Fermented Rice Gruel Derived Short-Chain Fatty Acids Modulate Triglyceride Homeostasis in Drosophila melanogaster

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Abstract: Traditional fermented foods are increasingly recognized for their probiotic microorganisms and bioactive metabolites that contribute to host metabolic regulation. Fermented rice gruel (Kanji), a traditional household cereal-based fermented food consumed throughout South Asia, remains insufficiently explored with respect to its microbiological diversity and metabolic functionality. The present study aimed to investigate the occurrence of *Lactobacillus species* in fermented rice gruel and to evaluate the role of short-chain fatty acids (SCFAs) produced by these microorganisms in modulating lipid metabolism using Drosophila melanogaster as an experimental model. Phytochemical screening, microbial enumeration, Fourier Transform Infrared (FTIR) spectroscopy of fermented rice gruel, triglyceride estimation, and behavioral-based physical activity assays were employed in *Drosophila melanogaster*. The findings demonstrate that fermented rice gruel supports probiotic-level growth of *Lactobacillus plantarum* and contains SCFA-associated functional groups that are associated with reduced lipid accumulation and enhanced physical activity. These findings underscore the functional and nutritional significance of traditional rice-based fermented foods. The abundant presence of *Lactobacillus plantarum* in fermented rice gruel and its association with short-chain fatty acid (SCFA) synthesis, along with the regulatory influence of SCFAs on lipid metabolism in experimental models, highlight the potential of affordable, home-based fermented foods in enhancing dietary quality and supporting metabolic health.

Keywords: Fermented rice gruel, Probiotics, Short-chain fatty acids, Lipid metabolism, *Drosophila melanogaster*

1. 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 [1]. 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 [2]. *Lactobacillus 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 [3]. 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 [4].

Drosophila melanogaster serves as a well-established model organism for investigating diet-induced metabolic alterations and obesity-associated behavioural changes due to its conserved lipid metabolic pathways and rapid life cycle [5]. The present study integrates phytochemical, microbiological, biochemical, and behavioural analyses to elucidate the role of *Lactobacillus 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), being an inexpensive and widely consumed cereal-based food, has the potential to improve nutritional quality through natural fermentation and probiotic enrichment. Simultaneously, the metabolic benefits associated with probiotic *Lactobacillus plantarum* and short-chain fatty acid endorse the lipid homeostasis and overall metabolic health. Thus, the present study provides a scientific framework linking traditional dietary practices with global sustainability and health objectives.

2. Materials and Methods

2.1. 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 [6].

2.2. Phytochemical profiling of fermented rice gruel

Phytochemical tests were performed using standard protocols [7]. Tests included qualitative analysis for alkaloids, flavonoids, tannins, glycosides, proteins, carbohydrates, saponins, and resins. Observations were recorded based on color changes or precipitate formation. 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 [8].

2.3. Microbial identification and characterization

Lactic acid bacteria were isolated using de Man, Rogosa and Sharpe (MRS) agar and identified based on colony morphology [9]. Viable counts were expressed as CFU/mL.

2.4. Physical activity analysis and Estimation of TG levels in experimental models

Adult *Drosophila melanogaster* were maintained under standard laboratory conditions and fed fermented gruel-supplemented diet for 21 days [10]. 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 experiment done as triplicates. Climbing assays, and open-field locomotor activity were assessed following established protocols [11]. Triglyceride content was analyzed to assess metabolic changes [12].

3. Results and Discussion

3.1. Phytochemical profiling of Fermented Rice Gruel

Table 1. Phytochemical Analysis of Fermented Gruel

Phytochemical	Result
Alkaloids	+
Flavonoids	-
Tannins	+
Glycosides	+
Proteins	+
Carbohydrates	+
Saponins	-
Resins	-

Phytochemical screening of the fermented rice gruel (Kanji) revealed the presence of multiple bioactive compounds generated or enhanced during the fermentation process Table 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 [13].

3.2. FTIR Spectral Evidence of SCFA-Associated Functional Groups

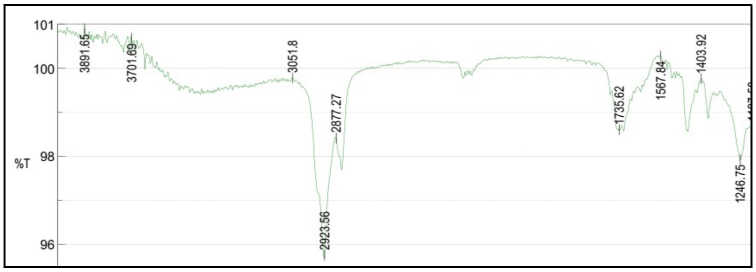

Fig. 1. FTIR analysis of Fermented Gruel

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 2920-2850 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 (Fig. 1). These spectral features align with earlier reports linking *Lactobacillus plantarum* mediated fermentation to acetate and propionate production in cereal substrates [14].

3.3. 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 o in the fermented gruel. The high viable count and stable colony morphology of *Lactobacillus plantarum* observed in this study strongly support 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 [15]. 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 2).

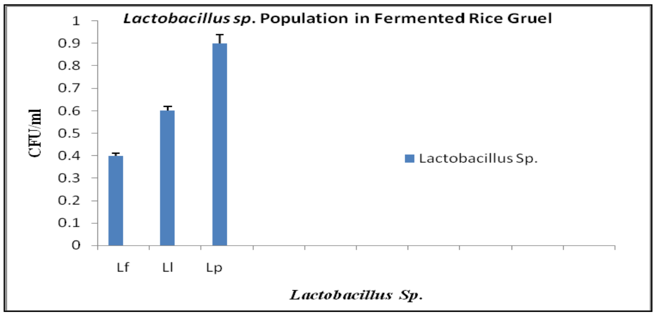

Fig. 2. *Lactobacillus Sp*. Population in fermented rice gruel

3.4. Behavioral Performance, Physical Activity and Triglyceride Levels

3.4.1. Climbing Assay and Open-Field Locomotor Activity

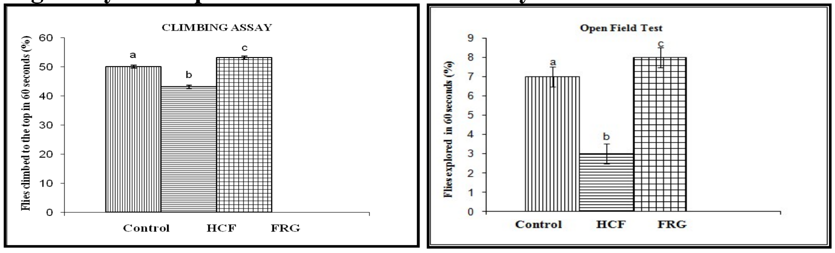

Fig. 3 (a & b). Climbing Assay and Open-Field Locomotor Activity

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 (Fig. 3 (a & b)) [16].

3.4.2. Triglyceride (TG) Estimation in Drosophila Experimental Groups

Triglyceride quantification was carried out in control and experimental groups of *Drosophila 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 ± SD for three biological replicates per group. The High carbohydrate and fat diet (HCF) group exhibited higher triglyceride concentrations of 77.2µg/mg, whereas flies supplemented with fermented gruel extract (SCFA group) showed a significant reduction in total triglyceride content of 46.2 2µg/mg ($p < 0.05$) compare to other experimental groups (Fig.4). 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. In particular, propionate and butyrate have been shown to suppress hepatic fatty acid synthesis by downregulating acetyl-CoA carboxylase and fatty acid synthase enzymes. Moreover, acetate serves as an energy substrate that promotes mitochondrial activity, further contributing to lipid [17].

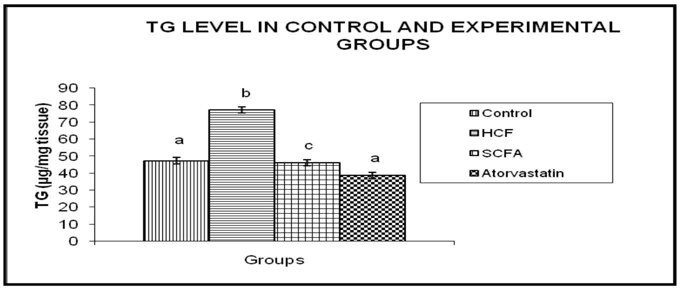

Fig. 4. Triglyceride levels in experimental groups

The findings are consistent with recent evidence demonstrating that metabolic flexibility in *Drosophila melanogaster* is highly responsive to dietary fatty acid composition. The significant reduction in triglyceride levels following fermented gruel supplementation suggests that SCFA-rich traditional foods can beneficially modulate lipid metabolism and mitigate fat accumulation. The physiological relevance of these metabolites was further validated through triglyceride estimation in Drosophila, where fermented gruel extract supplementation resulted in marked lipid lowering. Collectively, these results indicate that fermentation-derived SCFAs, particularly acetate and propionate, act as metabolic modulators by reducing triglyceride accumulation and enhancing lipid oxidation [18].

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 [18,19].

The microbiological results further validate the fermentative potential of rice gruel, as evidenced by the robust growth of *Lactobacillus 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 [15]. 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 [20].

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, indicating improved neuromuscular coordination and reduced diet-induced lethargy. These behavioral improvements are consistent with recent evidence demonstrating that short-chain fatty acids modulate energy expenditure, enhance mitochondrial oxidative activity, and improve muscle function and physical performance [17]. In contrast, flies subjected to a high carbohydrate-fat (HCF) diet exhibited reduced mobility, reflecting obesity-associated physiological and metabolic 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 [4]. 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 [5].

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 [19].

5. Conclusion

The present investigation establishes the nutritional and functional significance of traditional rice-based fermented foods, with particular emphasis on fermented rice gruel (Kanji). The findings demonstrate that natural fermentation enriches rice gruel with metabolically active *Lactobacillus plantarum* and short-chain fatty acids, which collectively exert beneficial effects on host metabolism. Integrated phytochemical, microbiological, biochemical, and behavioral analyses confirm that fermented rice gruel facilitates SCFA-driven modulation of lipid metabolism and improves locomotor performance in *Drosophila melanogaster*. These results substantiate its role as a functional food with probiotic attributes and metabolic health–promoting properties. Beyond its biological effects, the study provides scientific evidence supporting the broader relevance of traditional fermented foods within global sustainability frameworks. The enhancement of nutritional quality, bioavailability, and lipid regulatory capacity highlights the contribution of fermented rice gruel toward advancing food quality and dietary resilience. Furthermore, the observed improvements in metabolic health and lipid homeostasis emphasize the value of incorporating traditional fermented foods into sustainable nutrition models and public health initiatives aimed at improving metabolic health and overall well-being.

Conflict of Interest: The Authors declare no conflict of interest.

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