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Selective Microbial Modulation and Metabolic Reprogramming in Kefir Supplemented with Torch Ginger (*Etlingera elatior*) Tea

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Kefir is a functional fermented dairy product whose health-promoting properties can be enhanced through fortification with plant-derived bioactive compounds. *Etlingera elatior* (torch ginger) represents a promising botanical source due to its rich phytochemical content. This study aimed to evaluate the effects of torch ginger tea (TGT) supplementation on the physicochemical, microbial, and metabolic characteristics of kefir. A comparative analysis between control kefir (CK) and TGT-supplemented kefir was conducted using an integrated approach, including physicochemical and sensory evaluations, as well as metagenomic and untargeted LC-MS metabolomic analyses. The results showed that TGT significantly increased moisture and ash content while reducing total soluble solids and color intensity. The microbial community remained stable, with no significant differences observed in alpha and beta diversity. However, TGT selectively modulated the microbiota by increasing the abundance of beneficial lactic acid bacteria and suppressing non-lactic acid bacteria. Metabolomic analysis further revealed a clear separation between treatments and identified key differential metabolites, including fatty acids and caffeine as a plant-derived marker. These findings highlight plant–microbe interactions as a key mechanism influencing fermentation dynamics and metabolic outcomes, supporting the potential of TGT as a functional ingredient for the development of innovative kefir-based products.

1. Introduction

Kefir is a functional fermented dairy product widely recognized for its high nutritional value and distinctive sensory characteristics (Alraddadi, Ross, & Powell, 2023; Guzel-Seydim et al., 2011). It contains a complex microbial consortium composed of lactic acid bacteria (LAB), yeasts, and occasionally acetic acid bacteria embedded within a polysaccharide matrix known as kefiran (Marques Soutelino et al., 2025; Prado et al., 2015). During fermentation, these microorganisms produce a variety of bioactive metabolites that contribute to multiple health benefits, including improved gut microbiota balance, immune modulation, and metabolic regulation (Ahmed et al., 2013; Kim et al., 2019; Rosa et al., 2017). In response to the growing consumer demand for health-oriented foods, there has been a global trend toward the development of functional fermented dairy products higher abundance with plant-derived bioactive compounds (Granato et al., 2018; Kandyliari et al., 2023). Among these, torch ginger (*Etlingera elatior*), a native Indonesian plant, has attracted considerable attention due to its rich content of phenolic compounds, flavonoids, and aromatic constituents with strong antioxidant and antimicrobial activities (Ismail & Ridzuan, 2023; Naufalin, Setyawardani, & Gunawan, 2024b).

Previous studies have demonstrated that the incorporation of plant-based ingredients into kefir can significantly influence its physicochemical properties, microbial composition, and sensory characteristics (Ramos et al., 2017; Uruc et al., 2022). Bioactive compounds derived from plants, particularly phenolics, flavonoids, and volatile compounds, are known to interact with fermentation processes and modulate microbial growth by either stimulating beneficial LAB or inhibiting undesirable microorganisms (Amini, Mousavi, & Mousavi, 2024; Chan et al., 2018; Mutmainah et al., 2024). Given that kefir represents a highly complex microbial ecosystem with dynamic interspecies interactions, understanding its microbial structure using metagenomic approaches has become increasingly important In parallel, metabolomic profiling offers valuable insights into the chemical transformations occurring during fermentation and

enables the identification of bioactive metabolites formed throughout the process (Azi et al., 2021; Cui et al., 2023; Ma et al., 2024). Therefore, integrating microbial and metabolic analyses is essential to comprehensively understand kefir fermentation and product quality.

Despite the promising potential of plant-enriched kefir, most existing studies remain limited to conventional analyses, including physicochemical evaluation, culture-based microbiological methods, and basic sensory assessment (Marques Soutelino et al., 2025; Ziarno et al., 2021). In particular, the application of torch ginger in the form of tea in kefir systems has been scarcely explored. Furthermore, limited studies have investigated how such herbal additions influence microbial community dynamics at a deeper level using next-generation sequencing approaches. A significant knowledge gap also exists in linking microbial shifts with metabolite profile changes resulting from interactions with plant-derived bioactive compounds (Azi et al., 2021; González-Orozco et al., 2023). Consequently, studies integrating physicochemical, microbiological, metagenomic, and metabolomic analyses within a single kefir system are still scarce, leaving the underlying mechanisms of plant–microbe interactions insufficiently understood.

The concentration of torch ginger tea used in this study was optimized at 5% (v/v) based on preliminary experiments and previous findings reported by Naufalin, Rizki, & Arsil (2024a) and Naufalin et al. (2026), which demonstrated that torch ginger tea is a rich source of phenolic compounds and natural antioxidants with established functional benefits. This supplementation level was strategically selected to provide sufficient bioactive compounds for functional enhancement while simultaneously maintaining acceptable sensory characteristics and normal kefir fermentation kinetics. Preliminary dose-response observations indicated that higher concentrations (>5% v/v) tended to produce excessively strong herbal flavors that could negatively impact consumer acceptance, whereas lower concentrations (<5% v/v) were less effective in modulating the microbial community and enhancing the functional properties of the fermented product. Consequently, a supplementation level of 5% (v/v) was determined to be optimal for comprehensively evaluating the physicochemical, sensory, microbial, and metabolomic characteristics of torch ginger-enriched kefir.

Therefore, this study aimed to evaluate the impact of torch ginger (*Etlingera elatior*) tea on kefir by comparing control kefir with kefir supplemented with torch ginger tea. A comprehensive set of analyses was performed, including physicochemical properties (pH, viscosity, syneresis, total titratable acidity, color, and proximate composition), total lactic acid bacteria, and sensory characteristics. In addition, metagenomic analysis was employed to characterize microbial community composition, while metabolomic profiling using LC-HRMS was conducted to identify treatment-related metabolite variations (Cui et al., 2023; Ma et al., 2024).This integrative approach provides deeper insights into the interactions between plant-derived bioactive compounds and kefir microbiota and their effects on product quality. The findings of this study are expected to contribute to the scientific understanding of fermented dairy systems and support the development of innovative kefir-based functional beverages utilizing local Indonesian plant resources (Naufalin et al., 2024b).

2. Materials and Methods

2.1. Materials

Fresh cow milk was obtained from the Experimental Farm of the Faculty of Animal Husbandry, Universitas Jenderal Soedirman, Purwokerto, Central Java, Indonesia. Kefir grains containing lactic acid bacteria (*Lactobacillus kefiri* and *Lactococcus lactis*) and yeasts (*Saccharomyces kefir*) were supplied by the Department of Food Technology, Universitas Jenderal Soedirman.

Kecombrang (*Etlingera elatior*) flowers were sourced from a local producer in Banyumas, Central Java, Indonesia, and used as the main plant-based ingredient in this study. The flowers were processed into kecombrang tea following the method described by Naufalin et al. (2024b) with slight modifications. Skim milk powder (Indoprima) and sucrose were purchased from Merck (Germany). All chemicals used were of analytical grade. De Man, Rogosa, and Sharpe (MRS) agar was used for lactic acid bacteria enumeration.

2.2. Preparation of Torch Ginger Tea

Torch ginger (*Etlingera elatior*) flowers and stems were processed into tea infusion following the method of Naufalin et al. (2024) with modifications. Fresh flowers and stems were thoroughly washed under running water to remove impurities, and any damaged parts were discarded. The cleaned flowers were reduced to approximately 2 mm particle size using a grinder, while stems were cut into segments of approximately 1 cm in length and further sliced to a thickness of approximately 3 mm using a knife. The prepared materials were spread evenly on a tray and dried in a cabinet dryer at 60 °C for 5 hours. After drying, 1 g of dried torch ginger simplisia was weighed and infused

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with 200 mL of hot water (approximately 90–100 °C) for 10 minutes to extract bioactive compounds. The resulting extract was filtered using sterile filter paper to remove solid particles, yielding a clear torch ginger tea infusion that was used for kefir supplementation.

2.3. Experimental Design

The study was arranged using a completely randomized design with one factor, namely the addition of torch ginger (*Etlingera elatior*) tea (TGT), at two levels: control kefir (CK) and kefir supplemented with torch ginger tea (TGT). Each treatment was produced in three independent batches (N=3).

2.4. Kefir Production Process

Fresh cow milk was pasteurized at 71–72 °C for 15 seconds and cooled to 30 °C. For the control kefir (CK) treatment, pasteurized milk was directly inoculated with kefir grains. For the torch ginger tea supplemented kefir (TGT) treatment, the cooled milk was supplemented with torch ginger tea infusion at a concentration of 5% v/v prior to inoculation. Kefir grains were then added to both treatments at a concentration of 5% (w/v). The mixtures were incubated at 30 °C for 24 hours under static conditions to allow fermentation to proceed. After fermentation, kefir grains were removed by filtration using sterile sieves. The kefir samples were gently homogenized and immediately subjected to analysis. When necessary, samples were stored at 0 °C for no longer than 24 hours prior to analysis to minimize post-fermentation changes.

2.5. Physicochemical Analysis

The pH of kefir samples was measured using a calibrated digital pH meter in accordance with the method described by AOAC (2006) Prior to measurement, the samples were gently homogenized to ensure uniformity. Viscosity was determined using a rotational viscometer following the procedure of Caesaron & Nintyas (2017) and Esteves, Onukwuba, & Dikici (2016), using 100 mL of kefir sample, and the measurement was recorded after the reading reached a stable value. Syneresis was evaluated by centrifuging 10 mL of kefir sample at 6000 rpm for 15 min, as described by Agustin & Putri (2013), and expressed as the percentage of whey separation relative to the initial sample volume.

Total titratable acidity (TTA) was determined by titration using 0.1 N NaOH with phenolphthalein as an indicator following the method of Suhaeni (2018), and the results were expressed as a percentage of lactic acid.

Color characteristics of kefir samples were measured using a color reader based on the CIE Lab* color system, including lightness (L*), redness–greenness (a*), and yellowness–blueness (b*), following the method reported by Wibawanti & Rinawidiastuti (2018).

Proximate composition analysis was conducted to determine the chemical characteristics of kefir, including moisture, ash, and carbohydrate contents, following AOAC (2006). Moisture content was measured using a halogen moisture analyzer according to Nielsen (2024), while carbohydrate content was calculated by difference.

2.6. Sensory Evaluation

Sensory evaluation was conducted using 35 semi-trained panelists. The attributes evaluated included color, aroma, texture, taste, and overall acceptance using a hedonic scale, following Khoiria & Bahar (2023). Kefir samples from both treatments were evaluated comparatively.

2.7. Metagenomic Analysis

Total microbial DNA was extracted from kefir samples using the Favorgren Biotech Corp DNA Extraction HE Mini Kit following the manufacturer's instructions. DNA quality and quantity were verified prior to sequencing. High-throughput sequencing of the hypervariable region V3–V4 of the bacterial 16S rRNA gene was conducted to characterize the microbial community composition of both control kefir (CK) and torch ginger tea supplemented kefir (TGT) samples.

Metagenomic data analysis was performed using the MicrobiomeAnalyst online platform (https://microbiomeanalyst.ca/). Operational taxonomic units (OTUs) were generated and classified using established taxonomic databases. Alpha diversity indices (Shannon, Simpson, and Chao1) were calculated to assess microbial richness and diversity. Beta diversity analysis was performed using Bray–Curtis distance matrices and principal coordinates analysis (PCoA) to evaluate differences in microbial community composition between treatments. Differential abundance analysis was conducted using appropriate statistical methods to identify taxa that differed significantly between CK and TGT. All parameters and statistical thresholds used for the metagenomic analysis are detailed in the results section and corresponding tables.

2.8. Metabolomic Analysis

Untargeted metabolomic profiling was conducted immediately after fermentation. Freeze-dried kefir samples

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(50 mg) were extracted using a methanol–acetonitrile solvent system (1:1, v/v), sonicated for 30 minutes, and centrifuged at 10,000 × g for 10 minutes. The supernatant was filtered through a 0.22 µm PTFE membrane prior to analysis.

Metabolomic profiling was performed using LC-HRMS with a Q Exactive Plus Quadrupole-Orbitrap instrument coupled to a Waters Acquity UPLC HSS T3 column. Mobile phases consisted of 0.1% formic acid in water (phase A) and 0.1% formic acid in methanol (phase B). Gradient elution was applied over 25 minutes with column temperature at 45 °C and injection volume of 2 µL. Mass spectrometric data were acquired in full-scan mode under both positive and negative electrospray ionization (ESI±) with optimized source parameters for comprehensive untargeted profiling.

Raw data were processed using Compound Discoverer (version 3.2; Thermo Scientific) for peak detection, alignment, and metabolite annotation against the mzCloud spectral database. Metabolite identifications were based on accurate mass matching with a stringent confidence threshold of mzCloud best match score ≥80%, without reliance on authentic reference standards. Preprocessed data were analyzed using MetaboAnalyst (version 6.0) with principal component analysis (PCA) and partial least squares–discriminant analysis (PLS-DA) for metabolomic visualization. Discriminant metabolites were identified using variable importance in projection (VIP) scores >1.0 combined with Student's t-test ($p < 0.05$). Model robustness was assessed through permutation testing and cross-validation. Pathway enrichment analysis was performed to identify biologically relevant metabolic processes.

2.9. Statistical Analysis

All data were expressed as mean ± standard deviation. Data normality was assessed prior to statistical testing. Differences between treatments were analyzed using an independent t-test at a 95% confidence level ($p < 0.05$). Statistical analyses for physicochemical, microbiological, and sensory data were performed using appropriate statistical software.

Metagenomic data were analyzed to assess microbial diversity and community structure, including alpha diversity indices and multivariate analysis. Metabolomic data were analyzed using multivariate approaches such as principal component analysis (PCA), followed by univariate analysis to determine significant differences between treatments.

3. Result

3.1. Physicochemical Properties of Kefir

The physicochemical properties of control kefir (CK) and kefir supplemented with torch ginger tea (TGT) are presented in Table 1. Overall, the addition of TGT resulted in measurable differences in several physicochemical parameters, indicating its influence on kefir characteristics. However, no significant differences were observed in pH ($p = 0.051$), temperature ($p = 0.927$), lightness (L*; $p = 0.194$), and yellowness (b*; $p = 0.116$) between treatments, suggesting that the fundamental properties of kefir remained stable.

In contrast, several parameters were significantly affected by TGT supplementation. Moisture content was significantly higher in TGT (89.57 ± 0.04%, 95% CI: 89.39–89.75%) compared to CK (88.99 ± 0.08%, 95% CI: 88.74–89.24%; $p < 0.001$, $d = -9.17$, very large effect). Similarly, ash content was significantly elevated in TGT (1.08 ± 0.01%, 95% CI: 1.03–1.13%) versus CK (0.88 ± 0.01%, 95% CI: 0.83–0.93%; $p < 0.001$, $d = -17.14$, very large effect). Conversely, total soluble solids (TSS) were significantly lower in TGT (5 ± 0°Brix) compared to CK (6 ± 0°Brix; $p = 0.026$), and redness (a) values were significantly reduced in TGT (1.6 ± 0.07, 95% CI: 1.45–1.75) relative to CK (1.7 ± 0.14, 95% CI: 1.44–1.96; $p = 0.039$, $d = 8.57$, very large effect).*

Table 1: Physicochemical Properties of Control Kefir (CK) and Kefir Supplemented with Torch Ginger (*Etlingera elatior*) Tea (TGT).

Parameter	CK (Mean ± SD)	95% (CI_CK)	TGT (Mean ± SD)	95% (CI_TGT)	p-value	Cohen's	Effect Size
pH	3.74 ± 0.02[a]	[3.68-3.80]	3.72 ± 0.01[a]	[3.68-3.76]	0.051	1.22	Large
TPT	6 ± 0[a]	[6.00-6.00]	5 ± 0[b]	[5.00-5.00]	0.026	∞*	Infinite**
Temperature	29 ± 0.14[a]	[28.74-29.26]	28.8 ± 0.14[a]	[28.54-29.06]	0.927	1.43	Large
L*	80.7 ± 0.14[a]	[80.44-80.86]	78.1 ± 0.07[a]	[77.89-78.31]	0.194	20.00	Very Large
a*,	1.7 ± 0.14[a]	[1.44-1.96]	1.6 ± 0.07[b]	[1.45-1.75]	0.039	8.57	Very large
b*	11.7 ± 0.14[a]	[11.44-11.96]	9.7 ± 0.07[a]	[9.49-9.91]	0.116	15.71	Very Large
Moisture contents	88.99 ± 0.08[a]	[88.74-89.24]	89.57 ± 0.04[b]	[89.39-89.75]	<0.001	-9.17	Very Large
Ash contents	0.88 ± 0.01[a]	[0.83-0.93]	1.08 ± 0.01[b]	[1.03-1.13]	<0.001	-17.14	Very Large

Data are presented as mean ± standard deviation (SD) with 95% confidence intervals [CI] (N=3). Differences between control kefir (CK) and torch ginger tea supplemented kefir (TGT) were analyzed using independent samples t-test. Effect sizes are reported as Cohen's d, where |d| < 0.2 = negligible, 0.2–0.5 = small, 0.5–0.8 = medium, ≥0.8 = large. Values with different superscript letters ([a], [b]) within the same row indicate statistically significant differences ($p < 0.05$).

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Overall, these findings demonstrate that the addition of TGT modified selected physicochemical attributes of kefir while maintaining its core characteristics, particularly acidity. The substantial effect sizes for moisture, ash, and color parameters (a*) indicate that these changes are not only statistically significant but also practically meaningful for product formulation and consumer perception.

3.2. Sensory Evaluation

The sensory evaluation results of control kefir (CK) and kefir supplemented with torch ginger tea (TGT), assessed by 35 semi-trained panelists using a 5-point hedonic scale, are presented in Table 2. Overall, the addition of TGT influenced selected sensory attributes. Color and taste attributes showed significant differences between treatments. For color, both CK and TGT received identical mean scores (2.00 ± 0.52^a vs. 2.00 ± 0.64^b; $p = 0.031$), though with overlapping 95% confidence intervals [CK: 1.82–2.18; TGT: 1.78–2.22], with a negligible effect size ($d = 0.00$). Similarly, taste scores were identical between treatments (3.00 ± 0.67^a vs. 3.00 ± 0.81^b; $p = 0.021$) with negligible effect size ($d = 0.00$), suggesting that while p-values indicate statistical significance due to low variability in responses, the practical differences are minimal.

In contrast, aroma and overall acceptance showed no statistically significant differences (aroma: 4.00 ± 0.83^a vs. 4.00 ± 0.76^a; $p = 1.000$, $d = 0.00$; overall acceptance: 4.00 ± 0.74^a vs. 3.00 ± 0.69^a; $p = 0.456$, $d = 1.40$). However, texture exhibited a striking non-significant difference ($p = 0.420$), with CK scoring substantially higher (5.00 ± 0.58^a, 95% CI: 4.80–5.20) compared to TGT (3.00 ± 0.92^a, 95% CI: 2.68–3.32), representing a very large effect size ($d = 2.67$). This discrepancy between statistical significance and practical magnitude reflects the complexity of interpreting sensory data with high variability in hedonic responses. Overall, despite some statistically significant differences in color and taste, the comparable overall acceptance scores suggest that TGT supplementation did not substantially reduce consumer preference for the product, though the texture attribute showed pronounced differences that warrant consideration in product development.

Table 2: Sensory Evaluation of Control Kefir (CK) and Kefir Supplemented with Torch Ginger (*Etlingera elatior*) Tea (TGT).

Parameter	CK (Mean ± SD)	95% (CI_CK)	TGT (Mean ± SD)	95% (CI_TGT)	p-value	Cohen's	Effect Size
Color	2.00 ± 0.52^a	[1.82-2.18]	2.00 ± 0.64^b	[1.78-2.22]	0.031	0.00	Negligible
Aroma	4.00 ± 0.83^a	[3.71-4.29]	4.00 ± 0.76^a	[3.74-4.26]	1.000	0.00	Negligible
Texture	5.00 ± 0.58^a	[4.80-5.20]	3.00 ± 0.92^a	[2.68-3.32]	0.420	2.67	Very large
Taste	3.00 ± 0.67^a	[2.77-3.23]	3.00 ± 0.81^b	[2.72-3.28]	0.021	0.00	Negligible
Overall acceptance	4.00 ± 0.74^a	[3.75-4.25]	3.00 ± 0.69^a	[2.76-3.24]	0.456	1.40	Very Large

Data are presented as mean ± standard deviation (SD) with 95% confidence intervals [CI] (N=35 panelists). Sensory attributes were evaluated on a 5-point hedonic scale (1 = dislike very much, 5 = like very much). Differences between control kefir (CK) and torch ginger tea supplemented kefir (TGT) were analyzed using independent samples t-test. Effect sizes are reported as Cohen's d, where $d < 0.2$ = negligible, 0.2–0.5 = small, 0.5–0.8 = medium, ≥0.8 = large. Values with different superscript letters (^{a}, ^{b}) within the same row indicate significant differences ($p < 0.05$).

3.3. Metagenomic Profile of Kefir

3.3.1. Alpha Diversity

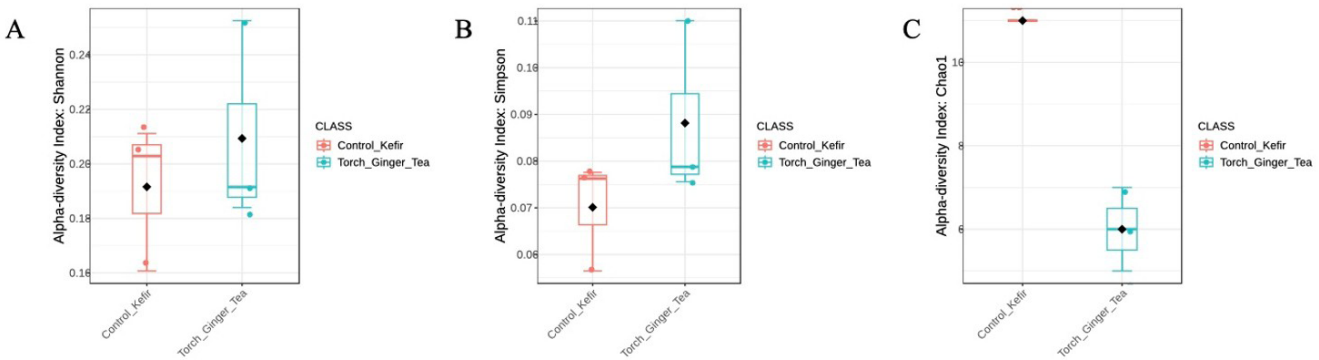

Figure 1: Alpha Diversity of Kefir Samples based on (A) Shannon, (B) Simpson, and (C) Chao1 Indices. No Significant Differences ($p > 0.05$) were Observed between Control Kefir (CK) and Kefir Supplemented with Torch Ginger Tea (TGT), Indicating that TGT did not Alter Microbial Diversity, Dominance, or Richness.

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The alpha diversity of microbial communities in control kefir (CK) and kefir supplemented with torch ginger tea (TGT) is presented in Figure 1, based on Shannon, Simpson, and Chao1 indices. No significant differences ($p > 0.05$) were observed between CK and TGT across all diversity metrics. These results indicate that microbial diversity, richness, and dominance were comparable between treatments, suggesting that the addition of TGT did not significantly alter the overall microbial community structure.

3.3.2. Beta Diversity

Beta diversity analysis based on Bray–Curtis distance is presented in Figure 2 using principal coordinates analysis (PCoA). A tendency of separation between control kefir (CK) and kefir supplemented with torch ginger tea (TGT) was observed along the primary axis. However, no significant difference was detected between treatments (PERMANOVA, $p > 0.05$).

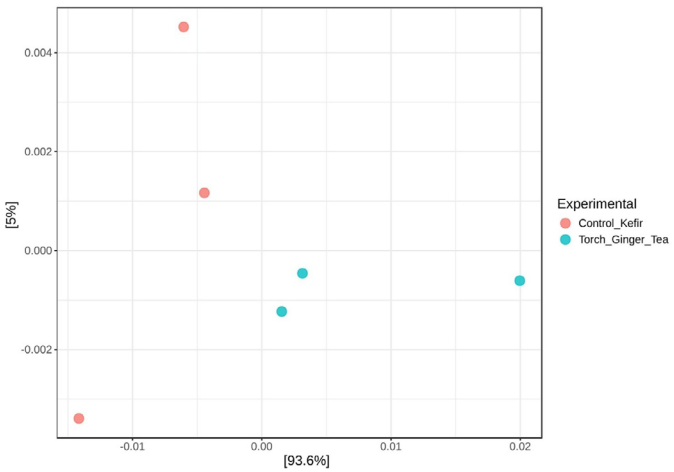

Figure 2: Beta Diversity Analysis of Kefir Samples based on Bray–Curtis Distance using Principal Coordinates Analysis (PCoA). A Tendency of Separation between Control Kefir (CK) and Kefir Supplemented with Torch Ginger Tea (TGT) was Observed, Although the Difference was not Statistically Significant (PERMANOVA, p > 0.05).

3.3.3. Taxonomic Composition

The taxonomic composition at the family level is presented in Figure 3. Both control kefir (CK) and kefir supplemented with torch ginger tea (TGT) were predominantly dominated by Lactobacillaceae, accounting for more than 90% of the total microbial community. Minor proportions of other families, including Streptococcaceae, Enterococcaceae, and Enterobacteriaceae, were detected at low abundances. Overall, no noticeable differences in microbial composition were observed between CK and TGT, indicating that TGT supplementation did not alter the dominant microbial structure of kefir.

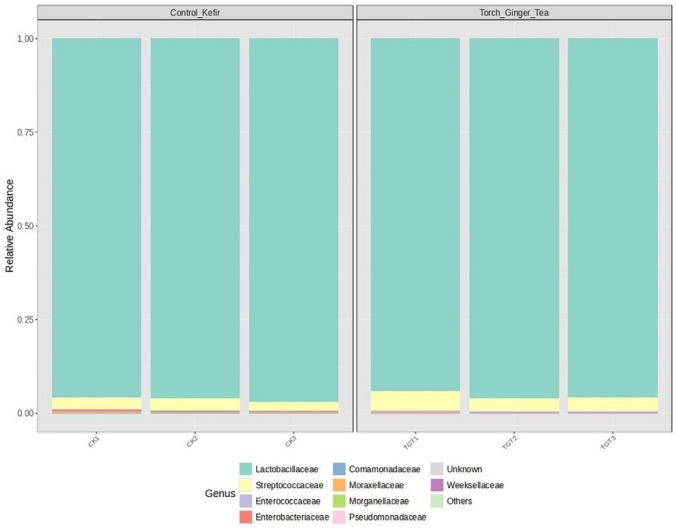

Figure 3: Taxonomic Composition of Microbial Communities at the Family Level in Control Kefir (CK) and Kefir Supplemented with Torch Ginger Tea (TGT), Presented as Relative Abundance.

3.3.4. Differential Abundance

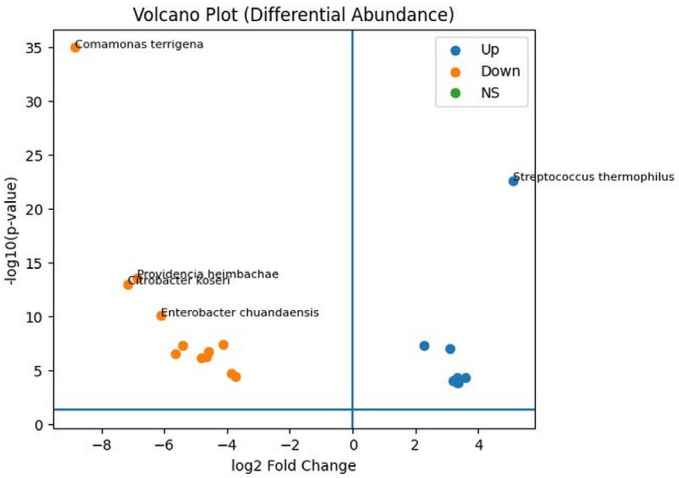

Figure 4: Volcano Plot of Differentially Abundant Bacterial Taxa between Control Kefir (CK) and Kefir Supplemented with Torch Ginger Tea (TGT).

Differential abundance analysis identified 24 significantly different taxa (FDR < 0.05) between CK and TGT (Figure 4; Table 3). TGT samples showed higher abundance of LAB *Streptococcus thermophilus*, *Lactococcus raffinolactis*, and *Limosilactobacillus fermentum*, while CK showed higher abundance of non-LAB taxa including *Comamonas terrigena* and *Enterobacteriaceae*-related species. These results indicate that TGT selectively promoted beneficial

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bacteria and suppressed undesirable taxa.

Volcano plot showing differentially abundant bacterial taxa between control kefir (CK) and kefir supplemented with torch ginger tea (TGT). The x-axis indicates log2 fold change and the y-axis represents –log10(p-value). Significantly altered taxa are highlighted.

Table 3: Differentially Abundant Bacterial Taxa between Control Kefir (CK) and Kefir Supplemented with Torch Ginger Tea (TGT).

Taxa (Genus/Species)	log2FC	logCPM	p-value	FDR
Comamonas terrigena	-8.8452	10.658	1.0986E-35	4.7242E-34
Streptococcus thermophilus	5.1045	11.554	2.4448E-23	5.2564E-22
Providencia heimbachae	-6.8608	8.7034	2.8367E-14	4.066E-13
Citrobacter koseri	-7.1609	8.9965	1.0556E-13	1.1348E-12
Enterobacter chuandaensis	-6.0995	7.9675	8.6947E-11	7.4774E-10
Klebsiella pneumoniae	-4.1417	10.315	3.9386E-8	2.8227E-7
Enterococcus italicus	2.2881	11.347	4.8836E-8	2.9046E-7
Stenotrophomonas hibiscicola	-5.4279	7.331	5.4039E-8	2.9046E-7
Lactococcus raffinolactis	3.0852	9.373	9.3108E-8	4.4485E-7
Vagococcus fluvialis	-4.5947	6.6036	2.1236E-7	9.1315E-7
Elizabethkingia anophelis	-5.6292	7.5176	2.8552E-7	1.1161E-6
Klebsiella electrica	-4.6429	6.6311	6.4393E-7	2.2879E-6
Phytobacter diazotrophicus	-4.8196	6.7862	6.9169E-7	2.2879E-6
Scandinavium goeteborgense	-3.8766	6.0156	1.9511E-5	5.9927E-5
Acinetobacter qingfengensis	-3.7169	5.8981	4.45E-5	1.2757E-4
Streptococcus macedonicus	3.6016	6.5004	4.9839E-5	1.3246E-4
Limosilactobacillus fermentum	3.3212	6.2973	5.2367E-5	1.3246E-4
Leuconostoc falkenbergense	3.211	7.7608	1.0674E-4	2.55E-4
Streptococcus vestibularis	3.3161	6.2923	1.5007E-4	3.2975E-4
Lactococcus chungangensis	3.3537	6.3188	1.5337E-4	3.2975E-4

Differentially abundant bacterial taxa between CK and TGT identified using EdgeR analysis. Log2FC values were calculated as log2(TGT/CK); therefore, positive values indicate higher abundance in TGT, while negative values indicate higher abundance in CK. Significance was determined at FDR < 0.05.

3.4. Metabolomic Profile of Kefir

A total of 94 metabolite features were initially detected using LC-MS analysis. Following data preprocessing, including filtering of low-variance and low-abundance features, 84 metabolites were retained for further analysis. The processed data were subsequently subjected to multivariate analysis and differential metabolite analysis.

3.5. Multivariate Analysis of Metabolite Profiles

Principal Component Analysis (PCA) showed a clear separation between control kefir (CK) and kefir supplemented with torch ginger tea (TGT) (Figure 5). PC1 and PC2 explained 75.7% and 18.5% of the total variance, respectively. The samples formed distinct and non-overlapping clusters, indicating significant differences in metabolite profiles between treatments, with good consistency observed within each group. These results suggest that the addition of torch ginger tea significantly altered the metabolomic composition of kefir.

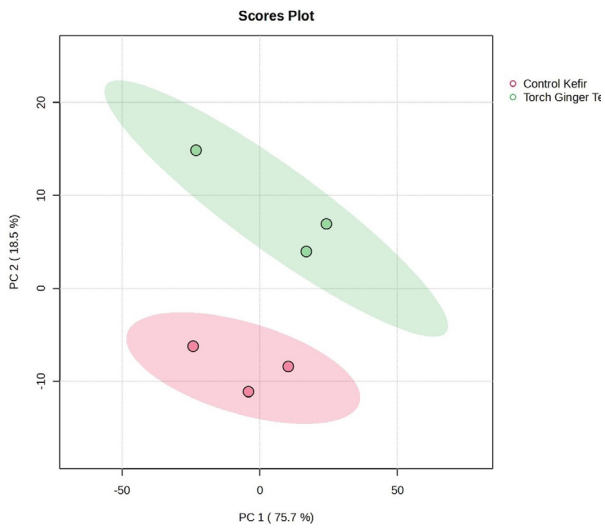

Figure 5: Plot Skor Principal Component Analysis (PCA) Yang Menunjukkan Pemisahan Jelas Profil Metabolit Antara Kefir Kontrol (CK) Dan Kefir Dengan Penambahan Teh Kecombrang (TGT).

3.5. Identification and Classification of Metabolites

Metabolomic analysis identified several metabolites that

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differed significantly between control kefir (CK) and kefir supplemented with torch ginger tea (TGT) (Figure 6; Table 4). Metabolites such as γ-linolenic acid ethyl ester, (R)-3-hydroxy myristic acid, and 4-ethoxy ethylbenzoate showed higher abundance in CK. In contrast, caffeine and NP-011548 showed higher abundance in TGT. These findings indicate that the addition of torch ginger tea altered the metabolite profile of kefir.

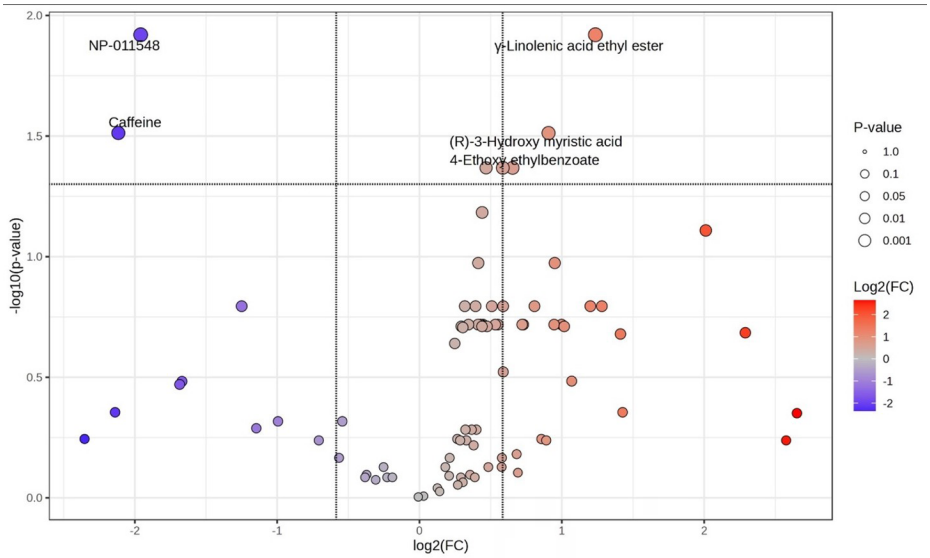

Figure 6: Volcano Plot of Differential Metabolites between Control Kefir (CK) and Torch Ginger Tea (TGT).

Volcano plot showing significantly altered metabolites between CK and TGT based on log2 fold change (log2FC) and statistical significance (–log10 p-value). Metabolites located on the right side (positive log2FC) indicate higher abundance in CK, while those on the left side (negative log2FC) are show higher abundance in TGT. The horizontal dashed line represents the significance threshold ($p < 0.05$), and the vertical dashed lines indicate fold change thresholds. Selected key metabolites are labeled. Log2FC values were calculated based on the ratio of CK to TGT.

Table 4: Differential Metabolites between Control Kefir (CK) and Torch Ginger Tea (TGT).

Metabolite Name	Fold Change (FC)	log2(FC)	Adjusted p-value	–log10(p)
NP-011548	0.2572	-1.9589	0.0120	1.9201
γ-Linolenic acid ethyl ester	2.3551	1.2358	0.0120	1.9201
Caffeine	0.2307	-2.1157	0.0307	1.5125
(R)-3-Hydroxy myristic acid	1.8746	0.9066	0.0307	1.5125
4-Ethoxy ethylbenzoate	1.5738	0.6543	0.0428	1.3683
Log2FC was calculated as log2(CK/TGT); therefore, positive values indicate higher abundance in CK, while negative values indicate higher abundance in TGT.				

3.6. Major Metabolites in Kefir

Major metabolites in kefir were identified based on their average normalized intensity across all samples. Lactic acid and its derivatives, including DL-lactic acid and L-(+)-lactic acid, were the most dominant metabolites, indicating active lactic acid fermentation. Other abundant metabolites included palmitic acid, amino alcohol derivatives, and carbohydrates such as lactose and maltose, reflecting contributions from lipid metabolism and residual milk sugars. These results highlight that kefir metabolite profiles are primarily characterized by organic acids, fatty acids, and carbohydrate-related compounds.

Table 5: Major Metabolites Identified in Kefir Samples based on Mean Intensity.

Metabolite Name	Category	Mean Intensity
DL-Lactic Acid	Organic acid	9.08×10^9
L-(+)-Lactic acid	Organic acid	8.85×10^9
Palmitic Acid	Fatty acid	6.50×10^9
2-Amino-1,3,4-octadecanetriol	Amino alcohol / lipid derivative	2.28×10^9
L-Iditol	Sugar alcohol	1.43×10^9
Creatine	Nitrogen compound	6.85×10^8
Methylmalonic acid	Organic acid	5.30×10^8
α-Lactose	Carbohydrate	4.41×10^8
6-Hydroxycaproic acid	Organic acid	3.67×10^8
D-(+)-Maltose	Carbohydrate	3.61×10^8
Mean intensity values were calculated from normalized metabolomics data across all samples		

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3.7. Differential Metabolite Analysis

Differential metabolite analysis, with log2FC values calculated based on the ratio of CK to TGT, revealed several metabolites that differed significantly between control kefir (CK) and kefir supplemented with torch ginger tea (TGT) (Figure 6; Table 4). A total of five metabolites showed significant differences (adjusted p-value < 0.05), indicating that the addition of torch ginger tea influenced specific components of the kefir metabolome.

Metabolites with positive log2FC values, including γ-linolenic acid ethyl ester, (R)-3-hydroxy myristic acid, and 4-ethoxy ethylbenzoate, showed higher abundance in CK. In contrast, metabolites such as caffeine and NP-011548, which exhibited negative log2FC values, showed higher abundance in TGT. The distribution of these metabolites is clearly illustrated in the volcano plot (Figure 6), while detailed statistical values are presented in Table 4. Overall, these results indicate that the addition of torch ginger tea selectively modulated specific metabolites without inducing broad changes in the overall metabolite composition.

4. Discussion

The incorporation of torch ginger tea (TGT) into the kefir matrix resulted in multidimensional effects on its physicochemical, microbial, and metabolic characteristics. Although the overall acidity remained relatively stable, TGT induced notable physicochemical changes, including increased moisture and ash content, accompanied by reduced total soluble solids (TSS) and color intensity. Metagenomic analysis revealed that these changes did not significantly affect alpha and beta diversity, highlighting the resilience of the kefir microbiome. However, selective microbial modulation was evident, as indicated by the higher abundance of specific lactic acid bacteria (LAB) and a concurrent reduction in non-LAB populations. Furthermore, metabolomic analysis demonstrated a clear metabolic shift, as reflected by distinct separation in principal component analysis (PCA) and the presence of specific differential metabolites, confirming the dynamic response of the kefir ecosystem to plant-derived bioactive compounds (Azi et al., 2021; Cui et al., 2023).

The observed physicochemical modifications can be attributed to the interaction between plant-derived bioactive compounds and the milk matrix during fermentation. The increase in moisture and ash content in TGT-treated kefir likely reflects the incorporation of water-soluble phytochemicals and mineral components naturally present in torch ginger extracts (Naufalin et al., 2024b). In contrast, the reduction in TSS and changes in color parameters may be associated with molecular interactions between phenolic compounds, flavonoids, milk proteins, and exopolysaccharides. These interactions can influence protein network structure, water-holding capacity, and pigment stability under acidic fermentation conditions driven by LAB activity (Marques Soutelino et al., 2025; Uruc et al., 2022).

Despite the introduction of phytochemical stressors from TGT, the microbial ecosystem of kefir remained remarkably stable, as evidenced by the absence of significant differences in alpha and beta diversity. The microbial community was consistently dominated by Lactobacillaceae across treatments, reinforcing the concept that kefir is a highly stable and self-regulating microbial consortium. This stability may be attributed to complex microbial interactions, including cross-feeding and niche partitioning within kefir grains, which buffer the community against external perturbations and maintain fermentation functionality (Alraddadi et al., 2023; Du et al., 2021).

Although overall diversity remained stable, TGT exerted a strong selective effect at the taxonomic level. A higher abundance of beneficial LAB, including *Streptococcus thermophilus*, *Lactococcus raffinolactis*, and *Limosilactobacillus fermentum*, was observed, alongside a reduction in non-LAB and opportunistic bacteria. This selective modulation is likely driven by the antimicrobial properties of phenolic compounds present in torch ginger, which can disrupt membrane integrity in susceptible microorganisms while allowing tolerant LAB to thrive (Chan et al., 2018; González-Orozco et al., 2023). Additionally, certain plant-derived compounds may act as substrates or growth-promoting factors for LAB, further enhancing their proliferation and contributing to improved safety and functional quality of kefir.

These microbial shifts were closely reflected in the metabolomic profile, as demonstrated by the clear separation observed in PCA. The addition of TGT altered specific metabolic pathways, leading to significant differences in key metabolites, including fatty acid derivatives such as γ-linolenic acid ethyl ester and the detection of caffeine as a plant-derived marker. The higher abundance of these metabolites highlights the strong interplay between microbial activity and plant-derived compounds, where bioactive

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precursors are biotransformed by LAB into diverse functional metabolites (Ma et al., 2024). This metabolic reprogramming reflects the combined contribution of intrinsic microbial metabolism and enzymatic transformation of plant compounds, resulting in a more complex biochemical profile compared to conventional kefir (Cui et al., 2023; Walsh et al., 2016).

Overall, these findings demonstrate that the addition of TGT induces a coordinated plant–microbe interaction, leading to selective microbial modulation and targeted metabolic shifts that ultimately influence kefir quality. This integrative multi-omics approach provides deeper mechanistic insight into how plant-derived bioactive compounds interact with fermentation ecosystems. The study highlights a novel strategy for modulating kefir fermentation through botanical supplementation, offering significant potential for the development of functional fermented dairy products with enhanced health-promoting properties (Azi et al., 2021; Sumarmono et al., 2023).

5. Conclusion

In conclusion, the supplementation of torch ginger (*Etlingera elatior*) tea (TGT) in kefir resulted in notable modifications in physicochemical properties and induced a distinct shift in metabolic profiles, as evidenced by clear separation in principal component analysis and the presence of specific differential metabolites. Despite the introduction of plant-derived bioactive compounds, the overall microbial community structure remained stable, with no significant changes observed in alpha and beta diversity. Importantly, TGT selectively modulated the microbial community by promoting the growth of beneficial lactic acid bacteria while suppressing non-lactic acid bacteria. This selective modulation reflects a dynamic plant–microbe interaction, in which bioactive compounds influence fermentation pathways and metabolic outcomes without disrupting the stability of the kefir ecosystem. Through the integration of physicochemical, metagenomic, and metabolomic approaches, this study provides novel mechanistic insights into multi-omics interactions in plant-enriched fermented dairy systems. These findings highlight the potential of TGT as a functional ingredient for the development of innovative fermented dairy products with enhanced functional properties.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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