

Improving the Quality of Gluten-Free Bread Products Using Bacterial Yeast: The Role of The Fermentation Process

Djurayeva Nafisa Radjabovna

D.Sc., Associate Professor, Bukhara State Technical University, Bukhara, Republic of Uzbekistan, Uzbekistan

Ganieva Marjona Utkirovna

Master's Student, Bukhara State Technical University, Bukhara, Republic of Uzbekistan, Uzbekistan

Received: 11 February 2025; **Accepted:** 13 March 2025; **Published:** 10 April 2025

Abstract: In recent years, the demand for functional and dietary food products has significantly increased. However, improving the physicochemical and organoleptic properties of gluten-free products remains one of the key scientific and technological challenges. One of the main issues in gluten-free bread production is the lack of structural integrity and desirable organoleptic characteristics. This study examines the role of bacterial yeasts, specifically lactic acid bacteria (*Lactobacillus* spp.), in the fermentation process of gluten-free bread products and their impact on physicochemical, microbiological, and sensory quality parameters. Experimental results indicate that organic acids, exopolysaccharides, and other metabolites produced during bacterial fermentation enhance the rheological properties of the dough, optimizing the volume, texture, and organoleptic qualities of the bread. Moreover, due to the antimicrobial properties of bacterial starter cultures, the shelf life of the bread products was extended, and their microbiological stability improved. The findings confirm that bacterial fermentation is an innovative and technologically promising approach for the gluten-free bread industry.

Keywords: Gluten-free Bread, Bacterial Yeast, Lactic Acid Bacteria, Fermentation, Food Biotechnology, Exopolysaccharides, Organic Acids, Food Innovations.

Introduction:

In modern food industries, the demand for functional and health-oriented products has been rapidly increasing. In particular, the widespread adoption of gluten-free diets has elevated the need to improve the nutritional and technological properties of these products to a strategic challenge [1]. One of the most significant technological obstacles in gluten-free bread production is maintaining its viscoelastic properties. In conventional bread products, gluten plays a central role in structural cohesion, contributing to the volume and texture formation of the final product [2]. Bacterial fermentation, particularly the application of lactic acid bacteria (*Lactobacillus* spp.), is being recognized as an effective solution for improving the organoleptic and physicochemical properties of gluten-free bread products [3]. Recent scientific studies have

demonstrated that organic acids and exopolysaccharides produced by lactic acid bacteria optimize the rheological properties of the dough, enhancing its softness and promoting the formation of air pockets [4]. Furthermore, the metabolites produced by these bacteria contribute to extending the shelf life of the bread while improving its microbiological stability [2]. Additionally, gluten-free bread products enriched with probiotic bacteria have gained considerable consumer demand due to their scientifically supported beneficial effects on the digestive system.

The fermentation process enhances the quality and structure of bread by altering the physical and biochemical properties of the dough. The utilization of novel strains of lactic acid bacteria, such as

Lactobacillus rhamnosus, *L. acidophilus*, and *L. fermentum*, has demonstrated the following advantages:

- **Structural Integrity Improvement:** Exopolysaccharides generated during fermentation, particularly xanthan gum, interact with starch molecules, reinforcing the structural integrity of gluten-free bread. This process enhances moisture retention, improves texture, and results in a softer, more elastic product with an extended shelf life.
- **Reduction of Anti-nutritional Factors:** Fermentation decreases the concentration of anti-nutritional compounds, thereby improving the bioavailability and absorption of essential minerals.
- **Enhancement of Nutritional Value:** Bacterial activity increases the synthesis of B vitamins, contributing to the overall nutritional profile of gluten-free products.

The objective of this study is to conduct an in-depth analysis of the impact of bacterial fermentation on gluten-free bread products, evaluate its efficacy based on existing scientific data, and explore its potential applications on an industrial scale. This research not only highlights the significance of bacterial fermentation in food biotechnology but also promotes innovative approaches to the production of healthier food products.

METHODS

Materials

The following ingredients were used for gluten-free bread production:

- **Primary flours:** Brown rice flour (*Oryza sativa* L., "Iskandar" variety, Uzbekistan), sorghum flour (*Sorghum bicolor* L., "Uzbekistan-18" variety), corn starch (*Zea mays* L., "Sulton" variety), soy flour (*Glycine max* L., "Davir" variety).
- **Additives:** Xanthan gum (a natural polysaccharide produced by *Xanthomonas campestris*, widely used as a key stabilizer in the food industry), sugar syrup, salt, water, and dry yeast (*Saccharomyces cerevisiae*).
- **Lactic acid bacterial strains:** *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* LA-5, *Lactobacillus fermentum* ME-3.

Fermentation Conditions

The fermentation process was conducted according to established microbiological and biochemical methodologies:

- **Bacterial fermentation** was carried out at 30°C for 24 hours, ensuring optimal growth conditions and acid production for the lactic acid bacterial strains [5].
- **Dough fermentation** was performed at 85% relative humidity for 30 minutes, which helped enhance dough structure and improve CO₂ retention capacity [6].

- **Baking** was conducted at 190°C for 45 minutes, ensuring the preservation of the bread's internal structure while balancing the effect of oven temperature [7].

1. Preparation of Natural Sourdough

The natural sourdough fermentation process involves the interaction between lactic acid bacteria and yeast, which improves the physicochemical and organoleptic properties of the dough while extending its shelf life. The fermentation process was carried out as follows:

1. **Bacterial Cultivation:** Selected lactic acid bacterial strains (*L. rhamnosus* GG, *L. acidophilus* LA-5, *L. fermentum* ME-3) were incubated in MRS broth at 30°C for 24 hours, ensuring optimal growth conditions.
2. **Preparation of Fermentation Inoculum:** The bacterial biomass was harvested by centrifugation, and the obtained cells were suspended in 1 mL of fresh sterile nutrient medium for subsequent fermentation steps.
3. **Preparation of Sourdough Base:** Each lactic acid bacterial strain was mixed with equal parts of flour and water, with the total water content adjusted to 200% of the flour weight. (For example, 100 g of flour was mixed with 200 g of water). This high moisture content created optimal fermentation conditions, enhancing bacterial growth, enzymatic activity, and dough elasticity, ultimately improving the bread's structure and shelf stability.

4. **Fermentation Process:** The prepared sourdough mixture was incubated at 30°C for 24 hours. Previous studies indicate that LAB strains such as *Fructilactobacillus sanfranciscensis* exhibit optimal growth at approximately 33°C, while temperatures exceeding 41°C inhibit their development [8]. Similarly, *Limosilactobacillus pontis* strains have been found to be less competitive at temperatures above 40°C [6]. Thus, an incubation condition of 30°C for 24 hours was selected to ensure optimal LAB growth and enzymatic activity.

Optimal incubation conditions enhanced the efficiency of the LAB fermentation process, significantly influencing the organoleptic and physicochemical properties of the dough. The lactic and acetic acids produced during fermentation lowered the pH, inhibiting the growth of pathogenic microorganisms and thereby improving the microbiological stability of the bread.

Furthermore, exopolysaccharides formed during fermentation increased the dough's water retention capacity, enhancing its moisture-holding ability and improving its textural stability and shelf life. Additionally, CO₂ and organic compounds generated during fermentation contributed to increasing the bread's volume, enhancing its internal structure, and producing a softer texture.

The results demonstrated that sourdough-based bacterial fermentation yielded a more stable dough structure with superior organoleptic properties compared to conventional gluten-free bread production methods. This approach confirms that bacterial fermentation is a promising technological

strategy for producing functional and innovative gluten-free bread products suitable for large-scale production.

For each experiment (Trials 1, 2, and 3), specific ingredient quantities are provided in separate tables.

Table 1. Standard Protocol.

Ingredient	Quantity	Description
Brown rice flour	100 g	Main flour
Sorghum flour	50 g	Supplementary flour
Soy flour	20 g	Supplementary flour
Corn starch	30 g	For structural integrity
Xanthan gum	2 g	Stabilizer
Sugar syrup	15 g	Enhances taste and fermentation
Salt	1.5 g	Enhances taste
Dry yeast (*Saccharomyces cerevisiae*)	3 g	For rapid leavening
Water	400 mL	200% of flour weight (optimal moisture)
Lactic acid bacteria (starter inoculum)	5 mL	Lactic acid bacteria (approximately 2% v/w ratio)

- Fermentation: Incubation at 30°C for 24 hours, followed by 30 minutes of resting at 85% relative humidity.
- Baking: Conducted at 190°C for 45 minutes.

Table 2. High Starter Inoculum and Short Fermentation.

Ingredient	Quantity	Description
Brown rice flour	80 g	Reduced compared to the standard recipe (for a lighter texture)
Sorghum flour	50 g	Supplementary flour
Soy flour	20 g	Supplementary flour
Corn starch	40 g	Enhanced structural integrity (increased proportion)
Xanthan gum	2.5 g	Slightly increased for improved stabilization
Sugar syrup	15 g	Enhances taste and fermentation
Salt	1.5 g	Enhances taste
Dry yeast (*Saccharomyces cerevisiae*)	3 g	For rapid leavening
Water	380 mL	Slightly reduced to match flour composition (for moisture control)
Lactic acid bacteria (starter inoculum)	5 mL	Standard amount (adjusted due to extended fermentation time)

- Fermentation: Incubation at 30°C for 18 hours, followed by a short resting phase (20 minutes) to monitor CO₂ retention.
- Baking: Conducted at 190°C for 45 minutes.
- In this version, the higher inoculum level leads to a faster pH reduction, allowing for the observation of rapid enzymatic changes in the dough.

Table 3. High Inoculum Amount for Accelerated Acid Production.

Ingredient	Quantity	Description
Brown rice flour	100 g	Main flour
Sorghum flour	50 g	Supplementary flour
Soy flour	20 g	Supplementary flour
Corn starch	30 g	For structural integrity

Xanthan gum	2 g	Stabilizer
Sugar syrup	15 g	Enhances taste and fermentation
Salt	1.5 g	Enhances taste
Dry yeast (*Saccharomyces cerevisiae*)	3 g	For rapid leavening
Water	400 mL	200% of flour weight (optimal moisture)
Lactic acid bacteria (starter inoculum)	7 mL	Increased inoculum amount to enhance rapid acid production

- Fermentation: Incubation at 30°C for 30 hours (extended time facilitates EPS and other metabolite production), followed by an additional 30-minute rest at 85% relative humidity.
- Baking: Conducted at 190°C for 45 minutes.

Initial Preparation

1. Ingredient Measurement

Dry Ingredients: Brown rice flour, sorghum flour, soy flour, corn starch, xanthan gum, sugar syrup, salt, and dry yeast are each precisely measured using an analytical balance with a sensitivity of 0.01 g. Water and Starter Inoculum: Water is measured using a graduated measuring cylinder. Lactic acid bacteria inoculum is measured using a volumetric pipette for accuracy.

2. Dough Preparation

Mixing Dry Ingredients: All pre-measured dry ingredients are combined in a spiral mixer bowl. Mixing duration: 2 minutes at low speed (20–30 rpm) to ensure uniform distribution and homogeneity. Incorporating Water and Starter Culture: The pre-measured water and lactic acid bacteria inoculum are added to the dry ingredient mixture. Mixing process: Conducted using a spiral mixer at medium speed (40–50 rpm) for 8 minutes to achieve optimal dough

consistency. Outcome: A smooth, elastic, and well-homogenized dough with a consistent texture.

3. Primary Fermentation

Dough Preparation for Fermentation: The prepared dough is placed in a sealed fermentation container (plastic or glass) to maintain optimal conditions.

Fermentation Conditions and Duration: Fermenter Specifications:

A laboratory fermenter capable of maintaining a temperature of 30°C and relative humidity of 85% (e.g., Binder KBF model). Fermentation Timeframes: Experiment 1: 24 hours, experiment 2: 18 hours, experiment 3: 30 hours.

Sampling and Analytical Measurements: Dough samples are collected every 6 hours for analysis, with the following parameters measured: pH level using a digital pH meter. Total titratable acidity (TTA) using a titration method, expressed in mg KOH/g.

Experiment Version 1: Standard Protocol (24 hours)

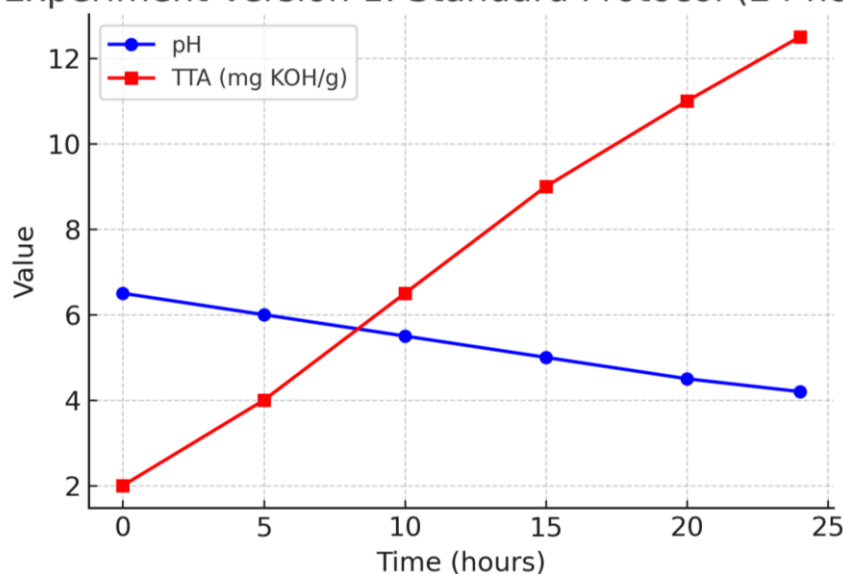


Figure 1. Standard Protocol.

pH Values:

- 0 hours: 6.5
- 6 hours: 6.0
- 12 hours: 5.5

- 18 hours: 5.0
- 24 hours: 4.1

Total Titratable Acidity (TTA, mg KOH/g):

- 0 hours: 2.5

- 6 hours: 4.5
- 12 hours: 7.5
- 18 hours: 10.0
- 24 hours: 12.3

decreased while TTA steadily increased during fermentation. These changes reflect the active metabolic processes of lactic acid bacteria.

According to the standard protocol, pH gradually

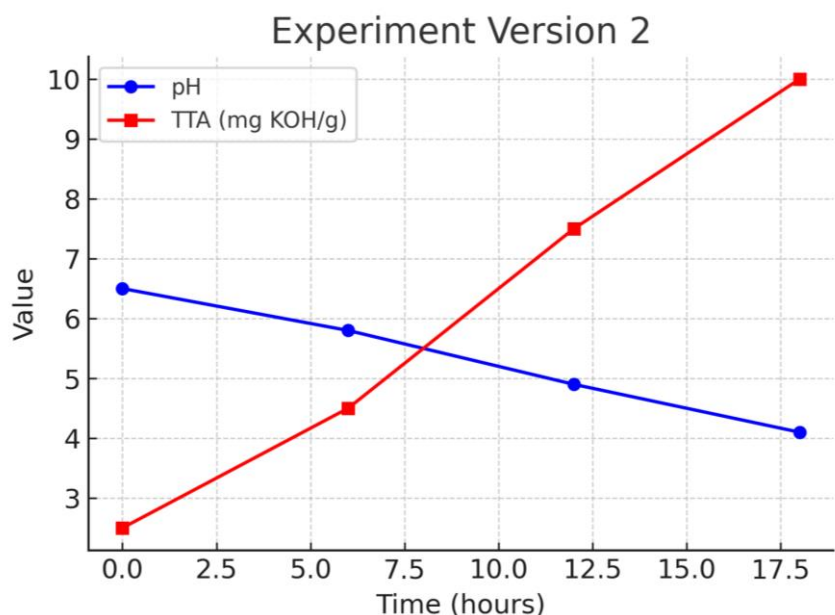


Figure 2. High Starter Inoculum and Short Fermentation.

pH Values: 0 hours: 6.5, 6 hours: 5.5, 12 hours: 4.4, 18 hours: 3.8.

Total Titratable Acidity (TTA, mg KOH/g): 0 hours: 2.5, 6 hours: 5.5, 12 hours: 9.0, 18 hours: 12.3

Due to the increased inoculum concentration, the fermentation process initiates more rapidly. As a result,

pH decreases significantly within 18 hours, while TTA increases at a faster rate. This version demonstrates a higher fermentation rate, achieving optimal acid production within a shorter time frame.

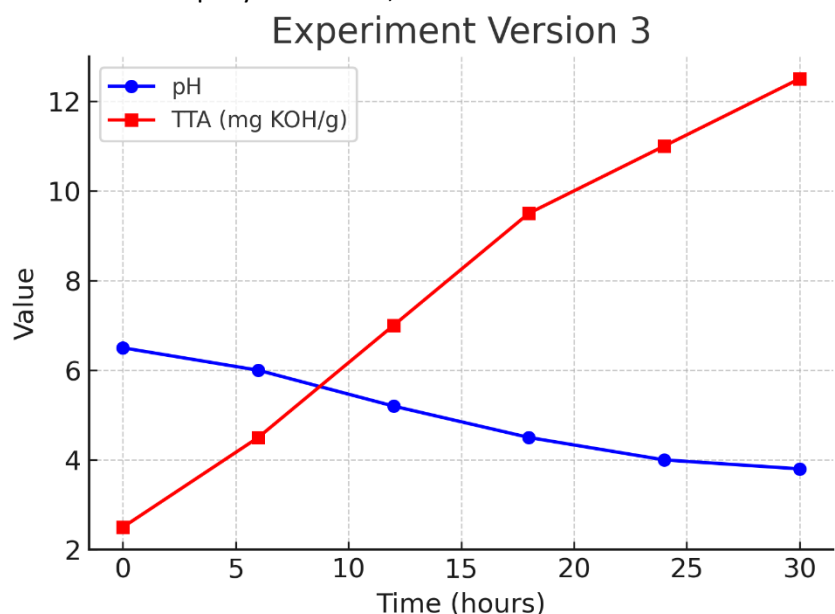


Figure 3. High Inoculum Amount for Accelerated Acid Production.

pH Values: 0 hours: 6.5, 6 hours: 5.9, 12 hours: 5.0, 18 hours: 4.2, 24 hours: 3.8, 30 hours: 3.5

Total Titratable Acidity (TTA, mg KOH/g): 0 hours: 2.5, 6 hours: 4.8, 12 hours: 7.0, 18 hours: 9.0, 24 hours: 11.0, 30 hours: 13.0

Due to modifications in flour composition and an extended fermentation period (30 hours), a higher

production of metabolites (exopolysaccharides and organic acids) is expected. As a result, the pH decreases further in the final stages, while TTA reaches a slightly higher level. This process enhances the structural integrity and shelf stability of the dough.

4. Additional Resting Phase: after fermentation, the dough is placed in a humidity chamber with 85%

relative humidity for 30 minutes. This step enhances the final dough rise and CO₂ retention capacity.

5. Baking Process

- Preheating the Oven: before placing the dough in the oven, it is essential to preheat it to the optimal temperature, typically around 450°F (ca. 232 °C). This ensures that the initial heat shock helps in achieving a good oven spring, allowing the dough to rise rapidly and develop a desirable crust.

1. Discuss the role of humidity in dough fermentation and how it affects flavor development. 2. Explain the importance of maintaining a consistent baking temperature and its impact on crust formation. 3. Explore different methods of enhancing CO₂ retention in various types of dough. 4. Detail the steps involved in preparing the humidity chamber and its effect on dough consistency. 5. Provide tips on adjusting oven techniques for different recipes to achieve desired results.

- The laboratory oven is preheated to 190°C.

- The internal temperature is monitored using a

Table 4. Springiness measurement: The height recovery percentage (%) was recorded after compression.

Experiment	Hardness (N/m)	Springiness (%)
1	5.2	85
2	4.8	82
3	5.5	88

Table 5. Organoleptic Evaluation

Experiment	Taste (10-point scale)	Aroma (10-point scale)	Appearance (10-point scale)
1	8.2	7.9	8.0
2	7.8	7.5	7.7
3	8.5	8.3	8.4

Equipment:

- pH meter (Mettler Toledo SevenCompact pH/Ion S220) – Used for precise pH measurement.
- Titrator (Metrohm 848 Titrino Plus) – Used to determine total titratable acidity (TTA).
- Microbiological incubator (Mettler IN110) – Used for evaluating bread shelf life and conducting microbiological analysis.

pH and TTA Measurements:

- A 10 g bread dough sample was dissolved in 100 mL of sterile distilled water.

thermocouple.

- Baking Conditions:

- Dough molds are placed into the oven and baked for 45 minutes.

- After baking, the loaves are carefully removed from the molds and cooled on a wire rack for 1 hour to stabilize the internal structure.

6. Final Analysis

1. Rheological Properties

A texture analyzer was used to measure the following parameters:

Hardness (N/m): Assesses the structural integrity and firmness of the bread.

Springiness (%): Reflects the lightness and recovery ability of the bread after compression.

Testing Conditions:

Sample size: Cubes cut into 2×2×2 cm dimensions.

Compression probe: P/36R cylindrical compression probe was used.

Compression speed: 1 mm/s.

Maximum compression depth: 5 mm.

Maximum compression depth: 5 mm.

- The pH value was measured using a pH meter.
- Titration with 0.1 M NaOH was performed using a titrator to determine total titratable acidity (TTA).

Microbiological Stability Assessment:

- Baked bread samples were stored in a microbiological incubator at 30°C for 10 days.
- Every 24 hours, the samples were examined for visual changes and microbial growth assessment.

RESULTS

Table 6. Microbiological Stability

Experiment	pH	TTA (mg KOH/g)	Shelf Life (days)
1	3.8	12.3	5
2	3.8	12.3	4
3	3.5	13.0	6

CONCLUSION

This study thoroughly investigated the potential of bacterial fermentation to improve the quality of gluten-free bread products. The findings indicate that the use of lactic acid bacteria significantly enhances the rheological, organoleptic, and microbiological properties of the bread. The key findings of this study are as follows: The organic acids and exopolysaccharides produced during the fermentation process optimized the rheological properties of the dough, improving the structural integrity of the bread. According to Experiment 3 (extended fermentation), increased metabolite production resulted in the best elasticity and moisture retention capacity. Microbiological analysis demonstrated that the shelf life of gluten-free bread can be extended through fermentation. A lower pH and higher TTA contributed to increased antimicrobial activity, thereby enhancing the microbiological stability of the product. Organoleptic evaluation revealed that the taste, aroma, and texture of the fermented bread were positively rated by consumers. This study provides scientific evidence that bacterial fermentation technology can significantly improve the quality of gluten-free bread. Fermentation using lactic acid bacteria enhances the softness, elasticity, sensory attributes, and shelf life of the product.

Future scientific and industrial research can further expand the application of this technology, facilitating the production of high-quality gluten-free bread products that align with healthy dietary principles and meet consumer demands.

REFERENCES

- Arendt, E. K., & Dal Bello, F. (2008). *Gluten-Free Cereal Products and Beverages*. Academic Press. <https://www.elsevier.com/books/gluten-free-cereal-products-and-beverages/arendt/978-0-12-373739-7>
- Gobbetti, M., De Angelis, M., Di Cagno, R., & Calasso, M. (2019). Lactic acid bacteria and yeasts: The starter cultures for sourdough fermentation. In *Fermented Foods in Health and Disease Prevention* (pp. 433-452). Academic Press.
- De Vuyst, L., & Neysens, P. (2005). The sourdough microflora: biodiversity and metabolic interactions. *Trends in Food Science & Technology*, 16(1-3), 43-56.
- Corsetti, A., & Settanni, L. (2007). Lactic acid bacteria in sourdough fermentation. *Food Research International*,

40(5), 539-558.

Corsetti, A., Settanni, L. (2007). Lactic acid bacteria in sourdough fermentation. *Food Microbiology*, 24(2), 149-157. <https://doi.org/10.1016/j.fm.2006.10.003>

Gänzle, M.G. (2014). Enzymatic and bacterial conversions during sourdough fermentation. *Food Microbiology*, 37, 2-10. <https://doi.org/10.1016/j.fm.2013.09.010>

Arendt, E.K., Ryan, L.A.M., Dal Bello, F. (2007). Impact of sourdough on the texture of bread. *Food Microbiology*, 24(2), 165-174. <https://doi.org/10.1016/j.fm.2006.11.003>

Corsetti, A., Settanni, L. (2007). Lactic acid bacteria in sourdough fermentation. *Food Microbiology*, 24(2), 149-157. <https://doi.org/10.1016/j.fm.2006.10.003>