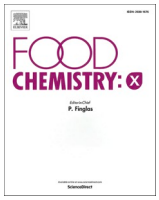
Food Chemistry: X 24 (2024) 102041

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Comparative effects of non-fermented and *Lacticaseibacillus paracasei*-fermented pomelo juice on gut microbiota composition and short-chain fatty acid production: An *in vitro* colonic model

Vernabelle Balmori [a,b], Marisa Marnpae [a,c], Kritmongkhon Kamonsuwan [a], Charoonsri Chusak [a], Uarna Nungarlee [c], Pavaret Sivapornnukul [d], Prangwalai Chanchaem [d], Sunchai Payungporn [d], Suvimol Charoensiddhi [e], Tanyawan Suantawee [a], Thavaree Thilavech [f], Sirichai Adisakwattana [a,*]

[a] *Center of Excellence in Phytochemical and Functional Food for Clinical Nutrition, Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand*
[b] *Department of Food Science and Technology, Southern Leyte State University, Sogod 6606, Southern Leyte, Philippines*
[c] *The Halal Science Center, Chulalongkorn University, Bangkok 10330, Thailand*
[d] *Center of Excellence in Systems Microbiology, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
[e] *Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand*
[f] *Department of Food Chemistry, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand*

ARTICLE INFO

Keywords:
Pomelo juice
Lacticaseibacillus paracasei
Gut microbiota
Short-chain fatty acids

ABSTRACT

Pomelo juice, especially from the Tubtim Siam cultivar, may offer prebiotic benefits by promoting beneficial gut bacteria. This study evaluated the impact of non-fermented and *Lacticaseibacillus paracasei* (*L. paracasei*)-fermented pomelo juice on gut microbiota using an *in vitro* colonic fermentation model. The *L. paracasei*-fermented juice significantly increased lactobacilli levels compared to the non-fermented juice, while both treatments similarly suppressed coliforms within 24 h. Microbiota analysis revealed increased richness and significant community shifts in both treatments. Moreover, the fermented juice demonstrated a greater decrease in the *Firmicutes/Bacteroidetes* ratio, indicating a greater impact on gut metabolism. Fermented juice promoted beneficial bacteria like *L. paracasei*, *Bifidobacterium longum*, and *Faecalibacterium prauznitzii* while inhibiting pathogens. These changes coincided with higher production of short-chain fatty acids (SCFAs), including acetic, propionic, and n-butyric acids. Therefore, fermenting pomelo juice with *L. paracasei* improves its ability to beneficially influence the gut microbiota, suggesting its potential for gut health enhancement.

1. Introduction

The human colon harbors a diverse gut microbiota composed of trillions of microorganisms that play a critical role in the metabolism of complex carbohydrates, which are otherwise indigestible by the host. This microbial fermentation process produces short-chain fatty acids (SCFAs), which impact energy homeostasis and various physiological functions (Wang, Yao, Lv, Ling, & Li, 2017). The gut microbiota is diverse with dominant phyla such as *Firmicutes* and *Bacteroidetes*, as well as subdominant phyla including *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia*. This diversity significantly influences host metabolism, physiology, and immune function. The microbiota also contributes to essential processes like synthesizing vitamin K and B-complex, strengthening the mucosal barrier against enteric pathogens, and modulating neurotransmitter signaling *via* the gut-brain axis (Sánchez et al., 2017). Emerging evidence suggests the gut microbiota composition, particularly the *Firmicutes/Bacteroidetes* (F/B) ratio, plays a key role in the pathogenesis of diverse disease states. Disruptions in this ratio have been linked to the development of various conditions, including cardiovascular diseases, cancer, diabetes, obesity, and even psychiatric disorders (Wang et al., 2017). A balanced gut microbiota, characterized by a specific composition of bacterial communities, is a

* Corresponding author.
E-mail addresses: charoonsri.c@chula.ac.th (C. Chusak), avaret.s@alumni.chula.ac.th (P. Sivapornnukul), sunchai.p@chula.ac.th (S. Payungporn), suvimol.ch@ku.th (S. Charoensiddhi), tanyawan.s@chula.ac.th (T. Suantawee), thavaree.thi@mahidol.ac.th (T. Thilavech), sirichai.a@chula.ac.th (S. Adisakwattana).

https://doi.org/10.1016/j.fochx.2024.102041
Received 11 August 2024; Received in revised form 21 November 2024; Accepted 22 November 2024
Available online 26 November 2024

critical determinant of human health and susceptibility to chronic diseases (Azad, Sarker, Li, & Yin, 2018). Oral probiotics exert their beneficial effects by modulating the gut microbiota composition, selectively enriching populations of commensal bacteria. This enrichment enhances intestinal epithelial barrier integrity and modulates cytokine production, ultimately strengthening the immune response (Azad et al., 2018). Strains of the *Lactobacillus* and *Bifidobacterium* genera significantly influence the gut microbiota composition, promoting a shift towards a more favorable profile. This shift is marked by an increase in beneficial phyla such as *Bacteroidetes* and *Verrucomicrobia*, while concurrently reducing *Firmicutes* (Azad et al., 2018). Among these, *Lacticaseibacillus paracasei* subsp. *paracasei* strain (*L. casei* 431®) has shown notable health benefits and a strong ability to survive the gastrointestinal tract. This strain is commonly used in functional foods and probiotic supplements due to its documented efficacy (Rizzardini et al., 2012). Clinical studies have demonstrated its positive impact on immune response, improvement in stool consistency for infants, and alleviation of symptoms in lactose-intolerant individuals (Vlieger et al., 2009).

Pomelo (*Citrus grandis* (L.) Osbeck), a citrus fruit in the Rutaceae family, is widely cultivated in Thailand. Cultivars like Kao-Yai, Thongdee, and Tubtim Siam exhibit distinct flavor profiles and characteristics. Pomelo fruits are rich in flavonoids, including neohesperidin, hesperidin, naringenin, naringin, and rutin, which contribute to their potent antioxidant properties (Mäkynen et al., 2013). Additionally, pomelo is a valuable source of dietary fiber, beta-carotene, terpenoids, and alkaloids. Fermenting pomelo juice, particularly the Tubtim Siam variety, with *L. paracasei* increases specific flavonoids (naringenin, naringin, hesperetin) (Balmori et al., 2023). This fermentation process enhances the juice's antioxidant and lipid-lowering effects. Notably, these flavonoids act as prebiotics, selectively fermented by gut bacteria, influencing gut microbiota composition and richness. This demonstrably benefits gut health by promoting intestinal barrier function, immune modulation, and production of beneficial metabolites (Ma & Chen, 2020). Additionally, pomelo's dietary fiber is fermented by gut bacteria, leading to the production of SCFAs that regulate host metabolism, immunity, and inflammation (Koh, De Vadder, Kovatcheva-Datchary, & Bäckhed, 2016). The present study hypothesizes that the combination of probiotic *L. paracasei* and the prebiotic components naturally present in pomelo, such as flavonoids and dietary fiber, may act synergistically to enhance gut microbiota modulation. Recent advancements in *in vitro* models utilizing human fecal samples have facilitated the evaluation of probiotics, prebiotics, and synbiotics on the gut microbiome. However, there is limited research investigating the effects of fruit juices and their lactic acid fermentation within simulated *in vitro* digestion and subsequent *in vitro* colonic fermentation models (da Silva et al., 2023). Therefore, this study aims to compare the impact of non-fermented pomelo juice and pomelo juice fermented with *L. paracasei* on gut microbiota modulation and metabolite production using an *in vitro* colonic fermentation model. It was hypothesized that *L. paracasei* fermentation of pomelo juice would beneficially modulate gut microbiota by promoting *Lactobacillus* proliferation and relative abundance, while concurrently suppressing pathogenic bacteria compared to the unfermented control. This modulation was expected to result in an altered F/B ratio and increased production of SCFAs, including butyrate, acetate, and propionate.

2. Materials and methods

2.1. Materials

The pomelo fruits (Tubtim Siam cultivar) were purchased from the Talaad Thai Fruit Market located in Pathum Thani Province, Thailand. The freeze-dried powder of L. *paracasei* subsp. *paracasei* CASEI 431 (Chr. Hansen A/S, Horsholm, Denmark) was obtained from Brenntag Ingredients (Thailand) Public Company Limited, Bangkok. Food-grade sodium bicarbonate was obtained from Thai Food and Chemical Co, Ltd. The α-amylase Type VI-B from porcine pancreas, pepsin from porcine gastric mucosa powder, bile extract, pancreatin from porcine pancreas, trypticase, resazurin, cysteine hydrochloride, hydrochloric acid, sodium hydroxide, and microminerals were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Amyloglucosidase from *Aspergillus niger* was purchased from Megazyme (Bray, Ireland). De Man–Rogosa–Sharpe (MRS) agar and Eosin Methylene Blue (EMB) agar were procured from Oxoid (Basingstoke, UK).

2.2. Preparation of non-fermented, and probiotic-fermented pomelo juices

Pomelo juice preparation followed a previously established protocol (Balmori et al., 2023). Briefly, Tubtim Siam pomelo fruits were peeled, and the pulp was separated from the rind and seeds. The pulp was then juiced using a low-speed extractor at a 4:1 pulp-to-water ratio (*v/v*). The resulting juice was filtered through cheesecloth to remove particulates. Subsequently, 5 % (*w/v*) sugar and 0.05 % (*w/v*) salt were added for taste adjustment. Pasteurization was performed at 80 °C for 30 s. The pasteurized juice was collected and stored at −20 °C (non-fermented juice). For fermented juice preparation, the pH of the non-fermented juice was aseptically adjusted to 6.0 with 1 M $NaHCO_3$. *L. paracasei* powder was aseptically added to establish an initial inoculum of 7 log CFU/mL. The mixture was incubated at 37 °C for 24 h. Following fermentation, the juice was immediately cooled and stored at −20 °C for subsequent analysis.

2.3. In-vitro gastrointestinal digestion

An *in vitro* digestion model mimicking the human digestive tract was employed to simulate the sequential processes occurring in the mouth (oral phase), stomach (gastric phase), and small intestine (intestinal phase). This static model was adapted from the methodology established by Minekus et al. (2014) (Minekus et al., 2014). Non-fermented and fermented pomelo juice samples (100 mL each) were aseptically transferred to glass bottles with screw caps. The digestion process commenced with the oral phase, where 2.5 mL of α-amylase solution (3.2 mg/mL in 0.2 M carbonate buffer, pH 7.0) was added to mimic salivary enzyme activity. This mixture was incubated in a shaking water bath at 37 °C for 1 min at 80 rpm. To simulate stomach conditions, the pH was then adjusted to 3.0 with 2 M HCl, followed by the addition of 10 mL of simulated gastric fluid containing pepsin (1 mg/mL in 0.02 M HCl). The gastric phase incubation continued for 30 min. Following the gastric phase, the intestinal phase was simulated by adjusting the pH to 7.0 with 6 M NaOH. Subsequently, 40 mL of a simulated intestinal fluid mixture containing pancreatin (2 mg/mL), bile extract (100 mg/mL), and amyloglucosidase (28 U/mL) prepared in 0.1 M $NaHCO_3$ solution was introduced. This mixture was incubated for an additional 2 h at 37 °C to mimic small intestine digestion. After the complete incubation period, samples were collected and immediately cooled to stop enzymatic reactions. A control digesta was prepared identically, substituting distilled water for the pomelo juice sample. Finally, all digested samples were freeze-dried and stored for further analysis.

2.4. In-vitro colonic fermentation

The *in vitro* colonic fermentation process employing human gut microbiota was conducted according to a previously established method (Charoensiddhi, Conlon, Vuaran, Franco, & Zhang, 2016). Fresh fecal samples were obtained from five healthy adults aged 25–35 years. All donors met the following criteria: no dietary restrictions, no history of gastrointestinal diseases, and no probiotic or antibiotic use for at least 3 months prior to donation. This research protocol received ethical approval from the Research Ethics Review Committee for Research Involving Human Research Participants at Chulalongkorn University (Certificate of Approval No. 090/65, Study Title No. 650032). Written informed consent was obtained from all fecal donors before

participation in the study. A 10 % (*w/v*) fecal slurry was prepared aseptically using 0.01 M phosphate-buffered saline (PBS, pH 7.2). The slurry was homogenized and maintained under constant stirring during inoculation into the fermentation test vessels as the inoculum. Anaerobic conditions were strictly maintained throughout the fermentation setup using a Bactron IV Anaerobic Chamber (Sheldon Manufacturing Inc., Cornelius, OR, USA).

The fermentation medium composition (per liter of distilled water) was as follows: 2.5 g trypticase, 125 μL micromineral solution (containing specified concentrations of calcium chloride, manganese chloride, cobalt chloride, and ferric chloride), 250 mL buffer solution (containing ammonium bicarbonate and sodium bicarbonate), 250 mL macromineral solution (containing sodium phosphate dibasic, potassium phosphate dibasic, and magnesium sulfate), and 1.25 mL resazurin solution (0.1 % *w/v*). A reducing solution containing cysteine hydrochloride, sodium sulfide nonahydrate, and sodium hydroxide was prepared, sterilized, and added to the fermentation medium before adjusting the pH to 7.2. This complete medium was then sterilized at 121 °C for 15 min. Freeze-dried digesta samples (0.8 g), derived from either non-fermented or fermented pomelo juice, were dissolved in 7.2 mL of fermentation medium and incubated in an anaerobic chamber at 37 °C for 1 h. The total volume was then adjusted to 8 mL using fecal slurry, incorporating the fecal inoculum to achieve a final digesta concentration of 10 % w/v. The inoculated mixtures were then incubated on an orbital shaker at 37 °C for 24 h. Following incubation, the colonic fermentation samples were centrifuged at 5000 rpm for 20 min at 4 °C. The supernatants were collected for SCFA analysis, while the pellet fractions were treated with the DNA/RNA shield (Zymo Research, Irvine, CA, USA) for subsequent microbiota analysis. All experiments were conducted under aseptic conditions to prevent contamination.

2.5. Viable lactobacilli and coliform counts

The quantification of viable lactic acid bacteria (LAB) and coliform populations following colonic fermentation was performed using the established standard plate count method (Balmori, Dizon, Barrion, & Elegado, 2019). Samples were plated onto MRS agar for the enumeration of LAB and onto EMB agar for the determination of coliform counts. Incubation occurred under anaerobic conditions at 37 °C for 48 h. The total number of viable cells (colony forming units per milliliter, CFU/mL) in the samples was calculated in accordance with standard microbial enumeration protocols.

2.6. Determination of microbiota composition

The isolation of DNA from colonic fermentation samples was performed using the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research, Irvine, CA, USA). The 16S ribosomal DNA gene was amplified using primers and protocols previously described by Jitvaropas, Mayuramart, Sawaswong, Kaewsapsak, and Payungporn (2022) (Jitvaropas et al., 2022). The resulting amplicons were barcoded *via* a 5-cycle PCR process utilizing barcode primers from the PCR Barcoding Expansion 1–96 (EXP-PBC096) kit (Oxford Nanopore Technologies, Oxford, UK). These amplicons were then purified using the QIAquick® PCR Purification Kit (QIAGEN, Hilden, Germany), and their concentrations were measured with the Qubit dsDNA HS Assay Kit and Qubit 4 fluorometer (Thermo Scientific, Waltham, MA, USA). The barcoded DNA libraries were pooled, subjected to end-repair, adaptor-ligated, and sequenced on the MinION Mk1C platform (Oxford Nanopore Technologies, Oxford, UK) using the R10.4 flow cell. Base calling was executed with the Guppy basecaller software v6.0.7 (Wick, Judd, & Holt, 2019), and sequencing read quality was evaluated using MinIONQC (Abenavoli et al., 2019). Following demultiplexing and adaptor trimming with Porechop v0.2.4, the reads were processed for clustering, refinement, and taxonomic classification *via* NanoCLUST (Rodríguez-Pérez, Ciuffreda, & Flores, 2021) with reference to the RDP database v11.5. The R software (Version 2023.09.1 + 494) was used to visualize bacterial abundance and conduct diversity analyses.

Table 1
Viable *lactobacilli* and coliform counts in non-fermented and fermented pomelo juice after 24 h of colonic fermentation.

Treatments	*Lactobacilli* (log CFU/mL)			Coliform bacteria (log CFU/mL)		
	0 h	24 h	ΔChange	0 h	24 h	ΔChange
Control	4.44 ± 0.04^{aA}	5.44 ± 0.10^{aB}	1.00 ± 0.17^{a}	5.14 ± 0.29^{aA}	6.75 ± 0.03^{aB}	1.61 ± 0.16^{a}
PJ-NF	5.12 ± 0.11^{bA}	6.17 ± 0.06^{abB}	1.05 ± 0.13^{a}	5.29 ± 0.22^{aA}	5.59 ± 0.03^{bA}	0.3 ± 0.01^{b}
PJ-F	5.45 ± 0.15^{bA}	7.15 ± 0.07^{bB}	1.7 ± 0.05^{b}	5.41 ± 0.05^{bA}	5.53 ± 0.06^{bA}	0.12 ± 0.04^{b}

Data are expressed as mean ± SEM, n = 3. Means with different capital letter superscripts (indicating time effects) at the same treatment are significantly different ($p < 0.05$). Means with different lowercase letter superscripts (indicating treatment effects) at the same time points are significantly different ($p < 0.05$). Abbreviations: PJ-NF: non-fermented pomelo juice; PJ-F: fermented pomelo juice.

2.7. Analysis of lactic and short-chain fatty acids (SCFAs)

The lactic acid content was quantified using an enzymatic colorimetric assay, following the manufacturer's protocol provided with the lactic acid test kit (BIOBASE, Shandong, China). The procedure for analyzing SCFAs in colonic fermented samples was adapted from a previous study of Marnpae et al. (2024) (Marnpae et al., 2024). Initially, the samples were centrifuged at 15,000 *g* for 10 min at 4 °C. Then, 0.4 mL of the supernatant was combined with 5 μL of 240 mM 2-ethylbutyric acid, serving as an internal standard at a final concentration of 3 mM. Subsequently, 80 μL of 50 % sulfuric acid was added and the mixture was acidified for 1 h at 4 °C. Following acidification, 0.4 mL of ethyl acetate was introduced and vortexed for 5 min. The mixture was then incubated at 4 °C for 10 min and centrifuged at 13,000 *g* for 5 min. The resulting samples were analyzed using a GC-2010 Plus gas chromatograph equipped with a flame ionization detector (GC-FID) (Shimadzu, Kyoto, Japan). The analysis utilized a DB-FATWAX UI chromatographic capillary column (30 m × 0.25 mm × 0.50 μm; Agilent, USA). The GC analysis conditions included an injection temperature of 250 °C, a split ratio of 1:50, and an injection volume of 0.5 μL. The column temperature was initially set at 105 °C for 3 min, then increased to 170 °C at a rate of 10 °C/min, and finally raised to 240 °C at 70 °C/min, maintained for 2 min. The detector temperature was set at 250 °C. Both internal (2-ethylbutyric acid) and external standards (acetic, propionic, n-butyric, i-butyric, n-valeric, and i-valeric acids) were used for quantitative analysis.

2.8. Statistical analysis

The data were presented as mean ± standard error of the mean (SEM). The study was conducted in 3 replications. For microbiota analysis, the Mann-Whitney *U* test was employed to determine the alpha diversity index for comparative analysis. Principal Coordinate Analysis (PCoA) was utilized, relying on Bray-Curtis dissimilarity distances, to elucidate sample clustering patterns. Beta diversity was evaluated using Permutational ANOVA (PERMANOVA). Bacterial abundances were quantified at the phylum, genus, and species taxonomic tiers and expressed as relative proportions. Log_2 fold changes were determined using the R software (Version 2023.09.1 + 494).

Data on viable microbial counts and SCFAs production underwent One-Way Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test to identify significant differences between groups. Significance was established at a threshold of $p < 0.05$. All statistical computations were performed using SPSS software (version 16.0, SPSS Inc., Chicago, IL, USA).

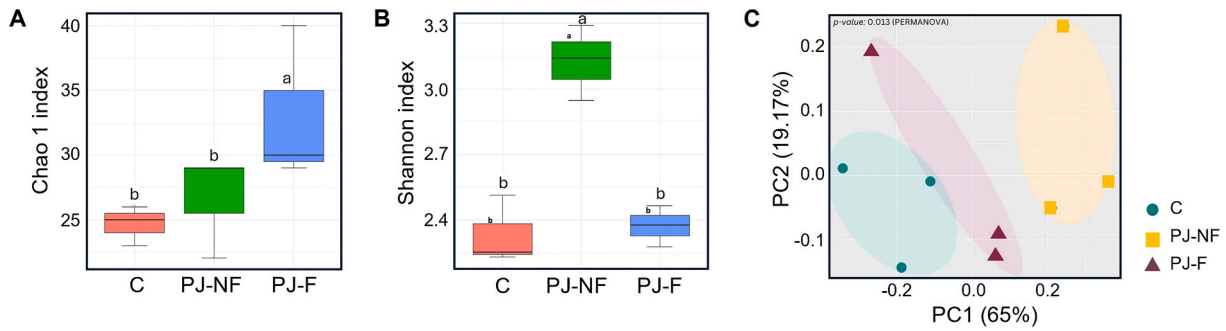


Fig. 1. Diversity indices of gut microbiota at 24 h of colonic fermentation: (A) Species richness (Chao1 index), (B) Species diversity (Shannon's index), (C) Beta-diversity (Bray-Curtis). Abbreviations: C: control; PJ-NF: non-fermented pomelo juice; PJ-F: fermented pomelo juice. Bars with different superscript letters are significantly different ($p < 0.05$, $n = 3$).

3. Results and discussion

3.1. Effect of pomelo juice and its fermentation on viable lactobacilli and coliform counts

At the outset of colonic fermentation (0 h), samples containing pomelo juice exhibited significantly higher viable *lactobacilli* counts compared to the control (Table 1). Following a 24-h colonic fermentation, a significant increase in *Lactobacillus* populations was exclusively observed in samples containing fermented pomelo juice. This increase is directly attributed to the inoculation of *L. paracasei* during the fermentation process. While the prebiotic properties of pomelo juice, such as its fiber and phytochemical content (Makkumrai, Huang, & Xu, 2021) may have contributed to overall microbial growth, the pronounced elevation of *Lactobacillus* is primarily due to the added probiotic strain. The prebiotic components serve as a nutritional source for beneficial gut bacteria, specifically promoting the growth of *Lactobacillus* species due to their unique metabolic pathways. This selective promotion translates to an increased viable count of *lactobacilli* within the gut. The addition of probiotic *L. paracasei* to the pomelo juice during fermentation likely contributed to the significantly higher abundance of *Lactobacillus* compared to the non-fermented control. The presence of *L. paracasei* likely exerted a substantial influence on the gut microbiota composition, promoting the growth of *Lactobacillus* species during the colonic fermentation process.

Following the observed increase in *Lactobacillus* populations (Table 1), both non-fermented and fermented pomelo juice samples displayed a significant reduction in coliform counts compared to the control group. Our findings suggest that pomelo juice may reduce coliform growth during colonic fermentation, likely because of its prebiotic compounds. One mechanism underlying this effect involves the limitation of nutrient availability for coliforms. This limitation likely arises

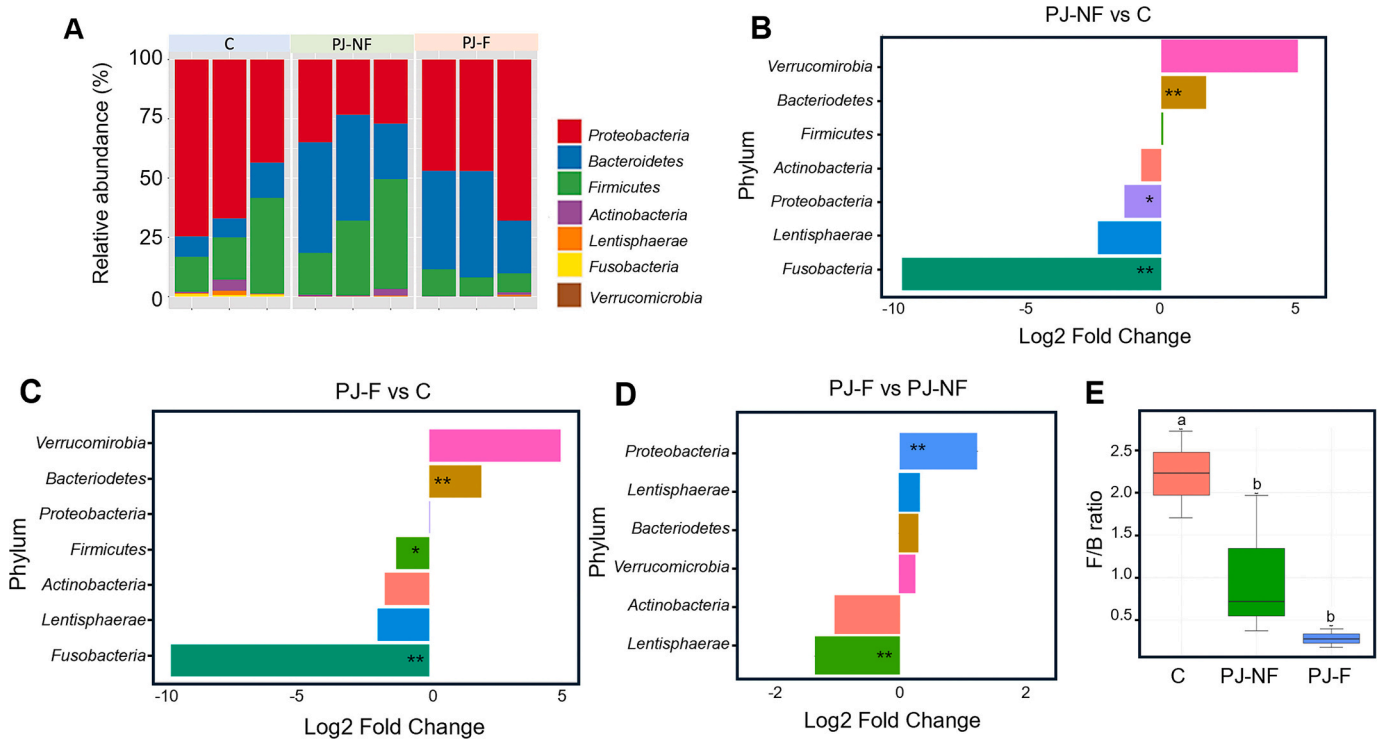


Fig. 2. Microbiota composition at phylum level at 24 h of colonic fermentation: (A) Relative abundance at the phylum level, (B) Log_2-fold change in microbiota composition of PJ-NF compared to control, (C) Log_2-fold change in microbiota composition of PJ-F compared to control, (D) Log_2-fold change in microbiota composition of PJ-F compared to PJ-NF, (E) *Firmicutes/Bacteroidetes* ratio. Means with different letters (E) are significantly different. Significance (log_2-fold change) was indicated by * and ** for $p < 0.05$ and $p < 0.01$, respectively (n = 3). Abbreviations: C: control; PJ-NF: non-fermented pomelo juice; PJ-F: fermented pomelo juice.

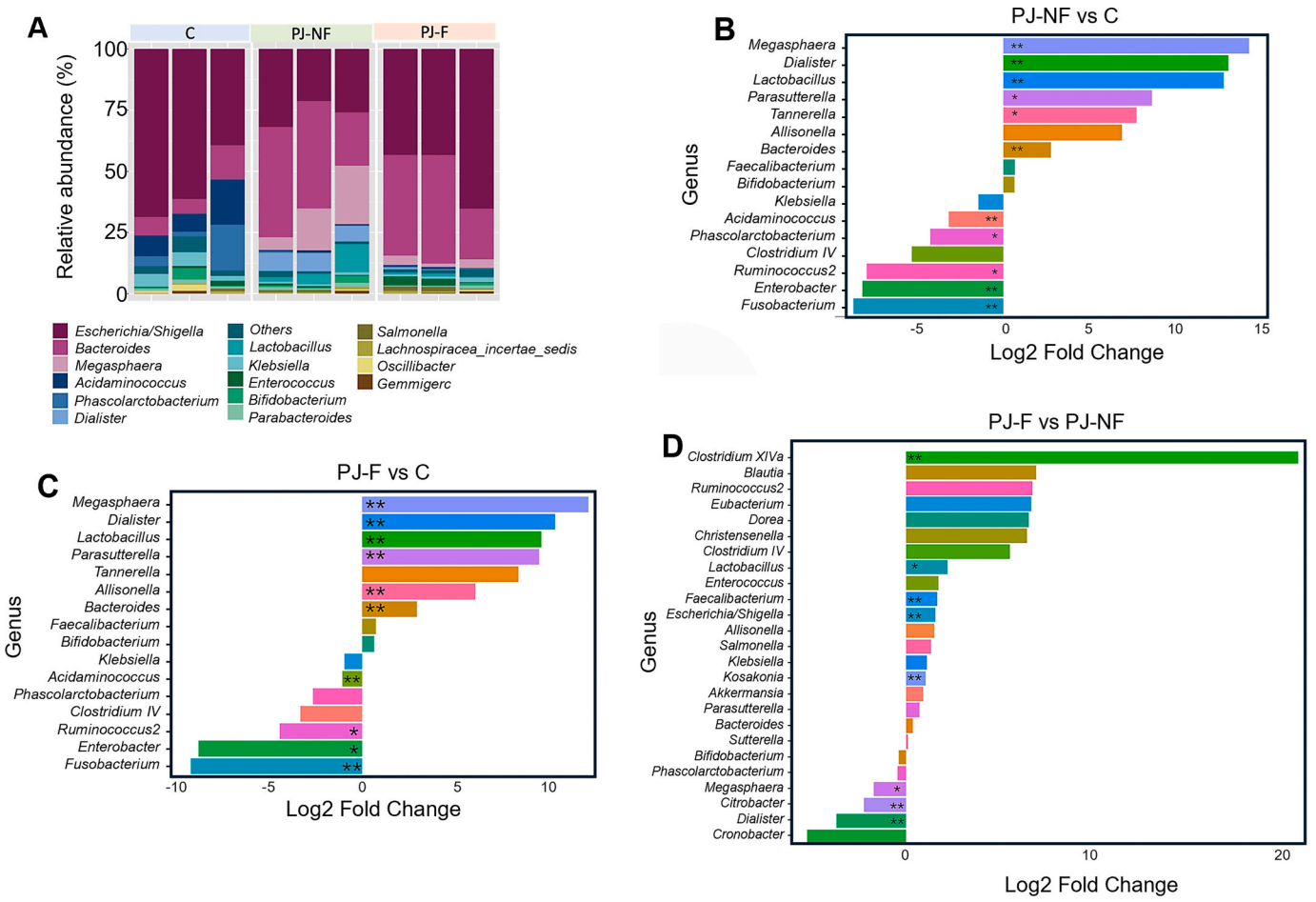


Fig. 3. Microbiota composition at genus level at 24 h of colonic fermentation: (A) Relative abundance at species level, (B) Log_2-fold change in microbiota composition of PJ-NF compared to control, (C) Log_2-fold change in microbiota composition of PJ-F compared to control, (D) Log_2-fold change in microbiota composition of PJ-F compared to PJ-NF. The significance was indicated by * and ** for $p < 0.05$ and $p < 0.01$, respectively ($n = 3$). Abbreviations: C: control; PJ-NF: non-fermented pomelo juice; PJ-F: fermented pomelo juice.

from the selective promotion of beneficial bacteria such as *Lactobacillus* through prebiotic activity (Dempsey & Corr, 2022). Furthermore, fermentation in pomelo juice appears to enhance its ability to suppress coliform growth. This effect likely stems from the increased abundance of *Lactobacillus* species observed in fermented samples. These bacteria engage in competitive exclusion with potentially pathogenic microbes, including coliforms (Azad et al., 2018). This competition centers around essential nutrients and adhesion sites on the intestinal epithelium.

3.2. *Effect of pomelo juice and its fermentation on gut microbiota composition*

3.2.1. *Microbiota diversity index*

Alpha diversity indices were used to assess variations in species richness and evenness within treatment groups. The Chao1 index indicated a significant increase in species richness in the fermented pomelo juice group compared to the control and non-fermented samples (Fig. 1A). This suggests a greater number of species present, including both observed and rare taxa. In contrast, the Shannon index revealed a higher bacterial diversity in the non-fermented juice group compared to both the control and fermented groups (Fig. 1B). The non-fermented group exhibited both high species richness and evenness, whereas the fermented group, despite a broader range of species, displayed lower evenness due to the dominance of certain taxa and the presence of rare species. These findings suggest distinct microbial diversity profiles shaped by the unique composition of each juice with prebiotic components such as dietary fiber and flavonoids potentially influencing community structure (Duque, Monteiro, Adorno, Sakamoto, & Sivieri, 2016).

Beta diversity analysis using the Bray-Curtis dissimilarity metric was employed to assess the bacterial community composition differences between treatments (Fig. 1C). Principal Coordinate Analysis (PCoA) based on these dissimilarities revealed a significant shift in the community structure for both non-fermented and fermented pomelo juice treatments compared to the control. Flavonoids in pomelo juice may have their concentration further increased during fermentation with *L. paracasei*. This suggests that both fermented and non-fermented pomelo juice treatments caused changes in the gut microbiota compared to the control. Interestingly, the non-fermented juice exhibited the greatest dissimilarity in species composition characterized by a broader range of bacterial taxa. This suggests a more unique microbial profile compared to both the control and fermented groups.

3.2.2. *Effect of pomelo juice and its fermentation on relative abundances of bacteria*

In vitro colonic models serve as robust laboratory tools for investigating digestion, addressing ethical concerns and interspecies microbiota variability inherent to *in vivo* studies, while offering methodological precision for systematic and high-throughput analyses. These models enable detailed characterization of digestion processes, microbiota dynamics, and microbial metabolite synthesis. Importantly, they complement *in vivo* studies by providing mechanistic insights and

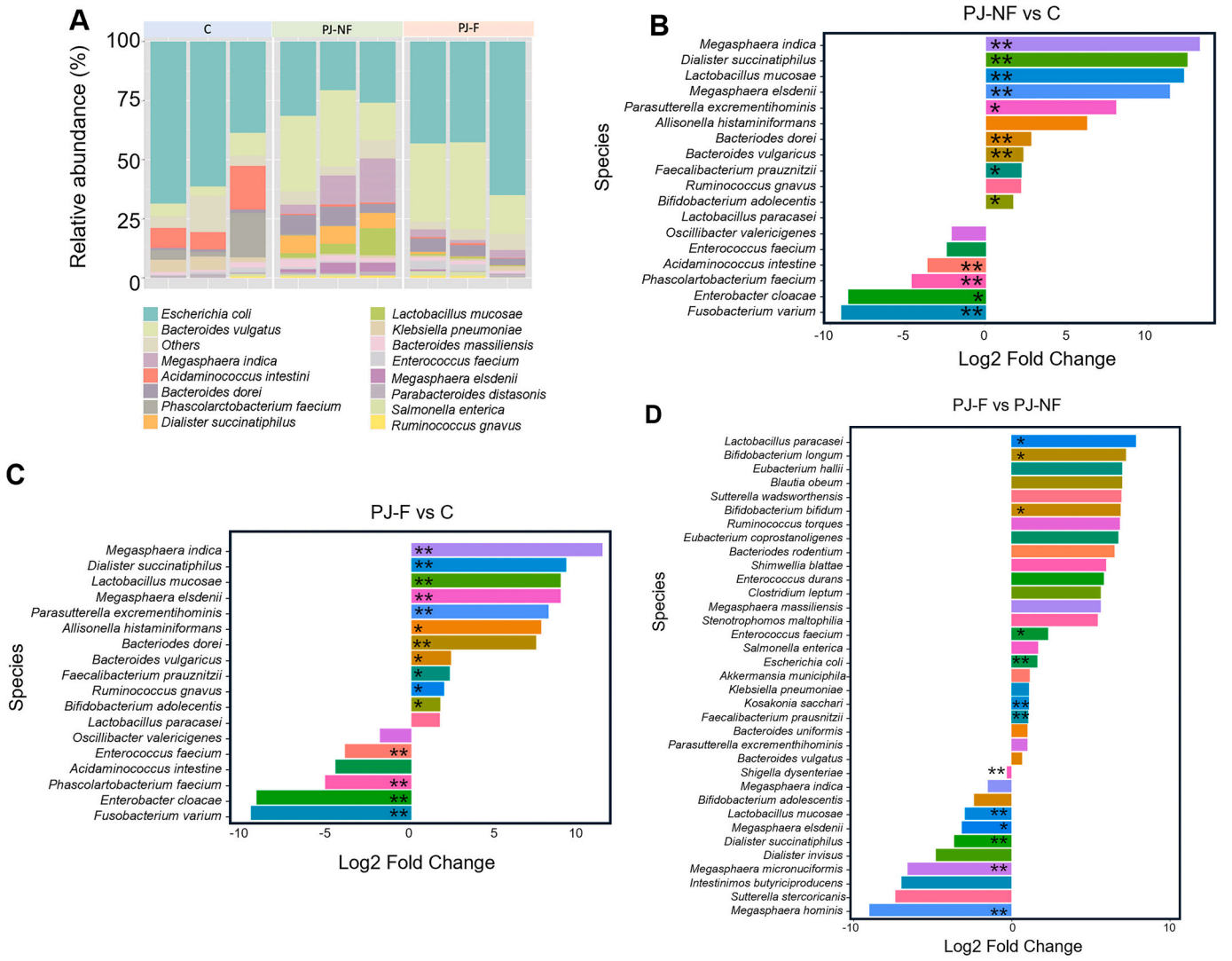


Fig. 4. Microbiota composition at species level at 24 h of colonic fermentation: (A) Relative abundance at species level, (B) Log_2-fold change in microbiota composition of PJ-NF compared to control, (C) Log_2-fold change in microbiota composition of PJ-F compared to control, (D) Log_2-fold change in microbiota composition of PJ-F compared to PJ-NF. The significance was indicated by * and ** for $p < 0.05$ and $p < 0.01$, respectively ($n = 3$). Abbreviations: C: control; PJ-NF: non-fermented pomelo juice; PJ-F: fermented pomelo juice.

informing experimental designs for validation under physiological conditions. Additionally, *in vitro* models are instrumental in evaluating the impact of prebiotics and probiotics on microbiota composition and their contributions to diet-derived metabolite production (Veintimilla-Gozalbo, Asensio-Grau, Calvo-Lerma, Heredia, & Andrés, 2021). Following 24 h of colonic fermentation, analysis revealed *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* as the dominant bacterial phyla (Fig. 2A). Compared to the control group, non-fermented pomelo juice significantly increased the abundance of *Bacteroidetes*, while concurrently reducing *Proteobacteria* and *Fusobacteria* (Fig. 2B). Fermented pomelo juice mirrored the increase in *Bacteroidetes* but exhibited a further significant decrease in *Firmicutes*, alongside reductions in *Proteobacteria* and *Fusobacteria* (Fig. 2C). Notably, fermented juice exerted a significantly stronger inhibitory effect on *Firmicutes* compared to the non-fermented variant. These observed shifts in the gut microbiota, particularly the pronounced rise in beneficial *Bacteroidetes* and decrease in *Firmicutes* resulted in a significant reduction in the F/B ratio for both pomelo juice treatments compared to the control (Fig. 2E).

Several factors such as changes in bacterial growth rates or viability might explain the observed shifts in the F/B ratio. Firstly, the presence of lactic acid, a product of fermentation, may create a less favorable environment for certain *Firmicutes* species. This acidic environment might inhibit their growth and contribute to their decrease in abundance. Additionally, the enrichment of viable *L. paracasei* in the fermented sample likely plays a further role. *Lactobacilli* are known to produce various antimicrobial compounds, including ethanol, fatty acids, hydrogen peroxide, and bacteriocins. These compounds have been demonstrated to target and inhibit specific pathogenic bacteria within the Firmicutes phylum (Tang, Huang, & Yao, 2023). This suggests that *L. paracasei* might further amplify the inhibitory effect on *Firmicutes* observed with lactic acid.

Citrus flavonoids, naturally present in pomelo juice, might further contribute to the observed decrease in *Firmicutes*. Pan et al. (2023) show that specific flavonoids such as naringenin and hesperidin can directly reduce *Firmicutes* bacteria in an *in vitro* model (Pan et al., 2023). Interestingly, our previous work reported an increase in these same flavonoids following fermentation of pomelo juice with *L. paracasei*. These findings suggest that fermentation may potentiate the prebiotic effects of pomelo juice by increasing the bioavailability or concentration of beneficial flavonoids (Balmori et al., 2023). The shift in gut microbiota composition was likely influenced by the combined effects of lactic acid, enriched viable *L. paracasei* and potentially citrus flavonoids in the

fermented pomelo juice. This shift, characterized by a lower F/B ratio, aligns with findings in studies on obesity and gut health. A higher F/B ratio is often associated with gut dysbiosis, a microbial imbalance linked to obesity and overweight. In line with the potential role of prebiotics and probiotics in F/B ratio, research demonstrates that supplementation with a combination of these elements can lead to reductions in this ratio among overweight and obese individuals (Oraphruek et al., 2023). This finding highlights the potential importance of investigating how dietary components potentially including pomelo interact with gut microbiota to influence this marker. *In vivo* research is crucial to confirm the observed impact of fermented pomelo on the F/B ratio in the context of obesity management and to elucidate the underlying mechanisms driving these potential changes.

Genus-level analysis (Fig. 3A) identified *Escherichia/Shigella* (*Proteobacteria*) as the dominant bacteria in both control and fermented pomelo juice, which deviates from typical gut microbiota composition. This finding is consistent with elevated levels of *Proteobacteria* reported in fermented beverages (Wu et al., 2021). The dominance of *Escherichia/Shigella* (*Proteobacteria*) in the *in vitro* gut model may result from the presence of bile salts favoring, which favor the growth of gram-negative bacteria (Ridlon, Harris, Bhowmik, Kang, & Hylemon, 2016). Further analysis of fold changes in microbiota composition revealed that pomelo juices increased the relative abundance of *Megasphaera, Dialister*, *Lactobacillus*, and *Parasutterella* genera compared to the control (Fig. 3B and C). Notably, *Megasphaera* and *Dialister*, native to the colon, contribute to gut health by producing SCFAs through the fermentation of complex carbohydrates (Shetty, Marathe, Lanjekar, Ranade, & Shouche, 2013). Some *Dialister* strains have been shown to reduce inflammation. A study found that eating whole grains increased *Dialister* levels, which was associated with better interleukin-6 levels (Martínez et al., 2013). Similarly, the observed increase in the *Parasutterella* genus aligns with its reported association with reduced low-density lipoprotein (LDL) levels in individuals consuming prebiotic-resistant potato starch (Bush & Alfa, 2020). These findings suggest that the prebiotic components in pomelo juices may have facilitated the growth of beneficial bacterial populations, contributing to gut health and metabolic benefits.

Fermented pomelo juice with *L. paracasei* demonstrated a significantly higher abundance of *Lactobacillus*, *Faecalibacterium, Clostridium, Escherichia/Shigella,* and *Kosakonia* genera, alongside a significantly lower abundance of *Megasphaera*, and *Dialister* genera, compared to the non-fermented juice (Fig. 3D). The elevated levels of *Faecalibacterium* are particularly noteworthy, as this bacterium is widely regarded as a marker of intestinal health. Reduced levels of *Faecalibacterium* are often linked to gastrointestinal diseases or disorders (Maioli et al., 2021). Furthermore, the increased abundance of *Clostridium* cluster XIVa in the fermented juice highlights potential anti-inflammatory benefits. This bacterial cluster is known for its role in maintaining intestinal health, primarily through the production of butyrate and other beneficial metabolites (Guo, Zhang, Ma, & He, 2020). These findings underscore the potential advantages of fermented pomelo juice over its non-fermented counterpart. The lower abundance of *Megasphaera* and *Dialister* genera in the fermented juice supports the hypothesis that the growth of these groups is primarily driven by the prebiotic properties of pomelo juice, rather than the addition of *L. paracasei*.

Colonic fermentation of both fermented and non-fermented pomelo juice significantly reduced the abundance of *Enterobacter* and *Fusobacterium* genera compared to the control (Fig. 3B and C). *Enterobacter (Enterobacteriaceae)* is a commensal gut bacterium, but includes opportunistic pathogens linked to urinary tract infection (UTI) and diarrhea (Baldelli, Scaldaferri, Putignani, & Del Chierico, 2021). Similarly, *Fusobacteria*, while part of the indigenous gut microbiota, is associated with localized and polymicrobial infections. Although its pathogenesis remains incompletely understood, recent studies suggest that *Fusobacterium* virulence factors contribute to chronic inflammation, obesity, and vascular invasion, potentially leading to thrombosis (Vodzak, 2023). The observed reduction in *Enterobacter* and *Fusobacterium* indicates potential health benefits from pomelo juice consumption, particularly for fermented variants.

Species-level analysis (Fig. 4A) revealed significant enrichment of beneficial bacteria in both non-fermented and fermented pomelo juice treatments compared to the control (Fig. 4B and C). Enriched species included *Parasutterella excrementihominis*, *Megasphaera inidica/elsdenii*, *Lactobacillus mucosae*, *Dialister succinatiphilus*, *Bifidobacterium adolescentis*, and *Faecalibacterium prausnitzii*. In contrast, a reduction in potential pathogens such as *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acidaminococcus intestinii*, and *Phascolarctobacterium faecium* was observed. The presence of *L. paracasei*, introduced as a starter culture during fermentation, was exclusively detected in the fermented juice group, confirming that it was not a native member of the initial microbial community. Although research specifically linking pomelo juice and gut microbiota is limited, existing studies suggest that citrus juices can promote the growth of *Lactobacillus* and *Bifidobacterium* species (Duque et al., 2016). These beneficial bacteria support gut health through mechanisms, such as SCFA production and antimicrobial activity *via* bacteriocin production, which suppresses pathogenic populations. The observed increase in *B. adolescentis* is particularly notable, as certain strains of this species have been shown to alleviate constipation, and some even produce gamma-aminobutyric acid (GABA), a neurotransmitter potentially influencing anxiety and depression (Duranti et al., 2020). This suggests additional benefits for host well-being, particularly for individuals with gastrointestinal disorders.

Similarly, the enrichment of *F. prausnitzii* may provide anti-inflammatory benefits. This bacterium produces butyrate, a SCFA activating G protein-coupled receptors (GPRs) that regulate gut changes linked to obesity and diabetes (Maioli et al., 2021). Conversely, a decrease in *F. prausnitzii* has been linked to chronic inflammation, particularly in obese individuals. The observed decrease in potential pathogens, including *E. faecium*, *K. pneumoniae*, *A. intestinii*, and *P. faecium*, is often associated with gut dysbiosis and inflammation. These pathogens can further promote their growth and virulence within the gut (Khan et al., 2021). Therefore, the shift in gut microbiota composition towards beneficial bacteria and away from potential pathogens in response to pomelo juice treatments suggests potential benefits for gut health.

L. paracasei fermentation significantly altered the bacterial profile of pomelo juice compared to the non-fermented juice (Fig. 4D), enriching beneficial genera such as *Bifidobacterium, Lactobacillus,* and *F. prausnitzii,* while reducing the abundance *Megasphaera and Dialister*. Notably, Notably, the fermented pomelo juice showed an increase in established probiotic species like *Bifidobacterium longum* and *Bifidobacterium bifidum*

Table 2
Lactic acid and short-chain fatty acid (SCFA) concentrations in non-fermented and fermented pomelo juice after 24 h of colonic fermentation.

Treatments	Lactic acid (mM)	SCFAs (mM)						
		Acetic acid	Propionic acid	n-Butyric acid	Iso-butyric acid	Iso-valeric acid	Valeric acid	Total SCFAs
Control	17.62 ± 1.80^{a}	50.73 ± 3.08^{a}	0.23 ± 0.01^{a}	0.71 ± 0.08^{a}	0.47 ± 0.03^{a}	0.08 ± 0.00^{a}	0.03 ± 0.00^{a}	69.87 ± 0.13^{a}
PJ-NF	35.45 ± 1.68^{b}	58.81 ± 0.86^{a}	21.5 ± 02.94^{b}	0.93 ± 0.13^{a}	0.12 ± 0.02^{b}	0.02 ± 0.00^{b}	0.03 ± 0.01^{a}	116.86 ± 1.05^{b}
PJ-F	79.97 ± 0.89^{c}	93.33 ± 2.70^{b}	13.39 ± 0.43^{c}	1.22 ± 0.02^{b}	0.12 ± 0.00^{b}	0.01 ± 0.00^{b}	0.04 ± 0.00^{a}	187.96 ± 0.96^{c}

Data are expressed as mean ± SEM, n = 3. Means with different lowercase letter superscripts (indicating treatment effects) in the same column are significantly different ($p < 0.05$). Abbreviations: PJ-NF: non-fermented pomelo juice; PJ-F: fermented pomelo Juice.

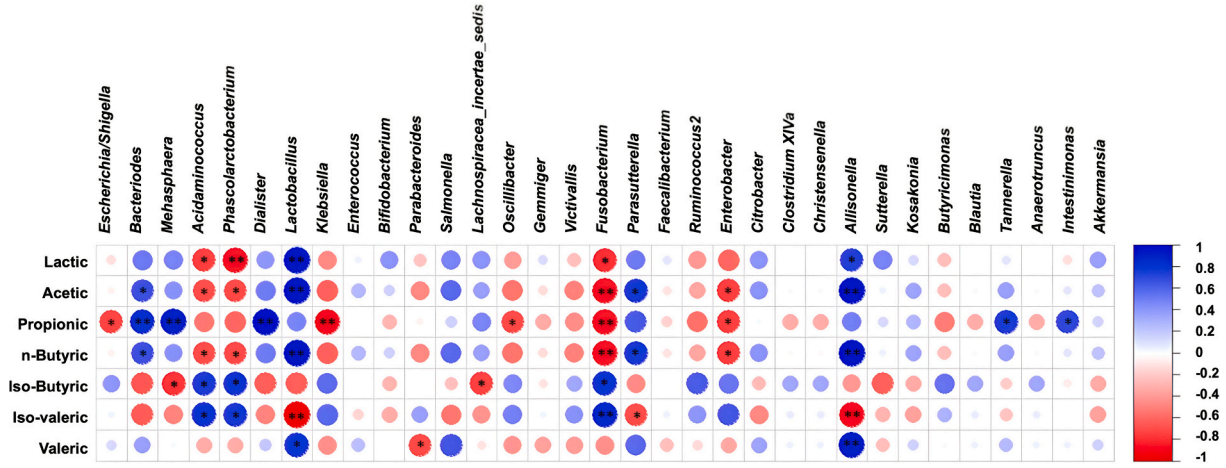


Fig. 5. Correlation analysis between bacterial groups, lactic acid, and short-chain fatty acids (SCFAs). The color intensity indicates the strength of the correlation (positive or negative). Asterisks indicate statistically significant correlations according to Spearman's correlation (*, $p < 0.05$), (**, $p < 0.01$), $n = 3$.

(Chen, Chen, & Ho, 2021; Wong & Odamaki, 2019), which are known for promoting gut health and combating infections. Additionally, *F. prausnitzii,* a butyrate-producing bacterium, was more abundant in the fermented juice. Butyrate has anti-inflammatory properties and strengthens the gut barrier, contributing to a healthy gut ecosystem (Silva, Bernardi, & Frozza, 2020). Conversely, lower levels o*f F. prausnitzii* are associated with various diseases, including inflammatory bowel disease (IBD) and colorectal cancer (CRC) (Maioli et al., 2021). The observed decrease in *Megasphaera* and *Dialister* Dialister in fermented juice suggests that their growth may be driven primarily by the prebiotic properties of pomelo juice itself, rather than the addition of *L. paracasei.* These findings highlight the potential of fermented pomelo juice to promote gut health by fostering a microbiota composition enriched in beneficial bacteria.

3.3. Effect of pomelo juice and its fermentation on lactic and SCFAs production

After 24 h of colonic fermentation, both non-fermented and fermented pomelo juice treatments exhibited significant increases in lactic acid (2.0-fold and 4.5-fold, respectively) and total SCFAs (1.7-fold and 2.7-fold, respectively) compared to the control (Table 2). This rise in SCFAs was mainly due to increased levels of acetic and propionic acids with a smaller but significant increase in butyric acid. SCFAs are essential metabolites produced by gut bacteria, and their levels were significantly higher following both pomelo juice treatments. Previous research indicates that probiotic and prebiotic supplementation can enhance colonic SCFA production, with effects varying depending on the specific strains and prebiotic types (Azad et al., 2018). In this study, metabolism by *Lactobacillus* and *Bifidobacterium*, which were promoted by the pomelo juice treatments, produced acetic and lactic acids from pomelo juice prebiotics. These fermentation products can serve as a carbon source for butyrate-producing bacteria, particularly *Faecalibacterium* spp., which may explain the observed increase in butyric acid after fermentation (Martín et al., 2023). Additionally, the prebiotic components and carbon source inherent in pomelo juice may have directly contributed to SCFA production by colonic microbiota (da Silva et al., 2023). Elevated SCFA levels, particularly butyrate, provide potential benefits for gut health. SCFAs serve as a primary energy source for colonic epithelial cells, with butyrate playing a crucial role. It helps maintain gut barrier integrity, prevents the translocation of harmful substances, and exerts anti-inflammatory effects (Silva et al., 2020).

3.4. Correlation analysis of bacterial groups, lactic acid, and SCFAs

Shifts in gut microbiota composition can influence nutrient availability and metabolite production. Our correlation analysis revealed strong positive associations between lactic acid and *Lactobacillus* abundance, while acetic acid correlated positively with both *Lactobacillus* and other bacteria such as *Bacteroides*, *Parasutterella*, and *Allisonella* (Fig. 5). *Lactobacillus*, known for metabolizing sugars present in pomelo juice (Dempsey & Corr, 2022), produces lactic acid as its primary metabolite. Propionic acid, another SCFA commonly found in the gut, showed positive correlations with bacteria known to utilize dietary fibers present in pomelo juice such as *Bacteroides*, *Megasphaera*, and *Dialister*. This aligns with literature indicating that *Bacteroides* spp. and certain *Megasphaera* species, such as *M. elsdenii*, generate propionic acid *via* acrylate and succinate pathways (Koh et al., 2016). Furthermore, flavanones in pomelo juice, including hesperidin and naringenin, are metabolized by gut bacteria such as *Bacteroides* and *Clostridium* into propionic and acetic acids (Pereira-Caro et al., 2015). Interestingly, butyric acid was positively correlated with *Lactobacillus* and *Allisonella*, being primarily produced by *Firmicutes* bacteria like *Faecalibacterium prausnitzii*, the main butyrate producer (Maioli et al., 2021). Conversely, potentially pathogenic groups like *Klebsiella*, *Fusobacterium*, *Enterobacter*, and *Phascolarctobacterium* exhibited negative correlations with SCFA production. This suggests inhibiting these bacteria might be beneficial for optimizing gut SCFA levels.

4. Conclusion

In vitro colonic fermentation demonstrated that both non-fermented and fermented pomelo juice possess prebiotic properties, as evidenced by the modulation of gut microbiota towards a healthier profile and increased SCFA production. The addition of *L. paracasei* during fermentation significantly amplified these beneficial effects, suggesting a synergistic interaction between the probiotic and prebiotic components of the juice. These findings highlight the potential of pomelo juice, especially in its fermented form, as a functional food for promoting gut health. However, further *in vivo* studies are warranted to elucidate the underlying mechanisms and confirm the observed benefits in human subjects.

CRediT authorship contribution statement

Vernabelle Balmori: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Marisa Marnpae:** Investigation. **Kritmongkhon Kamonsuwan:**

Investigation. **Charoonsri Chusak:** Investigation, Conceptualization. **Uarna Nungarlee:** Investigation. **Pavaret Sivapornnukul:** Investigation, Formal analysis. **Prangwalai Chanchaem:** Investigation, Formal analysis. **Sunchai Payungporn:** Supervision. **Suvimol Charoensiddhi:** Supervision, Methodology. **Tanyawan Suantawee:** Investigation, Conceptualization. **Thavaree Thilavech:** Writing – review & editing, Investigation, Formal analysis. **Sirichai Adisakwattana:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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.

Acknowledgement

This study received support from the 90th Anniversary of Chulalongkorn University Scholarship, Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University, and the National Research Council of Thailand (NRCT) under Grant Number N42A640325.

Data availability

Data will be made available on request.

References

Abenavoli, L., Scarpellini, E., Colica, C., Boccuto, L., Salehi, B., Sharifi-Rad, J., & Capasso, R. (2019). Gut microbiota and obesity: A role for probiotics. *Nutrients, 11*(11). https://doi.org/10.3390/nu11112690

Azad, M. A. K., Sarker, M., Li, T., & Yin, J. (2018). Probiotic species in the modulation of gut microbiota: An overview. *BioMed Research International, 2018*, 9478630. https://doi.org/10.1155/2018/9478630

Baldelli, V., Scaldaferri, F., Putignani, L., & Del Chierico, F. (2021). The role of Enterobacteriaceae in gut microbiota dysbiosis in inflammatory bowel diseases. *Microorganisms, 9*(4). https://doi.org/10.3390/microorganisms9040697

Balmori, V., Dizon, E., Barrion, A., & Elegado, F. (2019). Effect of adjunct inoculation of lactobacillus plantarum BS and pediococcus acidilactici 3G3 on the microbiological, physicochemical and sensory properties of fermented carabeef (Pindang damulag). *Food Research, 3*(1), 70–78.

Balmori, V., Marnpae, M., Chusak, C., Kamonsuwan, K., Katelakha, K., Charoensiddhi, S., & Adisakwattana, S. (2023). Enhancing phytochemical compounds, functional properties, and volatile flavor profiles of pomelo (Citrus grandis (L.) Osbeck) juices from different cultivars through fermentation with *Lacticaseibacillus paracasei*. *Foods, 12*.

Bush, J. R., & Alfa, M. J. (2020). Increasing levels of Parasutterella in the gut microbiome correlate with improving low-density lipoprotein levels in healthy adults consuming resistant potato starch during a randomised trial. *BMC Nutrition, 6*(1), 72. https://doi.org/10.1186/s40795-020-00398-9

Charoensiddhi, S., Conlon, M. A., Vuaran, M. S., Franco, C. M. M., & Zhang, W. (2016). Impact of extraction processes on prebiotic potential of the brown seaweed Ecklonia radiata by *in vitro* human gut bacteria fermentation. *Journal of Functional Foods, 24*, 221–230. https://doi.org/10.1016/j.jff.2016.04.016

Chen, J., Chen, X., & Ho, C. L. (2021). Recent development of probiotic Bifidobacteria for treating human diseases. *Frontiers in Bioengineering and Biotechnology, 9*, Article 770248. https://doi.org/10.3389/fbioe.2021.770248

Dempsey, E., & Corr, S. C. (2022). Lactobacillus spp. for gastrointestinal health: Current and future perspectives. *Frontiers in Immunology, 13*, Article 840245. https://doi.org/10.3389/fimmu.2022.840245

Duque, A. L. R. F., Monteiro, M., Adorno, M. A. T., Sakamoto, I. K., & Sivieri, K. (2016). An exploratory study on the influence of orange juice on gut microbiota using a dynamic colonic model. *Food Research International, 84*, 160–169. https://doi.org/10.1016/j.foodres.2016.03.028

Duranti, S., Ruiz, L., Lugli, G. A., Tames, H., Milani, C., Mancabelli, L., & Turroni, F. (2020). Bifidobacterium adolescentis as a key member of the human gut microbiota in the production of GABA. *Scientific Reports, 10*(1), 14112. https://doi.org/10.1038/s41598-020-70986-z

Guo, P., Zhang, K., Ma, X., & He, P. (2020). Clostridium species as probiotics: Potentials and challenges. *Journal of Animal Science and Biotechnology, 11*(1), 24. https://doi.org/10.1186/s40104-019-0402-1

Jitvaropas, R., Mayuramart, O., Sawaswong, V., Kaewsapsak, P., & Payungporn, S. (2022). Classification of salivary bacteriome in asymptomatic COVID-19 cases based on long-read nanopore sequencing. *Experimental Biology and Medicine (Maywood, N. J.), 247*(21), 1937–1946. https://doi.org/10.1177/15353702221118091

Khan, I., Bai, Y., Zha, L., Ullah, N., Ullah, H., Shah, S. R. H., & Zhang, C. (2021). Mechanism of the gut microbiota colonization resistance and enteric pathogen infection. *Frontiers in Cellular and Infection Microbiology, 11*, Article 716299. https://doi.org/10.3389/fcimb.2021.716299

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell, 165*(6), 1332–1345. https://doi.org/10.1016/j.cell.2016.05.041

Ma, G., & Chen, Y. (2020). Polyphenol supplementation benefits human health via gut microbiota: A systematic review via meta-analysis. *Journal of Functional Foods, 66*, Article 103829. https://doi.org/10.1016/j.jff.2020.103829

Maioli, T. U., Borras-Nogues, E., Torres, L., Barbosa, S. C., Martins, V. D., Langella, P., & Chatel, J. M. (2021). Possible benefits of Faecalibacterium prausnitzii for obesity-associated gut disorders. *Frontiers in Pharmacology, 12*, Article 740636. https://doi.org/10.3389/fphar.2021.740636

Makkumrai, W., Huang, Y., & Xu, Q. (2021). Comparison of pomelo (Citrus maxima) grown in China and Thailand. *Frontiers of Agricultural Science and Engineering, 8*, 335–352. https://doi.org/10.15302/J-FASE-2021391

Mäkynen, K., Jitsaardkul, S., Tachasamran, P., Sakai, N., Puranachoti, S., Nirojsinlapachai, N., & Adisakwattana, S. (2013). Cultivar variations in antioxidant and antihyperlipidemic properties of pomelo pulp (Citrus grandis [L.] Osbeck) in Thailand. *Food Chemistry, 139*(1–4), 735–743. https://doi.org/10.1016/j.foodchem.2013.02.017

Marnpae, M., Balmori, V., Kamonsuwan, K., Nungarlee, U., Charoensiddhi, S., Thilavech, T., Adisakwattana, & S.. (2024). Modulation of the gut microbiota and short-chain fatty acid production by gac fruit juice and its fermentation in in vitro colonic fermentation. *Food & Function, 15*(7), 3640–3652. https://doi.org/10.1039/D3FO04318E

Martín, R., Rios-Covian, D., Huillet, E., Auger, S., Khazaal, S., Bermúdez-Humarán, L. G., & Langella, P. (2023). Faecalibacterium: A bacterial genus with promising human health applications. *FEMS Microbiology Reviews, 47*(4). https://doi.org/10.1093/femsre/fuad039

Martínez, I., Lattimer, J. M., Hubach, K. L., Case, J. A., Yang, J., Weber, C. G., & Walter, J. (2013). Gut microbiome composition is linked to whole grain-induced immunological improvements. *The ISME Journal, 7*(2), 269–280. https://doi.org/10.1038/ismej.2012.104

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., & Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food - an international consensus. *Food & Function, 5*(6), 1113–1124. https://doi.org/10.1039/c3fo60702j

Oraphruek, P., Chusak, C., Ngamukote, S., Sawaswong, V., Chanchaem, P., Payungporn, S., & Adisakwattana, S. (2023). Effect of a multispecies synbiotic supplementation on body composition, antioxidant status, and gut microbiomes in overweight and obese subjects: A randomized, double-blind, placebo-controlled study. *Nutrients, 15*(8), 1863. https://doi.org/10.3390/nu15081863

Pan, L., Ye, H., Pi, X., Liu, W., Wang, Z., Zhang, Y., & Zheng, J. (2023). Effects of several flavonoids on human gut microbiota and its metabolism by *in vitro* simulated fermentation. *Frontiers in Microbiology, 14*, 1092729. https://doi.org/10.3389/fmicb.2023.1092729

Pereira-Caro, G., Borges, G., Ky, I., Ribas, A., Calani, L., Del Rio, D., & Crozier, A. (2015). In vitro colonic catabolism of orange juice (poly)phenols. *Molecular Nutrition & Food Research, 59*(3), 465–475. https://doi.org/10.1002/mnfr.201400779

Ridlon, J. M., Harris, S. C., Bhowmik, S., Kang, D. J., & Hylemon, P. B. (2016). Consequences of bile salt biotransformations by intestinal bacteria. *Gut Microbes, 7*(1), 22–39. https://doi.org/10.1080/19490976.2015.1127483

Rizzardini, G., Eskesen, D., Calder, P. C., Capetti, A., Jespersen, L., & Clerici, M. (2012). Evaluation of the immune benefits of two probiotic strains Bifidobacterium animalis ssp. lactis, BB-12® and *Lactobacillus paracasei ssp. paracasei,* L. casei 431® in an influenza vaccination model: A randomised, double-blind, placebo-controlled study. *The British Journal of Nutrition, 107*(6), 876–884. https://doi.org/10.1017/s000711451100420x

Rodríguez-Pérez, H., Ciuffreda, L., & Flores, C. (2021). NanoCLUST: A species-level analysis of 16S rRNA nanopore sequencing data. *Bioinformatics, 37*(11), 1600–1601. https://doi.org/10.1093/bioinformatics/btaa900

Sánchez, B., Delgado, S., Blanco-Míguez, A., Lourenço, A., Gueimonde, M., & Margolles, A. (2017). Probiotics, gut microbiota, and their influence on host health and disease. *Molecular Nutrition & Food Research, 61*(1). https://doi.org/10.1002/mnfr.201600240

Shetty, S. A., Marathe, N. P., Lanjekar, V., Ranade, D., & Shouche, Y. S. (2013). Comparative genome analysis of Megasphaera sp. reveals niche specialization and its potential role in the human gut. *PLoS One, 8*(11), Article e79353. https://doi.org/10.1371/journal.pone.0079353

da Silva, R. M., Santos, B. N., da Silva Oliveira, F. A., Filho, E. G. A., Fonteles, T. V., Campelo, P. H., & Rodrigues, S. (2023). Synbiotic Sapota-do-Solimões (Quararibea cordata Vischer) juice improves gut microbiota and short-chain fatty acid production in an *in vitro* model. *Probiotics and Antimicrobial Proteins.*. https://doi.org/10.1007/s12602-023-10178-z

Silva, Y. P., Bernardi, A., & Frozza, R. L. (2020). The role of short-chain fatty acids from gut microbiota in gut-brain communication. *Front Endocrinol (Lausanne), 11*, 25. https://doi.org/10.3389/fendo.2020.00025

Tang, H., Huang, W., & Yao, Y. F. (2023). The metabolites of lactic acid bacteria: Classification, biosynthesis and modulation of gut microbiota. *Microbial cell (Graz, Austria), 10*(3), 49–62. https://doi.org/10.15698/mic2023.03.792

Veintimilla-Gozalbo, E., Asensio-Grau, A., Calvo-Lerma, J., Heredia, A., & Andrés, A. (2021). *In vitro* simulation of human colonic fermentation: A practical approach towards models' design and analytical tools. *Applied Sciences, 11*(17), 8135. https://doi.org/10.3390/app11178135

Vlieger, A. M., Robroch, A., van Buuren, S., Kiers, J., Rijkers, G., Benninga, M. A., & te Biesebeke, R. (2009). Tolerance and safety of *Lactobacillus paracasei* ssp. paracasei in combination with Bifidobacterium animalis ssp. lactis in a prebiotic-containing infant formula: A randomised controlled trial. *The British Journal of Nutrition, 102*(6), 869–875. https://doi.org/10.1017/s0007114509289069

Vodzak, J. (2023). Fusobacterium Species. In S. S. Long (Ed.), *Principles and practice of pediatric infectious diseases* (6th ed., pp. 1033–1036.e1032). Philadelphia: Elsevier.

Wang, B., Yao, M., Lv, L., Ling, Z., & Li, L. (2017). The human microbiota in health and disease. *Engineering, 3*(1), 71–82. https://doi.org/10.1016/J.ENG.2017.01.008

Wick, R. R., Judd, L. M., & Holt, K. E. (2019). Performance of neural network basecalling tools for Oxford Nanopore sequencing. *Genome Biology, 20*(1), 129. https://doi.org/10.1186/s13059-019-1727-y

Wong, C. B., Odamaki, T., & Xiao, J.-Z. (2019). Beneficial effects of Bifidobacterium longum subsp. longum BB536 on human health: Modulation of gut microbiome as the principal action. *Journal of Functional Foods, 54*, 506–519. https://doi.org/10.1016/j.jff.2019.02.002

Wu, T., Chu, X., Cheng, Y., Tang, S., Zogona, D., Pan, S., & Xu, X. (2021). Modulation of gut microbiota by Lactobacillus casei fermented raspberry juice *in vitro* and in vivo. *Foods, 10*(12). https://doi.org/10.3390/foods10123055