

Short-Chain Fatty Acid Extracts from Fermented Rice Gruel Modulate Triglyceride Metabolism in *Drosophila melanogaster*

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Abstract

Traditional fermented foods are rich sources of probiotic microorganisms and bioactive metabolites that contribute to metabolic health. Fermented rice gruel (Generally referred as Kanji in Tamil) is a home-based fermented food widely consumed in South Asia, yet its microbiological and metabolic significance remains underexplored. The present study aimed to explore the presence of *Lactobacillus* species in fermented rice gruel further it investigates the role of short-chain fatty acids (SCFA) produced by the *Lactobacillus* species in lipid metabolism using *Drosophila melanogaster* as a model system. Phytochemical analysis, microbial enumeration, Fourier transforms infrared (FTIR) spectroscopy of fermented rice gruel, triglyceride estimation and behavioral based physical activity assays were employed in *D. melanogaster* experimental models. The results demonstrate that fermented rice gruel supports probiotic-level growth of *L. plantarum* and contains SCFA-associated functional groups that contribute to reduced lipid accumulation and improved physical activity. These findings highlight the functional and nutritional relevance of traditional fermented rice-based foods. This study aligns with Sustainable Development Goals of Zero Hunger, and Good Health and Well-Being by scientifically validating fermented rice gruel (Kanji) as a nutritionally functional, probiotic-rich traditional food. The rich existence of *Lactobacillus plantarum* and its association with short-chain fatty acid (SCFA) synthesis in fermented gruel and regulatory ability of SCFA on lipid metabolism in experimental models highlights the role of affordable home-based fermented foods in improving dietary quality and supporting metabolic health.

Keywords

Fermented rice gruel, *Lactobacillus plantarum*, Short-chain fatty acids, Lipid metabolism, *Drosophila melanogaster*

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Introduction

Fermented foods have been consumed for centuries due to their enhanced shelf life, improved digestibility, and health-promoting properties. Such foods are enriched with probiotic microorganisms, particularly lactic acid bacteria, which exert beneficial effects by modulating gut microbiota and host metabolism (FAO-WHO, 2001). Among fermented foods, fermented rice gruel, commonly known as Kanji, occupies a prominent place in traditional diets across South Asia and is recognized for its therapeutic and nutritional value (Tamang, 2022). *L. plantarum* is one of the most frequently isolated lactic acid bacteria from fermented cereal substrates and exhibits strong fermentative capacity, acid tolerance, and metabolic versatility (Jeygowri et al., 2015). This species efficiently converts dietary carbohydrates into organic acids such as acetate and propionate, which collectively form short-chain fatty acids (SCFAs). SCFAs play a critical role in regulating lipid metabolism, energy homeostasis, and obesity-related pathways by inhibiting lipogenesis and enhancing fatty acid oxidation (den Besten et al., 2013).

Drosophila melanogaster serves as a well-established model organism for investigating diet-induced metabolic alterations and obesity-associated behavioral changes due to its conserved lipid metabolic pathways and rapid life cycle (Brankatschk et al., 2018). The present study integrates phytochemical, microbiological, biochemical, and behavioral analyses to elucidate the role of *L. plantarum* isolated from fermented rice gruel in SCFA-mediated lipid modulation. In the context of global nutrition and health challenges, traditional fermented foods offer sustainable, culturally accepted solutions that address both food security and metabolic health. Fermented rice gruel (kanji), an inexpensive and widely consumed cereal-based food, has the potential to contribute to Sustainable Development Goal – Zero Hunger by improving nutritional quality through natural fermentation and probiotic enrichment. Simultaneously, the metabolic benefits associated with probiotic *L. plantarum* and short-chain fatty acid production support the Sustainable Development Goal- Good Health and Well-Being by promoting lipid homeostasis and overall metabolic health. Thus, the present study provides a scientific framework linking traditional dietary practices with global sustainability and health objectives.

Methodology

Preparation of Fermented Rice Gruel

Fermented rice gruel was prepared following a traditional natural fermentation protocol. Raw rice was thoroughly washed and boiled until complete gelatinization. After cooling to room temperature, the cooked rice was immersed in sterile distilled water and allowed to ferment naturally at ambient temperature (28 – 30°C) for 24 – 48 h without the addition of starter cultures. After fermentation, the liquid fraction was aseptically filtered and stored at 4°C for further analyses (Tamang, 2022).

Phytochemical Profiling of Fermented Rice Gruel

Phytochemical tests were performed using standard protocols (Harborne, 1998). Tests included qualitative analysis for alkaloids, flavonoids, tannins, glycosides, proteins, carbohydrates, saponins, and resins. Observations were recorded based on color changes or precipitate formation (Harborne, 1998). FTIR analysis was carried out to identify the functional groups present in the

fermented gruel extract. Samples were dried, mixed with KBr, and scanned in the range of 4000 – 400 cm^{-1} . Peaks were assigned to functional groups based on established databases (Iqbal, 2025).

Microbial identification and characterization

Lactic acid bacteria were isolated using de Man, Rogosa and Sharpe (MRS) agar and identified based on colony morphology (De Man et al., 1960). Viable counts were expressed as CFU/mL.

Physical Activity Analysis and Estimation of TG Levels in Experimental Models

Adult *D. melanogaster* was maintained under standard laboratory conditions and fed fermented gruel-supplemented diet for 21 days. Extracted SCFAs from fermented gruel were supplemented into the diet of *Drosophila* experimental groups as Control SCFA treated, high carbohydrate and fat diet (HCF), and Atorvastatin treated group. Each experimental group contains 10 flies and experiments are done as triplicates. Climbing assays, and open-field locomotor activity were assessed following established protocols (Liu et al., 2008). Triglyceride content was analyzed to assess metabolic changes.

Results and Discussion

Phytochemical Profiling of Fermented Rice Gruel

Phytochemical screening of the fermented rice gruel (Kanji) revealed the presence of multiple bioactive compounds generated or enhanced during the fermentation process (Table 1 and Figure 1). Alkaloids, tannins, glycosides, proteins, and carbohydrates were present in appreciable quantities, while flavonoids, saponins, and resins were absent. The appearance of creamy white coloration confirmed the presence of alkaloids, while the formation of a yellow color indicated tannins and proteins. The presence of carbohydrates and proteins in the fermented sample provides essential substrates for microbial metabolism, leading to the generation of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate (Harborne, 1998; Tamang, 2022; Koh, 2016).

Table 1. Phytochemical analysis of fermented gruel

Phytochemical	Result	Observation
Alkaloids	+	White creamy appearance
Flavonoids	-	No yellow coloration
Tannins	+	Yellow coloration observed
Glycosides	+	White coloration appeared
Proteins	+	Yellow coloration observed
Carbohydrates	+	Ring formation detected
Saponins	-	No foam formation
Resins	-	No color observed



Figure 1. Phytochemical analysis of fermented gruel

FTIR Spectral Evidence of SCFA-Associated Functional Groups

FTIR analysis of fermented rice gruel revealed characteristic absorption peaks corresponding to ester carbonyl (C=O), alkyl (C-H), and carboxylate functional groups. A prominent band around 1735 cm^{-1} was attributed to esterified carbonyl stretching, while peaks in the region of $2920\text{--}2850\text{ cm}^{-1}$ indicated aliphatic C-H groups. Additional bands corresponding to carboxylate stretching vibrations support the presence of organic acids, consistent with SCFAs synthesized during microbial fermentation (Figure 2). These spectral features align with earlier reports linking *L. plantarum* mediated fermentation to acetate and propionate production in cereal substrates.

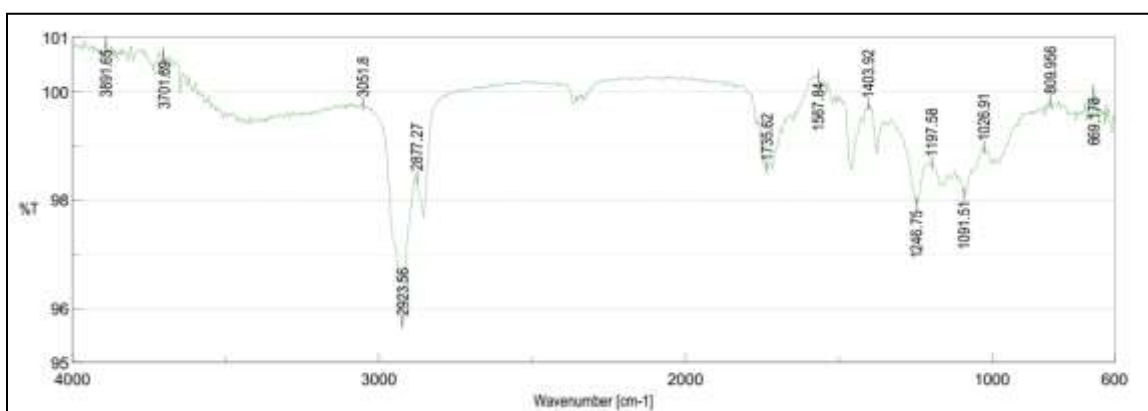


Figure 2. FTIR analysis of Fermented Gruel

Identification of *Lactobacillus* Species.

The isolates were Gram-positive, catalase-negative and capable of fermenting sugars, supporting their identification as *Lactobacillus* spp. Fermented rice gruel samples showed abundant growth on MRS agar plates. Colonies were circular, smooth, convex and cream white (Table 2).

Table 2. Biochemical Characteristics of Isolates

Test Parameter	Expected Result	Observed Result
Gram reaction	Positive	+
Catalase test	Negative	-
Glucose fermentation	Acid	+
Lactose fermentation	Acid	+
Sucrose fermentation	Acid	+
Mannitol fermentation	Variable	+/-

The CFU analysis showed maximum colony recovery at the 10^{-5} dilution, confirming a high population density of *L. plantarum* in the fermented gruel. The high viable count and stable colony morphology of *L. plantarum* observed in this study strongly supports its role as a primary fermentative organism responsible for SCFA generation in fermented rice gruel. *L. plantarum* is known to metabolize complex carbohydrates into organic acids such as lactate, acetate, and propionate, which subsequently influence lipid metabolism and energy homeostasis. Previous studies have demonstrated that *L. plantarum* isolated from fermented cereals exhibits superior carbohydrate fermentation efficiency and organic acid production compared to other lactic acid bacteria (Jeygowri et al., 2015). The presence of high CFU levels in the fermented gruel therefore provides a strong microbiological basis for subsequent FTIR-based detection of esterified fatty acids and SCFA-associated functional groups (Figure 3).

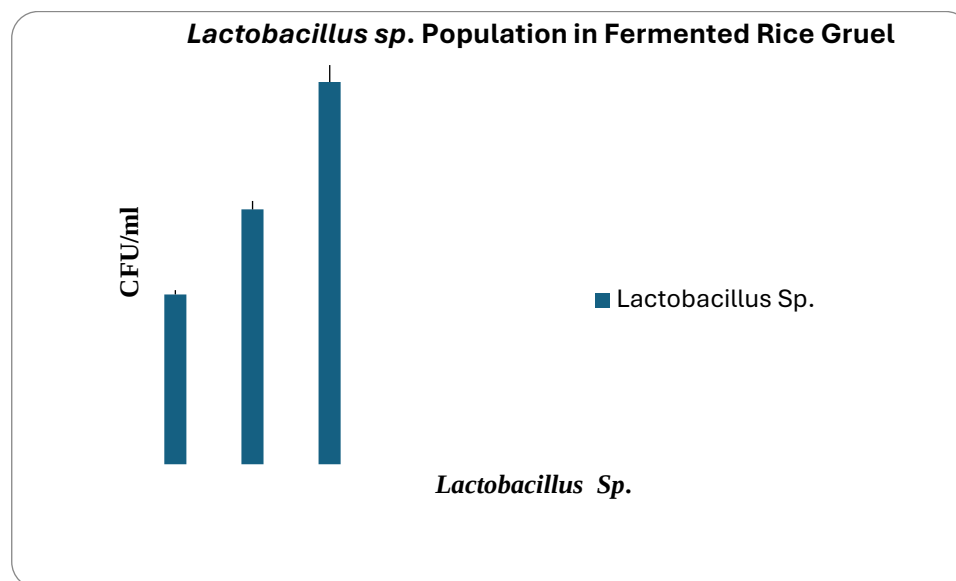


Figure 3. *Lactobacillus* Sp. population in fermented rice gruel

Behavioral Performance, Physical Activity and Triglyceride Levels

Fermented-gruel-supplemented flies exhibited improved climbing ability compared to high carbohydrate and fat diet group (HCF), indicating enhanced muscular coordination and physical activity. Reduced climbing performance in high carbohydrate and fat diet group is indicative of obesity-associated physical impairment. Open-field analysis revealed increased grid crossings in fermented-gruel-supplemented flies, reflecting improved spontaneous locomotor activity and reduced obesity-associated lethargy (Figure 4).

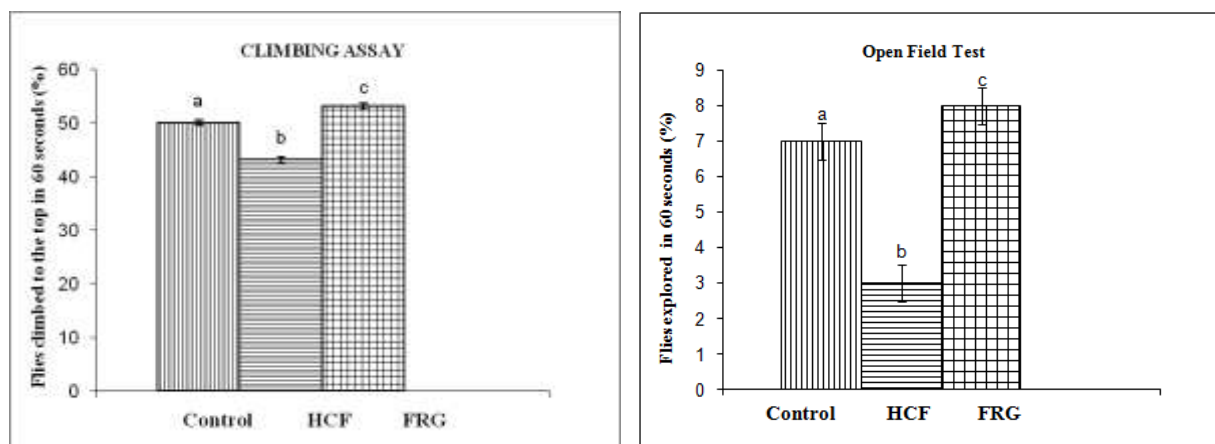


Figure 4. Climbing assay and open-field locomotor activity

Triglyceride quantification was carried out in control and experimental groups of *D. melanogaster* to evaluate the metabolic impact of fermented gruel-derived SCFAs. The assay was performed using an enzymatic colorimetric kit, and results were expressed as mean $\pm$ SD for three biological replicates per group. The High carbohydrate and fat diet (HCF) group exhibited higher triglyceride concentrations of 77.2 μ g/mg, whereas flies supplemented with fermented gruel extract (FG-SCFA group) showed a significant reduction in total triglyceride content of 46.2 μ g/mg ($p < 0.05$) compared to other experimental groups (Figure 5). This indicates that dietary supplementation with SCFA-rich Kanji extract exerts a lipid-lowering effect, likely by enhancing lipid oxidation and reducing lipid storage.

The observed decrease in TG levels in the experimental flies can be attributed to the metabolic activity of SCFAs, which are known to inhibit lipogenesis and promote fatty acid oxidation (Canfora, 2015). Propionate and butyrate have been shown to suppress hepatic fatty acid synthesis by downregulating acetyl-CoA carboxylase and fatty acid synthase enzymes (Den Besten et al., 2013). Moreover, acetate serves as an energy substrate that promotes mitochondrial activity, further contributing to lipid.

The findings agree with those of Brankatschk et al. (2018), who reported that metabolic flexibility in *Drosophila* is responsive to dietary fatty acid composition. The significant TG reduction following fermented gruel supplementation suggests that SCFA-rich traditional foods may beneficially modulate lipid metabolism and mitigate fat accumulation. This highlights their potential role as functional dietary agents for managing lipid-related metabolic disorders. The physiological relevance of these metabolites was validated through TG estimation in *Drosophila melanogaster*, where significant reductions in lipid levels were observed upon supplementation with fermented gruel extract. The cumulative results indicate that the SCFAs generated during fermentation particularly acetate and propionate may act as metabolic modulators, reducing triglyceride accumulation and enhancing lipid oxidation.

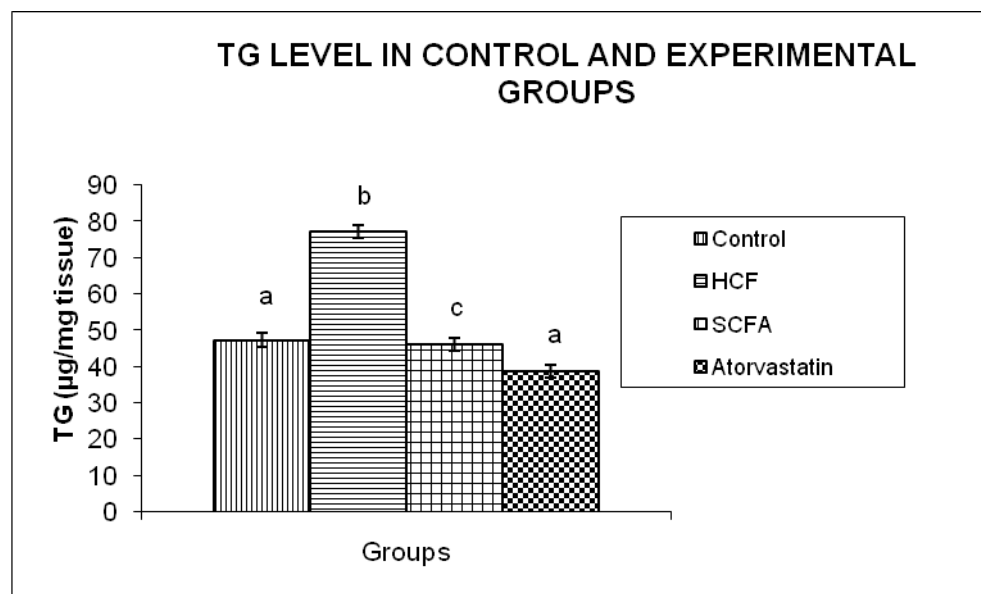


Figure 5. Triglyceride levels in experimental groups

The present study demonstrates that fermented rice gruel is a rich reservoir of bioactive phytochemicals, fermentative lactic acid bacteria, and short-chain fatty acid (SCFA)-associated functional groups, all of which collectively influence lipid metabolism and physiological performance in *Drosophila melanogaster*. The phytochemical analysis revealed the presence of alkaloids, tannins, glycosides, proteins, and carbohydrates, indicating that natural fermentation enhances the biochemical complexity of rice gruel. These components serve as substrates and signaling molecules that promote microbial growth and metabolic transformation during fermentation. Their availability is consistent with previous studies that reported the enrichment of bioactive compounds during cereal fermentation, which subsequently contributes to SCFA formation and improved nutritional value (Harborne, 1998; Tamang, 2022).

The microbiological results further validate the fermentative potential of rice gruel, as evidenced by the robust growth of *L. plantarum* indicated by high colony-forming units and characteristic biochemical reactions. This species is well documented for its ability to ferment complex carbohydrates into organic acids, including acetate, propionate, and lactate. The high population density observed aligns with earlier reports showing the dominance of *L. plantarum* in rice-based fermented foods and its superior metabolic efficiency in producing SCFAs that modulate host energy utilization (Jeygowri *et al.*, 2015). The presence of strong *Lactobacillus* growth provides a mechanistic basis for the FTIR-detected functional groups associated with SCFA biosynthesis.

The FTIR spectra revealed prominent absorption peaks corresponding to ester carbonyl, alkyl, and carboxylate functional groups, which are indicative of esterified fatty acids and organic acids typically produced during microbial fermentation. These spectral features support the biochemical evidence of SCFA generation in the fermented gruel. Similar findings have been documented in fermented cereal studies, where SCFA-associated functional groups were reported

as markers of microbial transformation and lipid-modulating potential of fermented foods (Koh et al., 2016).

The functional relevance of these biochemical and microbial attributes is supported by the *Drosophila* physiological assays. Flies supplemented with fermented gruel extract displayed improved climbing ability and enhanced open-field locomotion, suggesting better neuromuscular coordination and reduced diet-induced lethargy. These behavioral improvements are consistent with literature reporting that SCFAs can modulate energy expenditure, mitochondrial activity, and muscle function (Canfora et al., 2015). Conversely, flies subjected to a high carbohydrate-fat (HCF) diet exhibited decreased mobility, reflecting obesity-associated physiological decline.

The triglyceride assay provides direct metabolic evidence of SCFA action. Fermented-gruel-supplemented flies exhibited significantly reduced triglyceride levels compared to the HCF group, indicating decreased lipid accumulation and enhanced lipid turnover. SCFAs, particularly acetate and propionate, are known to inhibit lipogenic enzymes such as acetyl-CoA carboxylase and fatty acid synthase while stimulating mitochondrial oxidation pathways (Den Besten et al., 2013). These biochemical mechanisms align with the observed reduction in triglycerides and improved metabolic flexibility. The findings are also in agreement with reports showing that *Drosophila* lipid stores are highly responsive to dietary lipid composition and microbial metabolites (Brankatschk et al., 2018).

Collectively, the phytochemical richness, microbial composition, and SCFA-associated chemical signatures of fermented rice gruel provide complementary evidence for its lipid-modulating potential. The ability of fermented gruel to reduce triglyceride accumulation and enhance locomotor function demonstrates its functional role as a metabolic modulator. These results substantiate the nutritional and physiological relevance of traditional fermented foods and highlight their potential contributions to dietary strategies for improving lipid homeostasis and preventing metabolic disorders.

Conclusion

The findings of this study highlight the functional and nutritional relevance of traditional fermented rice-based foods, particularly fermented rice gruel (kanji). The results demonstrate that fermented gruel serves as a rich source of *Lactobacillus plantarum* and short-chain fatty acids, both of which contribute to favorable metabolic outcomes. The combined phytochemical, microbiological, biochemical, and behavioral evidence shows that fermented rice gruel supports SCFA-mediated regulation of lipid metabolism and enhances physical activity in *D. melanogaster*. These outcomes underline its potential as a functional food with probiotic and metabolic health benefits. Furthermore, the study provides scientific validation that directly aligns with global sustainability priorities. By demonstrating improvements in nutritional quality, biological accessibility, and lipid modulation, fermented rice gruel contributes to sustainable development goals of Zero Hunger through enhanced dietary value and food security. Its ability to promote metabolic health and reduce lipid accumulation supports sustainable development goals of Good Health and Well-Being. Collectively, these findings reinforce the relevance of integrating

traditional fermented foods into sustainable dietary patterns and public health strategies aimed at improving nutrition and metabolic well-being.

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