olive oil reflect the structural stability of medium-chain fatty acids and monounsaturated fatty acids. This finding aligns with systematic evaluations of antioxidant properties based on fatty acid structures, as documented in [40].

These results collectively validate the core principle that "fatty acid unsaturation determines oxidative stability" [41] . While polyunsaturated oils possess significant nutritional value, they require effective antioxidant protection during processing and digestion. In contrast, monounsaturated and saturated fats, due to their inherent structural stability, are more suitable as stable lipid sources in daily diets.

3.3 Antioxidant Capacity Analysis in the Colon

At 0h of colonic fermentation in **Figure 2 B**, all six lipids exhibited potent and remarkably similar antioxidant potential, with scavenging rates concentrated within a narrow range of 77.0% – 77.7%. This indicates that all tested lipid sources contain abundant and comparably active natural antioxidant components [42] .As fermentation time extended, significant differences emerged in antioxidant stability among different oils: Fish oil's radical scavenging rate plummeted from 77.02% to 58.63%, indicating its abundant omega-3 polyunsaturated fatty acids are highly susceptible to oxidative degradation in the colonic environment [43] .

In contrast, olive oil, camellia oil, and peanut oil maintained higher activity throughout fermentation, highlighting the antioxidant durability of monounsaturated

fats in the colonic environment. This indicates that the structural characteristics of such oils confer strong oxidative tolerance[30] . Coconut oil maintained optimal activity at 12 h but declined at 24 h, suggesting that even medium-chain fatty acid oils—known for stability—gradually deplete their antioxidant components during prolonged fermentation.

These findings confirm that the initial antioxidant capacity of oils depends on natural antioxidant content, while their stability during digestion is primarily governed by fatty acid unsaturation [44] . This discovery provides crucial evidence for evaluating the actual efficacy of functional oils in vivo.

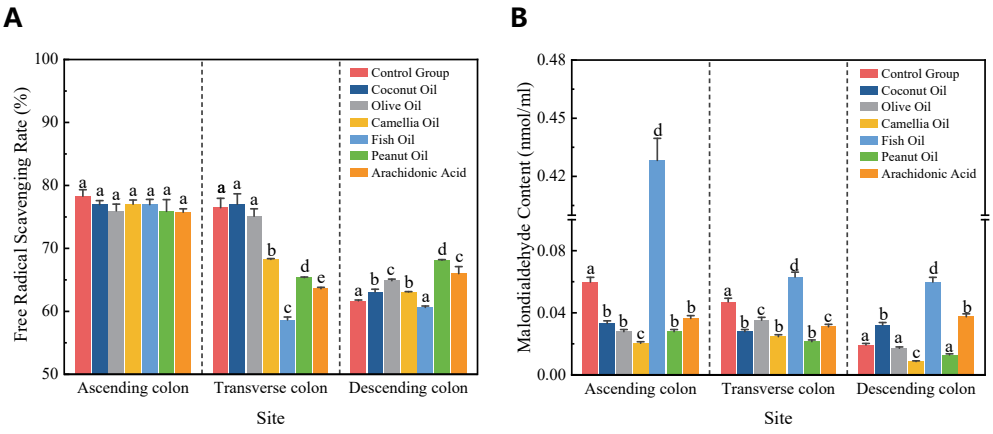


Figure 2. Oxidative stability and antioxidant activity of six dietary oils during colonic fermentation. (A) MDA content in ascending, transverse, and descending colon; (B) DPPH radical scavenging rate in ascending, transverse, and descending colon. Note: Results are expressed as mean ± standard deviation (n=3).

3.4 Gas Metabolism Analysis

The interplay between gas metabolism and gut function represents a complex and multifaceted research domain, highlighting the critical role of the gut microbiota

445 in health and disease [45] . This study evaluated the differential utilization of six lipids
446 by gut microbiota by measuring concentrations of key gas metabolites (H_2S, CO_2,
447 VOC, NO, H_2) produced in an *in vitro* gastrointestinal digestion model. Fish oil, ARA,
448 and peanut oil significantly promoted H_2S and VOC production, likely due to their
449 rich polyunsaturated fatty acids providing substrates for sulfate-reducing bacteria.
450 Conversely, olive oil and coconut oil exhibited the lowest H_2S generation.

451 **Figure 3** shows that coconut oil produced significantly higher H_2 yields than
452 other oils, supporting the theory that medium-chain fatty acids undergo rapid
453 microbial β-oxidation to generate hydrogen. This mechanism has been extensively
454 documented in gut microbial metabolism studies on [46] .Simultaneously, coconut oil
455 and camellia oil exhibited the highest NO production, suggesting their fermentation
456 may promote nitrate-reducing bacteria growth [47]. Regarding overall fermentation
457 intensity, camellia oil exhibited the highest CO_2 production, indicating its highest
458 microbial utilization. Olive oil and the control group showed the lowest CO_2
459 production, reflecting weaker fermentation activity [48].

460 In summary, this study categorizes six oils into three groups based on intestinal
461 gas metabolism characteristics: the high-activity gas-producing group (fish oil, ARA,
462 peanut oil), the high-hydrogen-producing group (coconut oil), and the mild
463 fermentation group (olive oil, camellia oil), offering a new perspective on
464 understanding the intestinal metabolic properties of different oils. This analysis
465 demonstrates that the molecular structure of oils not only influences their digestion

and oxidation but also indirectly determines their microbial metabolic fate in the colon, thereby exerting distinctly different effects on gut health across individuals [49].

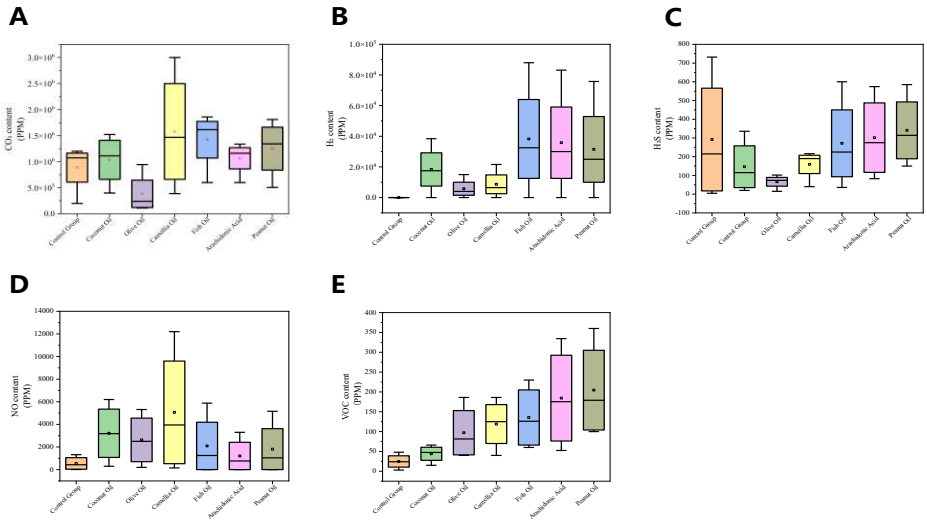


Figure 3. Gas production profiles of six dietary oils after colonic fermentation. (A) carbon dioxide (CO_2) concentrations at 12 h and 24 h; (B) Hydrogen (H_2) concentrations at 12 h and 24 h; (C) Hydrogen sulfide (H_2S) concentrations at 12 h and 24 h; (D) Nitric Oxide (NO) concentrations at 12 h and 24 h; (E) volatile organic compounds (VOC) concentrations at 12 h and 24 h. Note: Results are expressed as mean ± standard deviation (n=3).

3.5 16S rRNA Amplicon Sequencing Analysis

To elucidate the effects of different dietary lipids on the gut microbial ecosystem following colonic fermentation and to reveal potential mechanisms underlying the differential digestive fates of various lipids from a microbial perspective, this study assessed the microbial composition within the colon after *in vitro* fermentation of six lipids using 16S rRNA amplicon sequencing. Different lipid interventions remodeled the composition of the colonic microbiota. As shown in **Figure 4 A**(stacked bar chart of relative abundance at phylum level), all samples exhibited absolute dominance by

the Firmicutes and Bacteroidetes phyla, consistent with the characteristics of healthy adult colonic microbiot. However, lipid interventions significantly altered the relative abundance of specific phyla. Notably, the relative abundance of Proteobacteria and Desulfobacterota_I showed a marked upward trend in the fish oil (FO), ARA, and peanut oil (PO) treatment groups. This finding carries important functional implications: the Proteobacteria phylum encompasses numerous opportunistic pathogens, and its increased abundance is often regarded as a marker of gut dysbiosis [50];while the Desulfovibrionota phylum comprises known sulfate-reducing bacteria, a key microbial group responsible for endogenous hydrogen sulfide (H_2S) production in the gut [50] . This result directly corroborates, at the microbial taxonomic level, that these three oils belong to the "highly gas-producing" category in previous gas metabolism studies, particularly associated with the highest H_2S production. In contrast, the coconut oil (CO) group exhibited unique changes in the Firmicutes/Bacteroidetes ratio, while the olive oil (OO) and camellia oil (CaO) groups showed microbial compositions most similar to the blank control, with overall more stable structures consistent with their "mildly stable" phenotypes.

To elucidate the specific patterns of core microbial responders and key metabolites, we further generated cluster heatmaps for differentially abundant genera (genus level) and metabolites (**Figure 4 E**). The genus heatmap clearly reveals that all samples cluster into three major groups based on their microbial profiles, perfectly corresponding to the three lipid fates defined earlier by phenotype. The first cluster,

dominated by fish oil, ARA, and peanut oil samples, was characterized by a synergistic increase in the relative abundance of Desulfovibrio, Alistipes, and Bacteroides. The second cluster, dominated by coconut oil samples, exhibited enrichment of short-chain fatty acid-producing genera such as the NK4A136_group within the family Clostridiaceae. The third cluster, comprising olive oil, camellia oil, and most blank control samples, showed the least variation in bacterial composition. In the "strongly gas-producing" oil group, metabolites such as taurine, hypotaurine, and indole derivatives were significantly upregulated. Taurine serves as a key substrate for sulfite-reducing bacteria in H_2S production, with its accumulation and consumption directly linked to H_2S yield [51] . In contrast, the "high hydrogen-producing" coconut oil group exhibited changes in the relative abundance of fermentation intermediates like succinate and lactate, reflecting hydrogen-producing metabolic processes. Both oil type and colonic location jointly drive the differentiation of the overall microbial community structure.

To precisely identify microbial groups uniquely affected and those commonly influenced by different oil interventions, we constructed a Venn diagram based on operational taxonomic unit (OTU) levels (**Figure 4 C**). This diagram visually illustrates the number of significantly different OTUs unique to each oil treatment group and those shared among them compared to the blank control group.

Analysis revealed that fish oil (FO), arachidonic acid (ARA), and peanut oil (PO) shared the highest number of differentially abundant OTUs, forming a distinct core

shared set. Species annotation of OTUs within this set indicated they predominantly belonged to the genera Desulfovibrio (phylum Desulfovibrionota) and Escherichia-Shigella (phylum Proteobacteria). This finding exhibits high consistency and, from the perspective of a "core of differential species," strongly confirms that these three oils induce similar specific perturbations in the gut microbiota—namely, the co-enrichment of sulfate-reducing bacteria and potential opportunistic pathogens. This provides the most direct microbiological taxonomic evidence for their shared "highly gas-producing" (especially H_2S-producing) and potentially pro-inflammatory phenotypes [52] . In contrast, the coconut oil (CO) treatment group harbored a substantial number of unique differential OTUs. These OTUs predominantly annotated to butyrate-producing genera within the Lachnospiraceae and Ruminococcaceae families. This unique disturbance pattern aligns with the metabolic characteristics of coconut oil's medium-chain fatty acids [39], potentially driving distinct hydrogen-producing and acid-producing metabolic pathways, thereby supporting its independent classification as a "high hydrogen-producing type.". The olive oil (OO) and camellia oil (CaO) groups exhibited the lowest total numbers of differential OTUs and the highest proportion of shared OTUs with the blank control, further confirming their mildest impact on microbial ecology and relatively stable community structure.

Principal coordinate analysis (PCoA) based on Bray-Curtis distance (**Figure 4 E**) revealed a distinct separation trend along the first principal coordinate (PCo1, explaining 48.80% variance) among different sampling points. Although partial

545 overlap existed between samples, those from the fish oil, ARA, and peanut oil groups
546 tended to cluster in specific regions of the plot, distinguishing them from samples in
547 the coconut oil, olive oil, and camellia oil groups. This indicates that different oil
548 types exerted specific effects on the overall microbial community structure. Further
549 analysis revealed that samples from different colonic segments (ascending colon,
550 transverse colon, descending colon) did not fully separate along the PCo2 axis,
551 suggesting that in this study system, the effect of oil type may outweigh the physical
552 differences between colonic segments. However, some sample pairs sharing the same
553 oil type but differing in location (e.g., G-ascending-colon and G-descending-colon)
554 still exhibited discernible distances on PCo1, suggesting potential subtle spatial
555 heterogeneity in microbial responses. This may result from gradient changes in
556 physicochemical properties (e.g., pH, substrate concentration) of colonic contents
557 during transit. Statistical testing (PERMANOVA) further confirmed that lipid
558 treatment was a significant factor ($p < 0.05$) driving differences in microbial β-
559 diversity.

560 Spearman correlation analysis revealed that H_2S production was positively
561 correlated with the relative abundance of *Desulfovibrio* ($r = 0.82$, $p < 0.05$) and
562 *Escherichia-Shigella* ($r = 0.67$, $p = 0.06$), while H_2 production was positively
563 correlated with the Lachnospiraceae NK4A136 group ($r = 0.71$, $p < 0.05$). These
564 results provide statistical support for the functional linkages inferred from the
565 clustering analyses.

Integrated Discussion and Multi-Omics Correlation. The observed microbial disturbance patterns align with previous studies on polyunsaturated fatty acids (PUFAs). Fish oil and ARA, rich in omega-3 and omega-6 PUFAs, have been shown to potentially inhibit growth of certain beneficial bacteria at high doses, creating ecological advantages for opportunistic pathogens like Proteobacteria (Wu et al., 2011).Altered microbial structure directly impacts metabolic function: enrichment of the Desulfovibrionaceae phylum increased H_2S production [53] , while overall dysbiosis may further impair metabolism of bile acids, choline, and other substances. In summary, as the core executors of lipid colonic fermentation, the gut microbiota's specific structural and functional alterations serve as the key biological bridge linking different lipid chemical properties (unsaturation) to final digestive phenotypes (gas metabolism, oxidative stability) [13]. From a microbial perspective, the enrichment of sulfate-reducing bacteria (e.g.,*Desulfovibrio*) and opportunistic pathogens (e.g.,*Escherichia-Shigella*) observed in the high gas-producing oil groups is consistent with the known capacity of these taxa to utilize sulfur-containing compounds and produce H_2S-. Conversely, the minimal microbial perturbation observed in the mild fermentation groups (olive and camellia oils) may be attributed to the greater oxidative stability of monounsaturated fatty acids, which limits the generation of reactive lipid oxidation products that could otherwise serve as selective pressures on the microbial community-. These findings highlight the need for further studies to determine whether such microbial shifts translate into meaningful health outcomes *in*

vivo.

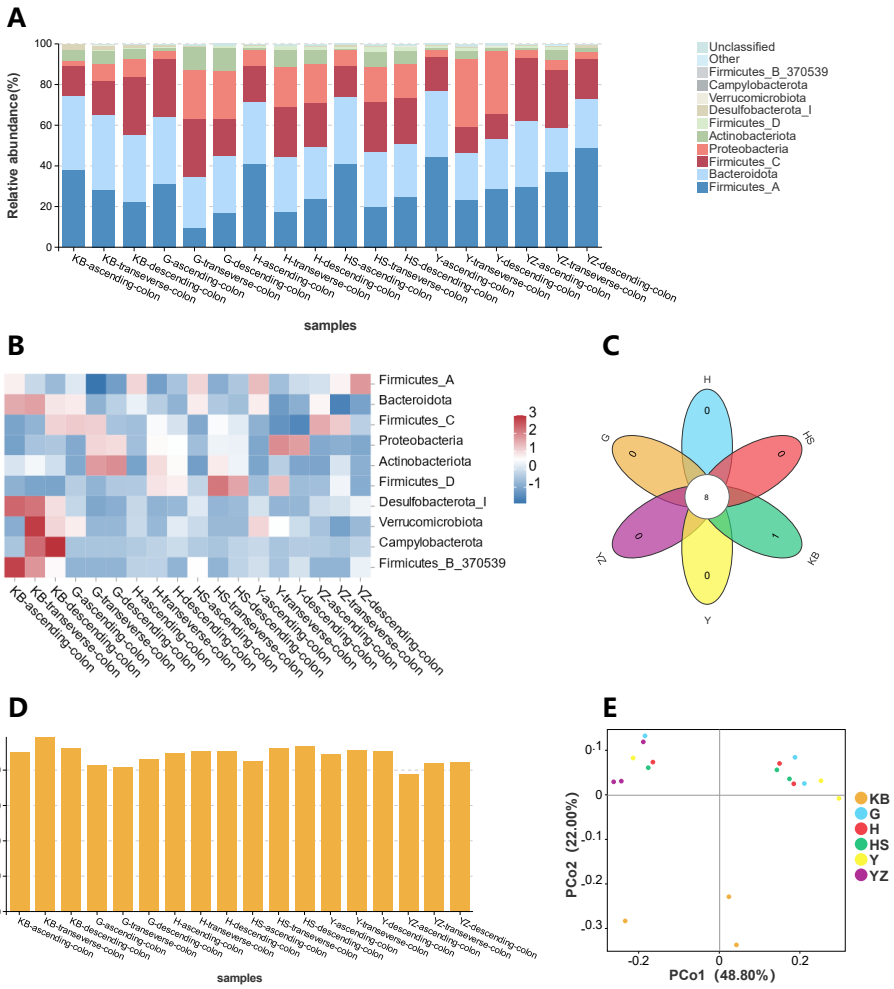


Figure 4. Gut microbiota analysis after colonic fermentation of six dietary oils. (A) Relative abundance of bacterial communities at the phylum level; (B) Heatmap of key differential genera; (C) Venn diagram showing unique and shared OTUs among oil groups compared to control; (D) Principal coordinate analysis (PCoA) plot based on Bray–Curtis distance; (E) Chao1 index. Note: Results are expressed as mean ± standard deviation (n=3). PERMANOVA test confirmed significant differences in β-diversity among oil groups ($p < 0.05$).

3.6 Metabolomics Analysis

To investigate differences in the metabolite profiles of dietary oils after fermentation by colonic microbiota, this study first employed non-targeted

metabolomics technology to analyze all samples. Principal Component Analysis (PCA) results revealed a trend of metabolic separation among groups, indicating potential differences within sample groups. As shown in **Figure 3**, the cumulative contribution rate of the first principal component (PC1) and second principal component (PC2) was 45.17%, reflecting the core variation in the data. Analysis results (**Figure 5 A**) revealed a distinct separation trend among samples from different oil treatment groups, indicating that oil type significantly altered the overall metabolite profile of the colonic fermentate (PERMANOVA, $p < 0.01$).Samples from the fish oil, ARA, and peanut oil groups clustered in relatively distinct regions within the PCA space, differentiating them from the coconut oil, olive oil, and camellia oil groups. This pattern of " " closely aligns with the "high gas production group" and "mild group" classifications based on gas metabolic characteristics in previous studies. Furthermore, some descending colon samples separated from other sites along the PC2 axis, suggesting that fermentation environments in distinct spatial locations of the colon may uniquely influence the composition of metabolic end products. This could result from gradients in pH, substrate concentration, and microbial community structure during colonic transit [54] .

To further identify key metabolite categories driving these overall differences, we performed Z-score normalization analysis on differentially abundant metabolites across groups (**Figure 5 B**). Results revealed characteristic metabolite category enrichments induced by different lipid interventions. In the fish oil, ARA, and peanut

oil groups (high-gas groups), relative abundances of organosulfur compounds and organic nitrogen compounds were significantly upregulated. These two classes are typically associated with microbial desulfurization and deamination of amino acids. Notably, the enrichment of organosulfur compounds directly corroborates the observation of highest hydrogen sulfide (H_2S) production in this group, as microbial metabolism of sulfur-containing amino acids (e.g., methionine, cysteine) constitutes the primary endogenous source of H_2S [55] .Concurrently, increased levels of aromatic metabolites (e.g., indoles, phenols) among organic nitrogen compounds were observed. These compounds are often produced by specific gut bacteria (e.g., Proteobacteria) through the metabolism of aromatic amino acids, and their accumulation may be associated with inflammatory gut conditions. This finding further corroborates the earlier 16S data showing elevated abundance of Proteobacteria in this group [56] .

In contrast, the coconut oil group (high hydrogen production group) showed significant enrichment of organic acids and their derivatives. This aligns with the group's unique high hydrogen (H_2) production, as hydrogen is a key byproduct of microbial fermentation of carbohydrates and certain amino acids in the colon, yielding acids like acetate and butyrate [57].In contrast, the olive oil and camellia oil group(mildly stable group) exhibited more stable relative abundance patterns of certain oxidized derivatives (e.g., hydroxylated fatty acids) within lipids and lipid-like molecules. This aligns with their highest oxidative stability and mildest gas-producing

properties during digestion, suggesting their parent fatty acids or primary oxidation products undergo limited further microbial conversion or metabolism in the colon [58,59].

K-means clustering analysis grouped differential metabolites into seven subclasses with unique expression patterns (**Figure 5 C**), revealing a systematic association between metabolic responses and oil type. Metabolites in Subclass 1 were significantly upregulated in the fish oil, ARA, and peanut oil groups. This subclass is rich in organic sulfur compounds (e.g., taurine) and aromatic amino acid metabolites. Its expression pattern directly correlates with the highest H_2S and VOC production phenotypes in this group, confirming that "highly gas-producing" oils collectively promote microbial sulfur amino acid metabolism and protein fermentation pathways [51](Hou, et al., 2025). Conversely, metabolites in Subclasses 4 and 7 exhibited the greatest stability in the olive oil and camellia oil groups. The mild variations in these lipid metabolism-related metabolites—such as certain fatty acid oxidation intermediates—corresponded with their higher oxidative stability and lower microbial disturbance, forming the metabolic foundation of the "mildly stable" pattern.

Subclass 2 was uniquely upregulated in the coconut oil group. This class includes substances such as short-chain fatty acid precursors, providing a metabolic explanation for its "high hydrogen production" characteristic: hydrogen is a byproduct of acid fermentation by carbohydrate-consuming microorganisms [57] , suggesting coconut oil may specifically drive this metabolic pathway.

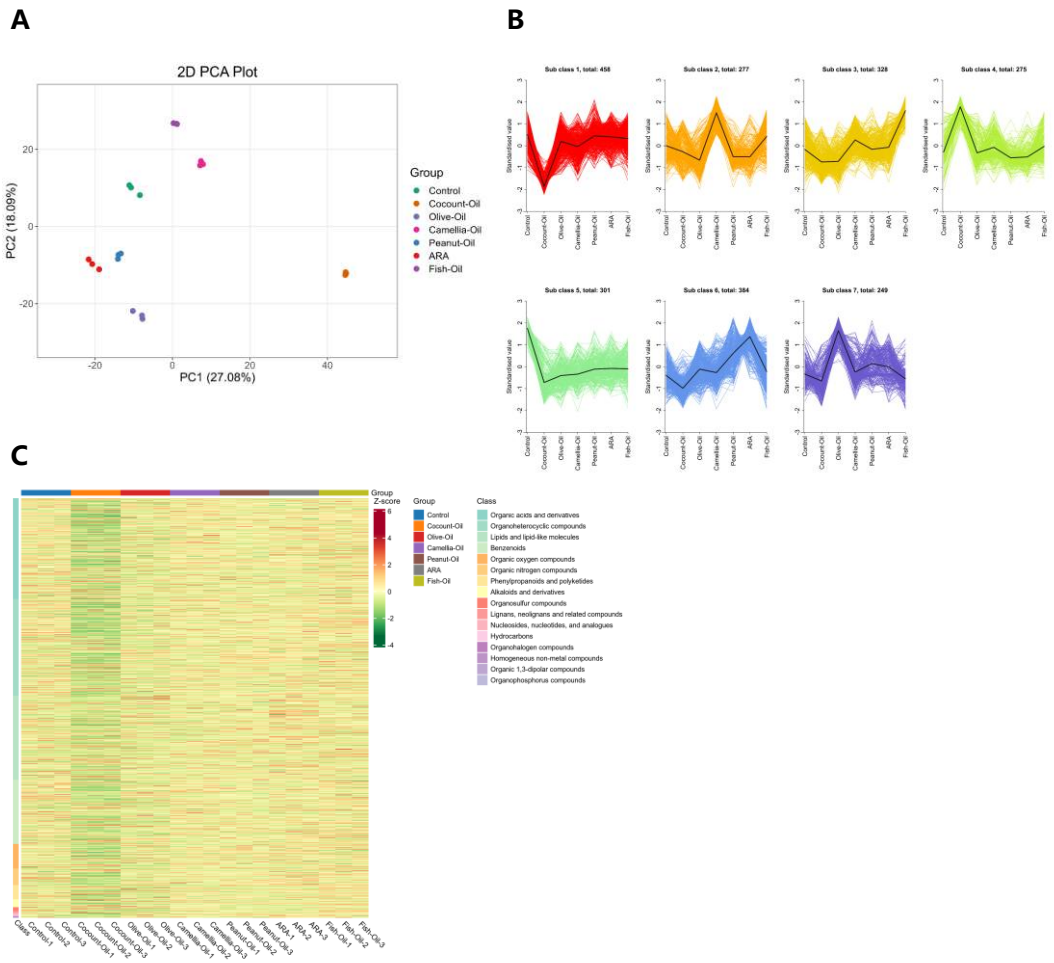


Figure 5.Metabolomic analysis of colonic fermentation broth after six dietary oils treatment. (A) Principal component analysis (PCA) score plot of all samples; (B) Z-score plot of differential metabolites classified by chemical categories;(C) K-means clustering analysis of differential metabolites showing seven distinct expression patterns. Note: PCA showed significant separation among oil groups (PERMANOVA, $p < 0.01$). Differential metabolites were selected with VIP > 1 and $p < 0.05$.

4. Conclusions

This study employed an *in vitro* gastrointestinal-colon continuous digestion model to systematically simulate and compare the complete digestion and fermentation processes of six dietary fats (coconut oil, olive oil, camellia oil, fish oil,

672 ARA, peanut oil), revealing distinct metabolic fates in the gastrointestinal tract.
673 Integrating multi-omics analysis revealed that the colonic fermentation characteristics
674 of fats are closely correlated with their fatty acid composition. Based on gas
675 metabolism profiles, oils were clearly classified into three categories: strong gas-
676 producing (fish oil, ARA, peanut oil), high hydrogen-producing (coconut oil), and
677 mildly stable (olive oil, camellia oil).During fermentation of strong gas-producing
678 oils, H_2S and VOC production peaked at 0.78 ppm and 0.48 ppm, respectively,
679 accompanied by a significant increase in the relative abundance of the
680 Desulfovibrionites phylum ($p<0.05$).At the phylum level, all oil interventions were
681 dominated by Firmicutes and Bacteroidetes, but strong gas-producing oils
682 significantly promoted the growth of Proteobacteria. At the genus level, strong gas-
683 producing oils increased the proportion of genera such as Vibrio desulfurizans, while
684 mildly stable oils exhibited microbial community structures most similar to the
685 control group. Further analysis revealed that the influence of oil type on microbial
686 community structure was stronger than the physical differences between distinct
687 colonic segments (ascending, transverse, descending colon). These alterations in
688 microbial community structure directly modulated the colonic metabolic environment,
689 consistent with the differences in oxidative stability and metabolite profiles exhibited
690 by different oils. This study demonstrates that dietary fats can influence colonic
691 fermentation gas and metabolite production by specifically modulating gut microbiota
692 structure, thereby generating distinct health effects. These findings provide scientific

basis for personalized fat selection tailored to different gut health conditions and offer new research perspectives for the precise development of functional fat products.

CRediT authorship contribution statement

Yating Wang: Methodology, Conceptualization, Writing-Original Draft, Visualization. Shuya Chen: Conceptualization, Investigation, Writing-review & editing. Jintian Chen: Investigation, Visualization.Xixian Lv: Methodology, Visualization, Writing-Original Draft. Yin Yin: Investigation, Writing-Original Draft. Wenxuan Yan: Supervision, Investigation. Zepeng Chang: Writing-review & editing, Investigation. Minjie Gao: Writing-review & editing, Supervision. Shiwei Song: Writing-review & editing, Methodology. Zhitao Li: Conceptualization, Supervision, Writing-review & editing, Funding acquisition, Project administration. Yuqin Wang: Validation, Visualization, Writing-review & editing, Methodology,Funding acquisition.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of competing interest

The authors declare no conflict of interests.

Data availability

The data that support the findings of this study are not publicly available due to commercial use. However, they are available from the corresponding author upon reasonable request.

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