



Improving teff sourdough bread quality through *Lactobacillus plantarum* fermentation

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ABSTRACT

Background: Sourdough fermentation involving yeasts and lactic acid bacteria (LAB) enhances dough rheology, texture, and sensory quality. *Lactobacillus plantarum*, known for its acidification capacity and metabolic versatility, has shown potential to improve the structure of gluten-free bread. Teff (*Eragrostis tef*), despite its nutritional richness, presents technological challenges due to its lack of gluten.

Objective: To evaluate the influence of *Lactobacillus plantarum* on the fermentation performance and bread quality of teff dough.

Novelty: This study provides the first comprehensive rheofermentometer-based evaluation of *L. plantarum* in teff sourdough, demonstrating quantitative improvements in gas production, dough development, and crumb structure.

Methods: Teff doughs were prepared with and without *L. plantarum* B4 sourdough. Fermentation behavior was assessed using a Rheofermentometer F3 (Chopin, France). Viscosity was measured with a Visco Basic Plus rotational viscometer (Fungilab, Spain). Crumb texture was evaluated using a Structurometer ST-2. All analyses were conducted in triplicate.

Results: Incorporation of *L. plantarum* B4 significantly increased total gas production (872 mL vs. 65 mL) and maximum gas height (47.9 mm vs. 7.5 mm), while shortening dough development time (2 h 3 min vs. 3 h). Crumb analysis revealed higher total and plastic deformation, indicating a softer, more desirable crumb.

Conclusion: *Lactobacillus plantarum* B4 markedly improved teff dough fermentation dynamics and bread quality, supporting its use as a functional microbial ingredient in the development of gluten-free bread.

Keywords: *Lactobacillus plantarum* B4, teff flour, sourdough bread, dough fermentation, gas retention, crumb texture, gluten-free bread



Graphical abstract: Improving teff sourdough with *Lactobacillus plantarum* B4.

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INTRODUCTION

Breadmaking is one of the oldest biotechnological processes, and fermentation plays a fundamental role in determining dough rheology, flavor, texture, and nutritional quality [1]. Sourdough fermentation relies on a symbiotic community of yeasts and lactic acid bacteria (LAB) that enhance the organoleptic, functional, and shelf-life properties of bread compared with conventional yeast-based fermentation [2–3]. LAB species produce organic acids, exopolysaccharides, and antimicrobial metabolites that improve dough stability, gas retention, and resistance to spoilage organisms [4–5].

Among the various LAB strains, *Lactobacillus plantarum* has emerged as one of the most versatile, owing to its ability to adapt to diverse environments, generate bioactive metabolites, and contribute positively to bread structure and flavor [6–8].

In gluten-free breadmaking, the absence of gluten proteins creates challenges in dough elasticity, gas-holding capacity, and crumb softness, often resulting in products with inferior texture and reduced consumer acceptance [9–10]. Teff (*Eragrostis tef*), a nutrient-dense, gluten-free grain native to Ethiopia, has gained global attention due to its high content of iron, calcium, dietary

fiber, and essential amino acids [11–12]. However, the lack of gluten limits its bread-making performance, often producing dense crumbs and poor gas retention [13–14]. Traditional Ethiopian teff-based products, particularly injera (a fermented flatbread), have demonstrated the technological potential of teff through natural fermentation processes [15–17]. Microbial fermentation represents a promising strategy for improving the functional and technological qualities of teff-based doughs [18–19].

Several studies have shown that sourdough fermentation with *L. plantarum* can improve crumb softness, increase gas production, and enhance sensory properties in gluten-free breads [20–22]. Co-fermentation strategies involving *L. plantarum* and *Saccharomyces cerevisiae* have shown synergistic effects in improving bread quality and digestibility [23]. The bacterium's acidification capability lowers pH, promoting enzymatic activity and protein solubility, while its exopolysaccharide production enhances dough viscosity and crumb elasticity [24]. Recent advances in gluten-free sourdough technology have highlighted LAB fermentation as a key approach to overcoming structural limitations in cereals lacking gluten networks [25,26]. However, research specifically examining teff dough fermented with *L. plantarum* remains limited. Notably, no previous studies have conducted a detailed rheofermentometer-based analysis of its fermentation behavior [27]. This study addresses this gap by providing the first quantitative assessment of gas production, dough development, and crumb structure in teff dough fermented with *L. plantarum*.

Therefore, this study investigates the role of *Lactobacillus plantarum* B4 in improving the fermentation dynamics and structural properties of teff sourdough bread. By comparing dough development, gas retention, and crumb deformation in breads with and without *L. plantarum*, this research provides new insights

into microbial-assisted optimization of gluten-free bakery products.

MATERIALS AND METHODS

Dough Preparation: Teff flour was obtained from teff harvested in Ethiopia in 2024. *Lactiplantibacillus plantarum* B4 was sourced from the Collection of Microorganism Cultures at the St. Petersburg Branch of the Research Institute of the Baking Industry.

Two sourdough cultures were prepared using teff flour: one inoculated with *L. plantarum* B4 and one uninoculated control. Equal proportions of teff flour and sterile water were mixed to obtain the desired sourdough yield. The starter culture containing *L. plantarum* B4 was then incorporated into a batch of teff dough for fermentation.

Two dough formulations were prepared: one containing *L. plantarum* B4 and one control without the bacterial strain. Sample 1 consisted of 80 g teff flour, 20 g sourdough (with *L. plantarum* B4), 2 g active dry baker's yeast, 1.9 g salt, 5 g vegetable oil, and 60 cm³ water. Sample 2 was prepared using the same formulation, except that 20 g of sourdough without *L. plantarum* B4 was used.

Determination of Rheological Properties: To determine the rheological properties of teff dough with and without *L. plantarum* B4 sourdough, a mixture of 80 g teff flour, 20 g sourdough (with or without *L. plantarum* B4), 2 g active dry baker's yeast, 1.9 g salt, 5 g vegetable oil, and 60 cm³ water was used. The dynamic viscosity of the dough was measured using a Visco Basic Plus rotational viscometer (Fungilab S.A., Spain) equipped with two spindles, R2 and R3, at different rotational speeds (RPM). Measurement parameters and viscosity values were displayed on the viscometer's LCD screen. Each spindle was immersed in the flour–water mixture to the indicated mark, and viscosity was recorded. The analysis was conducted in triplicate, and results are presented as mean ± SD.

Fermentation Measurements: Dough was prepared by combining 80 g teff flour, 20 g sourdough (with or without *Lactobacillus plantarum* B4), 2 g active dry yeast, 1.9 g salt, 5 g vegetable oil, and 60 cm³ water to form a homogeneous mixture. Fermentation was assessed using a Rheofermentometer (Chopin F3, France) with 150 g of dough at 28 °C for 180 min. Dough expansion and gas production were recorded continuously, from which maximum dough development, maximum gas release, total gas volume, and gas retention coefficient were determined. The experiment was conducted in triplicate.

Baking and Crumb Structure Analysis: Dough, prepared with the same ingredient proportions as for fermentation

analysis, was baked in a DiLa convection oven (Germany) at 180 °C for 45 min. The bread-crumbs structure was assessed using a Structurometer ST-2 (Russia) on 2 cm cubic samples compressed at 1 mm/s, measuring total deformation, plastic deformation, and elastic recovery. Experiments were conducted in triplicate.

RESULTS AND DISCUSSION

Rheological Properties of Teff Dough: The results of viscosity measurements for teff dough with sourdough containing or lacking *Lactobacillus plantarum* under various measurement conditions are presented in the table below.

Table 1. Dynamic viscosity coefficient of teff dough with sourdough with and without *Lactobacillus plantarum*.

Spindle speed (rpm)	Dynamic viscosity coefficient, mPa. s			
	spindle R2		spindle R3	
	Dough with <i>Lactobacillus plantarum</i> B4	Dough without <i>Lactobacillus plantarum</i> B4	Dough with <i>Lactobacillus plantarum</i> B4	Dough without <i>Lactobacillus plantarum</i> B4
6	732.3 ± 0.13	1099.3± 0.17	2619.8± 0.22	1667.2± 0.12
10	409.5± 0.11	642.5± 0.15	1834.3± 0.15	1225.6± 0.18
12	356.5± 0.15	528.5± 0.21	1575.8± 0.13	907.7± 0.16
20	325.4± 0.23	340.3± 0.14	1010.3± 0.16	535.4± 0.13
30	278.7± 0.21	229.4± 0.16	790.2± 0.17	521.6± 0.19
50	258.7± 0.16	156.5± 0.20	713.6± 0.23	393.8± 0.16
60	243.8± 0.14	122.8± 0.18	632.3± 0.14	299.5± 0.18
100	232.6± 0.12	53.7± 0.16	252.1± 0.12	195.9± 0.14

Across all spindle speeds, dough containing *Lactobacillus plantarum* B4 consistently showed higher dynamic viscosity than dough without the strain. For both spindles (R2 and R3), viscosity decreased as spindle speed increased, confirming shear-thinning behavior. The difference between treatments was most pronounced at low spindle speeds, where the viscosity of the inoculated dough was up to 2–3 times higher than the control.

The higher viscosity in dough with *L. plantarum* B4 suggests that fermentation by this strain enhances dough structure, likely through acidification and modification of

protein–starch interactions. The pronounced viscosity at lower shear rates indicates improved dough cohesiveness and water-binding capacity. As spindle speed increased, both doughs exhibited reduced viscosity, typical of non-Newtonian, shear-thinning systems; however, the inoculated dough maintained consistently higher resistance to flow. These findings indicate that *L. plantarum* B4 positively influences dough rheology, potentially improving handling properties and final product texture.

Total titratable acidity of sourdough: The results of titratable acidity measurements of sourdough over time,

with and without *Lactobacillus plantarum*, are presented in Figure 1.

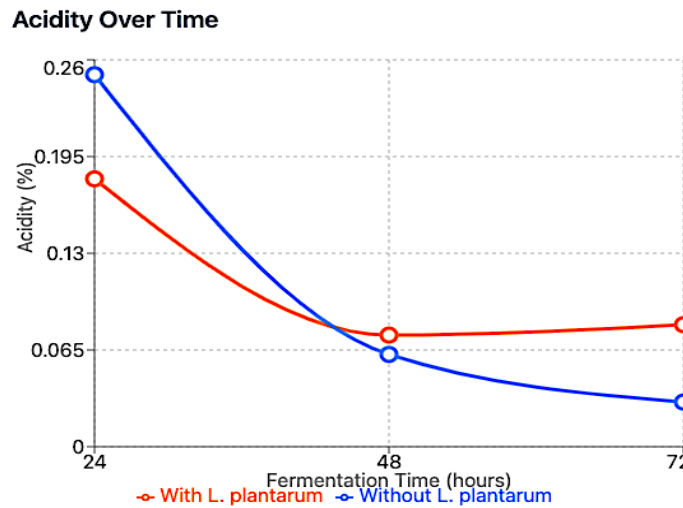


Figure 1. Acidity of sourdough with and without *Lactobacillus plantarum*

Acidity measurements of sourdough reveal a complex interaction between *Lactobacillus plantarum* B4 and the sourdough microbial ecosystem. Sourdough lacking *L. plantarum* B4 exhibited higher initial acidity compared to sourdough inoculated with *L. plantarum* B4, suggesting increased early activity of other acid-producing microorganisms in its absence. In contrast, *L. plantarum* B4 appears to moderate early acid production while sustaining acidification over prolonged fermentation. Dough without *L. plantarum* B4 showed a

rapid decline in acidity following the initial peak. These results indicate that the inclusion of *L. plantarum* B4 facilitates a more controlled and predictable acidity profile during sourdough fermentation, which is advantageous for consistent bread quality and flavor development.

Dough development measurement: Dough development in teff sourdough, with and without *Lactobacillus plantarum* B4, is shown in Figure 2.

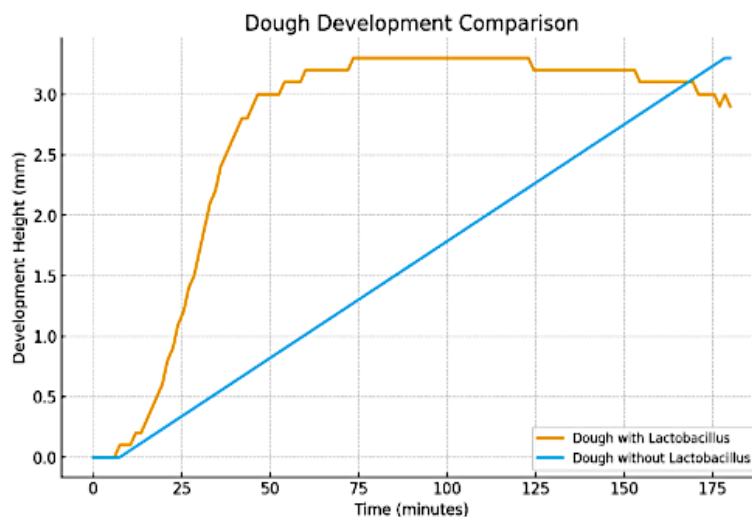


Figure 2. Teff dough development with the addition of sourdough with and without *Lactobacillus plantarum*

Dough development measurements (Figure 2) demonstrated clear differences between the samples. Dough containing *Lactobacillus plantarum* B4 showed a rapid increase in development height within the first 40 minutes, indicating accelerated network formation and enhanced extensibility. In contrast, dough without *L. plantarum* B4 exhibited minimal height increase after 180 minutes, reflecting slower hydration and reduced viscoelasticity. The accelerated development in the *L.*

plantarum B4-inoculated dough is likely due to acidification during fermentation, which lowers pH, enhances hydration, and promotes protein–protein interactions, thereby improving dough rheology and elasticity [28].

Gas release characteristics: The results of gas release measurements during teff dough fermentation, with and without *Lactobacillus plantarum* B4, are presented in Figure 3.

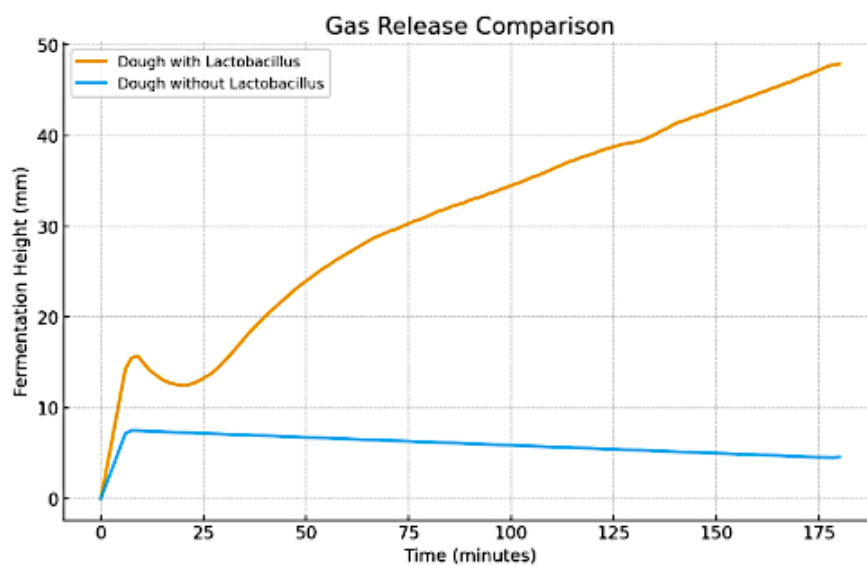


Figure 3. Dynamics of CO₂ emission during fermentation of teff dough with the addition of sourdough with and without *Lactobacillus plantarum*.

The gas-release measurements of teff dough during fermentation (Figure 3) demonstrate significantly greater CO₂ evolution in dough containing *Lactobacillus plantarum* B4, reaching a maximum gas height of 47.9 mm after 180 minutes. In comparison, the dough without *Lactobacillus plantarum* B4 reached only 7.5 mm over the same period, indicating minimal fermentation activity. These results highlight the synergistic role of the yeast–lactic acid bacterial interaction, in which *Lactobacillus* B4

enhances yeast performance by releasing amino acids and vitamins, thereby accelerating sugar metabolism [29]. The higher CO₂ evolution in the *Lactobacillus plantarum* B4-enriched dough reflects more efficient gas production.

Gas retention behavior: The results of gas retention measurements during teff dough fermentation, with and without *Lactobacillus plantarum* B4, are presented in Figure 4.

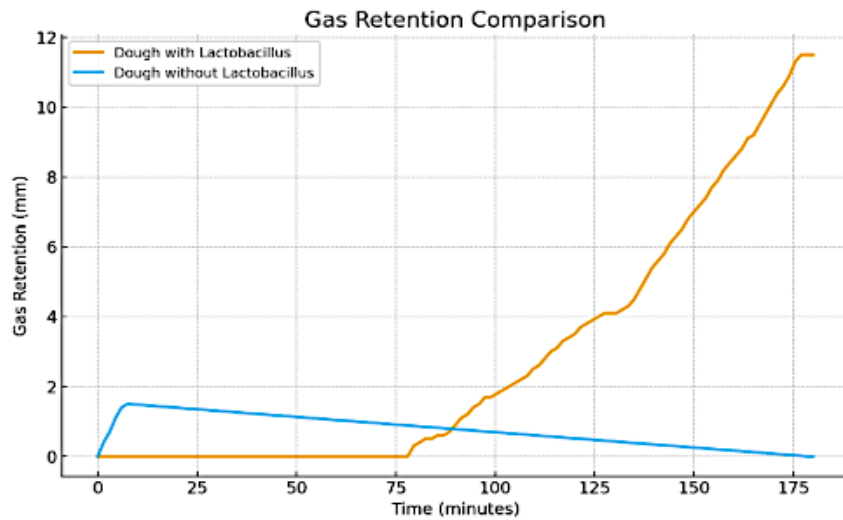


Figure 4. Carbon dioxide retention during fermentation of teff dough with the addition of sourdough with and without *Lactobacillus plantarum*

The gas retention analysis (Figure 4) shows that teff dough enriched with *Lactobacillus plantarum* B4 retained substantially more gas throughout fermentation, maintaining high dough stability. The difference between the direct and indirect development curves remained narrow, confirming efficient CO₂ entrapment within the dough matrix. In contrast, teff dough without *L. plantarum* B4 exhibited poor retention, with most gas escaping early in fermentation. This indicates weaker

structural integrity and reduced dough strength, which can lead to lower loaf volume in breadmaking. The structural reinforcement observed in *L. plantarum* B4 dough is likely due to acid-induced strengthening of protein cross-links [30].

Gas retention coefficient: The gas retention coefficient of teff dough during fermentation, with and without *Lactobacillus plantarum* B4, is presented in Figure 5.

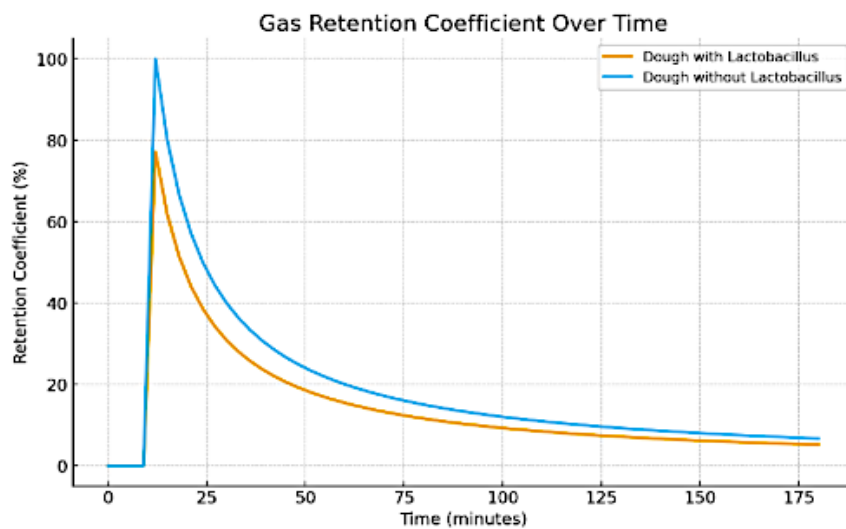


Figure 5. Gas retention coefficient during fermentation of teff dough with the addition of sourdough with and without *Lactobacillus plantarum*

The gas retention coefficient (Figure 5) quantifies the dough's ability to retain CO₂ during fermentation. Both doughs exhibited high gas retention (>90%), indicating that the dough matrix effectively confined the produced CO₂. The dough without *Lactobacillus plantarum* retained slightly more gas (92.3%) than the *Lactobacillus*-fermented dough (90.7%), suggesting a slight reduction in gas retention, potentially due to

acidification or bacterial proteolytic activity that affected protein structure. However, this modest decrease did not inhibit overall dough expansion or gas production.

Bread crumb structure: Figure 6 shows the structural deformation analysis results for teff bread produced from dough prepared with and without *Lactobacillus plantarum* B4.

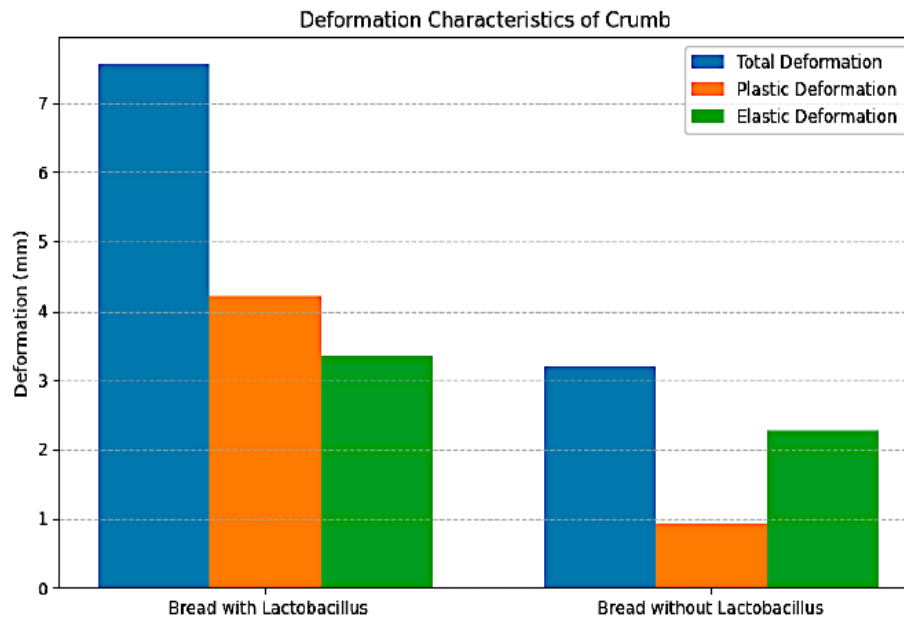


Figure 6. Structural deformation analysis of bread from dough with and without *Lactobacillus plantarum*

The structural behavior of teff bread enriched with *Lactobacillus plantarum* B4 differs markedly from that reported in previous studies of LAB-fermented gluten-free breads, which generally show reduced crumb deformation and increased firmness due to acidification-induced matrix strengthening and exopolysaccharide (EPS) production. In contrast, the present study observed a significant increase in both total and plastic deformation, indicating a considerably softer and more compressible crumb structure than the control and compared with typical values reported in the literature. The control (non-fermented) bread exhibited lower plastic deformation but slightly higher elastic recovery,

suggesting a firmer and more resilient crumb than is commonly found in non-sourdough gluten-free breads. These findings suggest that *L. plantarum* B4 modulates the teff crumb through strain-specific biochemical interactions distinct from those previously documented, providing novel evidence that certain LAB strains may soften rather than reinforce gluten-free crumb structures.

CONCLUSION

This study provides the first comprehensive rheofermentometer-based evaluation of teff dough fermented with *Lactobacillus plantarum* B4, offering quantitative evidence that advances current

understanding of gluten-free sourdough systems. Unlike previous work on rice, sorghum, and buckwheat—where LAB typically produce only modest increases in gas formation—*L. plantarum* B4 yielded substantially greater improvements in gas evolution, dough development, and crumb quality.

Fermentation with *L. plantarum* B4 produced a markedly high total gas volume (872 mL), a maximum gas release height of 47.9 mm, and a faster dough development time (2 h 3 min vs. 3 h), alongside significantly enhanced crumb softness. These combined improvements in gas production, CO₂ retention, fermentative efficiency, and texture are particularly notable for a gluten-free substrate and have not been previously reported for teff dough.

Overall, *L. plantarum* B4 markedly improved teff dough functionality, bread structure, and technological performance, surpassing outcomes reported in other gluten-free sourdough systems. Future studies should explore industrial-scale applications, multi-strain LAB strategies, and sensory evaluation to strengthen the link between instrumental data and consumer perception.

Conflict of interest statement: The authors declare no conflict of interest.

Abbreviations: LAB – Lactic acid bacteria; *L. plantarum* – *Lactobacillus plantarum*; CO₂ – Carbon dioxide; RPM – Revolutions per minute; SD – Standard deviation; cm³ – Cubic centimeter; EPS – Exopolysaccharides.

Contribution: G.A.Y., P.G., and A.D. performed the experimental work; G.A.Y. and V.S. carried out data processing and instrumental analyses using the Rheofermentometer F3, Structurometer ST-2, and Visco Basic Plus; M.S., E.K., M.I., and T.A. contributed to manuscript writing, formatting, and editing; and N.B. provided study organization and overall scientific supervision.

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