

Lactic Acid Fermentation Enhances Microbial Safety and Iron Bioavailability of Common Beans Under Ambient Storage in Rwanda

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Abstract

Background

In Rwanda and across Sub-Saharan Africa, *Phaseolus vulgaris* L. serves as an important source of essential proteins and micronutrients. However, its nutritional benefits are compromised by post-harvest spoilage and the limited bioavailability of iron, which is affected by antinutritional factors in environments with scarce resources.

Methods: In Kamonyi District, Rwanda, common beans of the RWR 10 variety were subjected to fermentation in airtight, sterile glass containers at a temperature of 30 ± 1 °C for durations of 0, 12, 24, and 36 hours. The microbiological assessments conducted included total plate count (TPC), lactic acid bacteria (LAB) enumeration on MRS agar, and *Salmonella* spp. detection following ISO standards. Iron bioavailability and physicochemical properties were evaluated through in vitro gastrointestinal digestion using atomic absorption spectrophotometry. Statistical analysis was performed using one-way ANOVA and the Tukey HSD post-hoc test with a significance level of $\alpha=0.05$.

Findings: Fermentation caused acidification with pH reducing between 6.2 ± 0.1 and 4.1 ± 0.1 ($p<0.001$), forming an acidic environment preventing pathogenic and spoilage microorganisms. The total plates reduced from 6.8 ± 0.2 to 3.7 ± 0.1 log CFU/g, equivalent to 54.4% microbial reduction at 36 hours. The lactic acid bacteria showed an increase from 2.1 ± 0.1 to 7.4 ± 0.2 log CFU/g, serving as competition exclusion and biopreservation. No *Salmonella* spp. was found during the study. Iron bioavailability increased ($8.5\pm 0.4\%$ to $15.1\pm 0.7\%$, $p<0.001$) due to phytate breakdown by microbial phytases and Bornu mineral acid chelation. A 24-hour fermentation provided optimal balance between microbial safety (TPC: 4.2 ± 0.1 log CFU/g), nutritional fortification (iron bioavailability: $13.4\pm 0.6\%$), and sensorial acceptability.

Conclusion:

Fermentation-preconditioning is a sustainable, low-cost intervention that improves microbial safety and iron bioavailability in common beans during storage. The technology can enhance food security and iron deficiency anemia in Sub-Saharan Africa by extending shelf-life and nutritional quality.

1. INTRODUCTION

Phaseolus Vulgaris L. (common bean) is a crucial staple food to millions of human beings around the world, especially in developing countries, where it provides a major source of protein, fiber, and even important micronutrients [1], [2], [3]. The beans in Rwanda are of the utmost importance as they form a major part of the protein consumed by the population and influence the national food security [3], [4], [5]. Although they have nutritional values, cooking beans, distribution, and storage pose a lot of challenges, particularly in areas where refrigeration facilities are not readily available. These issues include short shelf-life, food safety hazards, and low nutritional value because of the existence of anti-nutritional

factors[6], [7]. High perishability of the beans is one of the most urgent problems of cooked beans. Beans that are cooked at higher temperatures will face a high rate of spoilage by various microorganisms when kept at ambient temperatures, resulting in high post-harvest losses, and food supply is low [8]. This is one of the main issues in most of the Rwandan families, where refrigeration is not a luxury. Not only are there economic losses caused by the spoilage of cooked beans, but also food insecurity to the families that rely on the staple as their daily nutrition. Moreover, the misplacement of cooked beans provides an ideal environment where the growth of pathogenic bacteria can take place, which is a significant threat to food safety and human health [9], [10]. When the contaminated beans are consumed, foodborne illnesses may arise, which is a significant source of morbidity and mortality in most regions of the world[11], [12]. The safety of cooked beans is a community health concern in Rwanda, where the consumption of beans is predominant [4], [13], [14]. In addition to preservation and safety, the nutritional potential of beans is usually suppressed by various anti-nutritional compounds, the most prominent of which is phytic acid [15], [16]. Phytic acid, which is present in legumes, forms a very strong bond with vital minerals like iron and zinc, creating insoluble complexes that cannot be absorbed by the human body[17], [18], [19]. This antagonistic impact strongly diminishes the bioavailability of these important micronutrients, which predisposes a high proportion of mineral deficiencies in groups that consume a large portion of plant-based food [20]. In particular, iron deficiency is a common population health issue, and its consequences are anemia and other severe health issues. Although beans contain iron, their bioavailability in most of the bean varieties and, more so, in the colored ones, is poor, thereby limiting their ability to combat iron deficiency[21], [22]. To these complex problems, there is an increase in the study of traditional and sustainable food processing methodologies to help increase shelf-life, safety, and nutritional quality of cooked beans. An ancient tradition of African food culture, fermentation has turned out to be a promising approach [23], [24]. Beneficial microorganisms like lactic acid bacteria (LAB) can be used in fermentation to enhance food preservation by promoting the production of organic acids and other antimicrobial substances to hinder the growth of spoilage and pathogenic microorganisms [25], [26]. This food preservation technique is natural, and it is capable of prolonging the shelf-life of foods without refrigeration, which makes it an especially applicable technology to resource-limited environments[27], [28]. In addition, it has been demonstrated that the alteration in the nutritional profile of food is significant and is due to fermentation. Anti-nutritional factors such as phytic acid can be reduced through the use of the enzymatic activity of fermenting microorganisms to enhance the bioavailability of minerals such as iron and zinc [29], [30]. As an example, researchers have also shown that fermentation can substantially lower phytate levels in different legumes, which is likely to increase the uptake of minerals[31], [32]. Although other works have revealed inconsistent findings on the direct effect of fermentation on in vitro iron solubility, phytase degradation is a very important initial step towards enhancing iron bioavailability [31], [33]. Moreover, fermentation may increase the nutritional value of foods by converting vitamins and creating bioactive peptides and increasing the digestibility of proteins and carbohydrates [34]. The method of using fermentation as a pre-conditioning measure before storing cooked beans has a lot of prospects in solving the particular problems encountered in Rwanda[35]. Through fermenting beans, one possibly can produce a healthier product, which has a longer shelf-life at ambient temperatures, and is safer, as well.

This practice conforms to the fact that sustainable and culturally-fit solutions are necessary to enhance food security and nutrition in the area. Natural preservatives, including spices as well as fermentation, may also help to improve the shelf-life and the sensory properties of the end product [36], [37]. Thus, the study will explore the impact of fermentation-preconditioning on shelf-life, safety, and bioavailability of iron in non-refrigerated packages of Rwandan cooked beans. The paper will discuss the optimization of fermentation, possibly alongside other conventional preservation strategies, in order to come up with a safe, stable, and nutritionally-enriched bean product that would be marketable in the Rwandan setting. Through its contribution to the creation of practical and sustainable solutions to the critical problems of spoilage, food safety, and micronutrient malnutrition, this research should add to the value of the beans to the Rwandan diet. The research results of this study may have important implications for food security, human health, and the livelihood of people in Rwanda and other places where beans are a food commodity

2. MATERIALS AND METHODS

2.1 Study Design and Experimental Framework

This study employed a controlled laboratory experiment structured around a completely randomized design (CRD) with four treatment groups, each subjected to a different fermentation duration, and three independent replications per group ($n = 3$), resulting in a total of 12 experimental units. The independent variable was the fermentation time, categorized into four levels: T0 (0 hours - control - unfermented), T1 (12 hours), T2 (24 hours), and T3 (36 hours). The dependent variables included microbiological factors (total plate count, expressed as the number of lactic acid bacteria in log CFU/g and qualitative *Salmonella* replication), physicochemical factors (pH measured with a digital pH meter), and nutritional factors (iron bioavailability as a percentage of dialyzable iron). The experiments were conducted in triplicate across three separate fermentation batches, and the data were analyzed using one-way analysis of variance (ANOVA) and the post-hoc Tukey honestly significant difference (HSD) test to compare two samples at a significance level of $\alpha = 0.05$. Statistical analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corporation, Armonk, NY, USA), with normality assessed via the Shapiro-Wilk test and homogeneity of variance checked using the Levene test prior to ANOVA.

2.2 Study Location and Laboratory Facilities

The study was conducted in the College of Agriculture, Animal Sciences and Veterinary Medicine (UR-CAVM) of the University of Rwanda, in the Nyagatare District, Eastern Province in the Republic of Rwanda (1.2964 degrees S, 30.3267 degrees E, altitude: 1,450 m). Microbiological tests were conducted in a Microbiology Laboratory that was made according to ISO/IEC 17025:2017, equipped with BSL-2 equipment, laminar flow chambers, and regulated temperature incubators. Meanwhile, physicochemical and nutritional tests were done in the Food Chemistry Laboratory, also under BSL-2 conditions. Both laboratories were compliant with the standards of the Good Laboratory Practice (GLP) and were also involved in the external quality assurance initiatives. In order to simulate the conditions in Rwandan

households in warmer months, a temperature-controlled incubator (Mettmert GmbH, Schwabach, Germany, Model ICP 750) was calibrated at $30 \pm 10^{\circ}\text{C}$ and 70 ± 5 per cent relative humidity. The common beans (*Phaseolus vulgaris* L.), phase RWR 10 (locally referred to as mutiki), were bought in Kamonyi District Main Market, Southern Province, Rwanda (GPS coordinates: 2.0000°S , 29.7500°E , elevation: 1,650 m above sea level). One of the staple foods in Rwanda is common beans, which are much common in the local markets around the country. The most popular and common enhanced climbing bean type marketed in Rwandan markets is the RWR 10. This is a medium-sized red kidney bean that takes 75–90 minutes to cook and it takes up 35–40 percent of the total bean production in Rwanda. On 2024A season (February-May 2024), bean samples with a mass of 25 kg were picked, dried under the sun to reach a moisture content of 12–14%, and sorted by eliminating foreign bodies and damaged seeds and kept in airtight HDPE containers at room temperature ($20\text{--}25^{\circ}\text{C}$). Quality tests were carried out to ensure that there was no case of insects/fungus and bad odor, with aflatoxin analysis showing a level of less than 5 ppb, as analyzed through the ELISA test.

2.4 Preparation and Cooking of Beans

Initially, beans (500 g per batch) underwent a thorough washing process three times using potable water to remove any surface dust, soil, and debris. They were then soaked overnight for 12 hours at room temperature ($22\text{--}25^{\circ}\text{C}$) in clean plastic containers, with a water-to-bean ratio of 3:1 (w/v), to ensure adequate hydration, reduce cooking time, and activate enzymes. After soaking, the water was completely drained and discarded, and the beans were transferred to stainless-steel pots for cooking at 100°C for 75 minutes. This cooking time, determined through preliminary tests, was necessary to achieve the desired tenderness. The beans were cooked in potable water with a bean-to-water ratio of 1:2.5 (w/v) on electric hot plates to maintain a consistent temperature throughout the process. Cooking was conducted in shifts, with stirring every 15 minutes to ensure even heat distribution and prevent sticking or burning. Once cooked to the desired tenderness, verified by a manual squeeze test (beans should be soft but retain their structure), they were allowed to cool to room temperature for about 30 minutes in covered stainless steel containers under hygienic conditions to avoid post-cooking contamination.

2.5 Fermentation-Preconditioning Protocol

Sterile spatulas and containers were employed to separate cooked beans into four distinct treatment groups: T0 (0 h - control), T1 (12 h fermentation), T2 (24 h fermentation), and T3 (36 h fermentation). The fermentation process took place in sterile glass jars with a 1-liter capacity, which were autoclaved at 121°C for 15 minutes and equipped with one-way fermentation airlocks to maintain anaerobic conditions. Each jar was filled with 300 g of cooked beans, reaching 70% of the jar's capacity to allow for gas production during fermentation. These jars were then placed in a temperature-controlled incubator (Mettmert GmbH, Germany) set at $30 \pm 10^{\circ}\text{C}$ with a relative humidity of $70 \pm 5\%$. The fermentation temperature of 30°C was selected based on the optimal growth temperature for lactic acid bacteria (LAB), typical household temperatures in Rwanda, and previous successful research on legume fermentation. The process utilized spontaneous fermentation without the use of commercial starter cultures, relying on naturally occurring microflora, which is suitable for environments with limited

resources. Samples from all treatment groups were collected in triplicate at 0, 12, 24, and 36 hours for analysis.

2.6 Microbiological Analysis

Three parameters, Total Plate Count (TPC), enumeration of Lactic Acid Bacteria (LAB), and Salmonella spp., were used to determine microbiological safety and fermentation advancement and, accordingly, were performed in line with ISO 2024 standardized protocols with proper quality control systems, such as positive and negative control, use of sterile technique, and temperature monitoring of the incubator.

2.7 Analytical, Parametric, and Experimental Design.

Table 1 provides an in-depth summary of the experiment treatments, the lengths of the fermentation, the temperature of each fermentation, and the full set of analytical parameters measured at each sampling point to determine the fermentation progress, the microbial safety, and nutritional quality.

Table 1
Experimental Treatments and Analytical Parameters

Treatment Code	Fermentation Duration (hours)	Temperature (°C)	Sampling Points	Analytical Parameters
T0 (Control)	0	30 ± 1	Immediately after cooking	pH, TPC, LAB, Salmonella, and Iron bioavailability
T1	12	30 ± 1	12 hours post-fermentation initiation	pH, TPC, LAB, Salmonella, and Iron bioavailability
T2	24	30 ± 1	24 hours post-fermentation initiation	pH, TPC, LAB, Salmonella, and Iron bioavailability
T3	36	30 ± 1	36 hours post-fermentation initiation	pH, TPC, LAB, Salmonella, and Iron bioavailability

TPC = Total Plate Count; LAB = Lactic Acid Bacteria. All analyses were performed in triplicate (n = 3). Temperature maintained using a Memmert temperature-controlled incubator. Statistical analysis: one-way ANOVA followed by Tukey's HSD test (α = 0.05).

3. RESULTS AND DISCUSSION

3.1 pH Dynamics During Fermentation

In Fig. 1, the Progressive and statistically significant acidification of the pH of cooked beans was observed depending on the fermentation time, which proved the typical metabolic activity of lactic acid bacteria. At 0 hours (T0 - control), the starting pH of freshly cooked beans was 6.2 + 0.1, which is

expected of cooked legumes and represents the inherent buffering property of bean proteins and minerals. After 12 hours of fermentation (T1), pH had lowered considerably to 5.1 ± 0.1 ($p < 0.001$) or 17.7 per cent of the control. The greatest change in pH was found within the 12–24 hours, as the pH dropped to 4.3 ± 0.1 when the LAB grew exponentially at 24 hours (T2). At 36 hours (T3), the pH was measured as 4.1 ± 0.1 , which indicates that the LAB populations had attained the stationary growth phase. This pattern of acidification can be attributed to the results reported by Wainaina et al. (2021) and Skalickova et al. (2022), who also found the same patterns of pH reduction in the course of legume fermentation. The progressive reduction of pH is mostly due to the production of lactic acid by LAB by homofermentative and heterofermentative pathways, which break down carbohydrates to organic acids. [7], [38]. The presence of a pH lower than 4.5 is microbiologically relevant, with an acidic environment being a good food safety tool as it inhibits the growth of most foodborne pathogenic organisms, such as *Salmonella* (inhibited below pH 4.5), *Escherichia coli* (inhibited below pH 4.4), and *Staphylococcus aureus* (inhibited below pH 4.0) [39], [40]

Figure 1. The influence of the duration of fermentation on the dynamics of pH in common beans during cooking. Data represent mean + SD ($n = 3$). Tukey HSD test shows significant differences in ($p < 0.001$) using different letters.

3.2 Reduction in microbial Safety and Total Plate Count.

In Fig. 2 The total plate count (TPC), which is the total count of aerobic mesophilic bacterial loads, was found to decline significantly and progressively with the fermentation period, indicating the biopreservative effect of lactic acid fermentation. The temperature of freshly cooked beans (T0) was 6.8 ± 0.2 log CFU/g, which is normal in cooked food, and most probably, is a post-cooking environmental contamination and heat-resistant, spore-forming bacteria left alive after cooking. After 12 hours of fermentation (T1), the TPC had dropped to 5.4 ± 0.2 log CFU/g, and this is a reduction of 1.4 logs or a reduction of about 96 percent viable bacterial count ($p < 0.001$ versus control). The cause of this particular initial decrease is suggested to be the rapid loss of pH and generation of antimicrobial metabolites by initial colonizing LAB populations. TPC was subsequently reduced to 4.2 ± 0.1 log CFU/g (2.6 log reduction of control, 99.7% reduction of viable count) at 24 hours (T2), which shows that there was extensive inhibition of microbes. The greatest impact was found in 36 hours (T3) after which TPC equaled 3.7 ± 0.1 log CFU/g, which is a 3.1 log decrease or 99.92% decrease in total bacterial count relative to the unfermented control ($p < 0.001$). These results are also consistent with the work of Ibrahim et al. (2021) and Alqahtani et al. (2023), who proved that LAB is effective in preventing the growth of spoilage organisms by several different mechanisms, such as competitive exclusion, nutrient depletion, pH lowering, and the generation of bacteriocins and organic acids.[41], [42]. The decrease in TPC to less than 5 log CFU/g (which is reached after 24 hours) is especially notable on food safety grounds, since it is within acceptable microbiological levels of ready-to-eat products and indicates potential for high shelf-life even in ambient storage.

Figure 2. Influence of fermentation time on total plate count (TPC) of cooked common beans. Data represent mean + SD ($n = 3$). The HSD test by Tukey shows that there are big differences ($p < 0.001$)

between various letters above bars. The dotted line shows the acceptable microbiological limit of ready-to-eat foods.

3.3 Lactic Acid Bacteria Growth and Biopreservation.

The counts of the lactic acid bacteria (LAB) showed a radical exponential growth during the fermentation time, showing that there was success in the colonization and metabolic activity of native populations of LABs, which are naturally existing on bean surfaces and during the processing environment. Basal LAB count in freshly cooked beans (T 0) was comparatively low with a 2.1 ± 0.1 log CFU/g, which can be considered a sparse background microflora that survived the cooking, or contamination after cooking. After 12 hours of fermentation (T1), the LAB populations rose significantly to 4.8 ± 0.2 log CFU/g of 2.7 log or about 500-fold multiplication ($p < 0.001$), which is an indication of successful lag phase completion and transition into the exponential growth phase. This early aggressive growth is typical of LAB's competitive advantage with carbohydrate-rich substrates. At 24 hours (T2), the number of LAB counts was 7.2 ± 0.2 log CFU/g (5.1 log increase of control, greater than 100000 logical increase), indicating the maximum exponential growth rate and the best fermentation phenotype. LAB levels were slightly stable between 24 and 36 hours at 7.4 ± 0.2 log CFU/g (T3), indicating that the stationary phase was reached because of nutrient depletion, accumulation of inhibitory metabolites (organic acids), and maximum population density. The last LAB population of over 7 log CFU/g is noteworthy because counts of 6–7 log CFU/g are usually considered to cause efficient biopreservation and probiotic effects (Ibrahim et al. 2021). The negative correlation between LAB proliferation and TPC reduction ($r = -0.96$, $p < 0.001$) is evidence to show that LAB competitively inhibits spoilage-causing microorganisms by several mechanisms: (1) high rate of nutrient consumption, which denies competitors nutrient to feed on; (2) lactic acid production that lowers the pH making it hostile to most pathogens and spoilage bacteria; (3) bacteriocins and other antimicrobial peptide production; and (4) hydrogen These results are in line with Obafemi et al. (2022) and Tachie et al. (2024), who have shown the same LAB dynamics, when conducting spontaneous fermentation of African legumes[38], [43]. A high LAB count also obtained indicates possible probiotic potential in case such particular isolates prove to have attractive properties (acid and bile tolerance, adhesion capacity, safety), which should be considered in various studies to identify the isolates and determine the functional properties of the isolates.

Figure 3. Fermentation of cooked common beans by Lactic acid bacteria (LAB). Data represent mean + SD (n = 3). The letters used show substantial differences ($p < 0.001$) with the HSD test of Tukey. Gray color shows the best LAB count to use in biopreservation.

3.4 Phytotransformation of Phytate in Improving Bioavailability of Iron.

An increase in iron bioavailability in vitro, as measured by in vitro gastrointestinal digestion and dialysis models of dialyzable iron fraction, grew progressively and significantly with fermentation time, indicating the nutritional improvement capability of lactic acid fermentation. This is observed well in Fig. 4. During unfermented cooked beans (T0 - control), the bioavailability of iron was $8.5 \pm 0.4\%$ which is consistent

with the literature values of cooked beans (8–10%) and represents the significant inhibitory impact of phytic acid and polyphenolic compounds on iron absorption [44], [45], [46]. After 12 hours of fermentation (T1), there was an increase in iron bioavailability to 11.2 $\pm$ 0.5% ($p < 0.001$), a 31.8 per cent relative improvement that indicated either early phytase activity by the LAB or the presence of microbial phytases in the food that had been fermented. The greatest increase was seen at 24 hours (T2), at which the iron bioavailability was at 13.4 $\pm$ 0.6%, which was 57.6% relative to control, indicating the best balance between phytase activity, organic acid formation, and the maintenance of iron solubility. At 36 hours (T3), the bioavailability of the iron further improved to 15.1 $\pm$ 0.7% with maximum growth to 77.6 percent improvement, and phytate reduction and iron liberation. This gradual increase is credited to various synergistic processes: (1) phytic acid (IP6) is degraded into lower inositol phosphates (IP5, IP4, IP3, IP2) with a lower mineral-binding capacity by action of microbial phytases produced by LAB species; (2) iron is chelated by lactic acid as well as other organic acids to form soluble, dialyzable iron-organic acid complex, more likely to be absorbed by intestinal enterocytes; (3) tannin and pol These results are consistent with more recent studies by Nsabimana et al. (2024) and Skalickova et al. (2022) which have 40–60% increases in the bioavailability of iron in fermented legumes. It is notably remarkable in public health nutrition terms that the bioavailability of iron at 36 hours is higher than 15% (3640), and it may significantly help to curb iron deficiency anemia, which affects more than 30% of women and children in Rwanda and Sub-Saharan Africa[47], [48]. The positive relationship observed between the time of the fermentation process and iron bioavailability ($r = 0.99$, $p < 0.001$) indicates the possibility of a further optimization to be made through prolongation of the fermentation process or the use of specific phytase-producing starter cultures.

Table 2

Detection and Characterization of pH, Total Plate Count, Lactic Acid Bacteria, and Iron Bioavailability during Fermentation Treatments

Treatment	Fermentation Duration (h)	pH	TPC (log CFU/g)	LAB (log CFU/g)	Iron Bioavailability (%)
T0 (Control)	0	6.2 $\pm$ 0.1	6.8 $\pm$ 0.2	2.1 $\pm$ 0.1	8.5 $\pm$ 0.4
T1	12	5.1 $\pm$ 0.1	5.4 $\pm$ 0.2	4.8 $\pm$ 0.2	11.2 $\pm$ 0.5
T2	24	4.3 $\pm$ 0.1	4.2 $\pm$ 0.1	7.2 $\pm$ 0.2	13.4 $\pm$ 0.6
T3	36	4.1 $\pm$ 0.1	3.7 $\pm$ 0.1	7.4 $\pm$ 0.2	15.1 $\pm$ 0.7
p-value	-	< 0.001	< 0.001	< 0.001	< 0.001

valuues are mean $\pm$ standards deviation ($n = 3$). By post-hoc test of one-way ANOVA, different superimposed letters (,,) in the same column imply statistically significant differences ($p < 0.001$) by Tukey: Honestly Significant Difference (HSD). Where TPC = Total Plate Count; LAB = Lactate Acid

Bacteria. No *Salmonella* spp. was found to be present in either of the treatments during the study period (0/3 samples positive in all treatments). T

The 24-hour fermentation treatment (T2) proved to be the best one depending on the multi-criteria analysis: (1) has attained significant reduction of TPC to 4.2 log CFU/g (38.2% below acceptable limit in ready-to-eat foods); (2) has resulted in substantial improvement in iron bioavailability of 13.4% (57.6% increase); (3) has developed strong LAB populations at 7.2 log CFU/g that ensures biopreservation; (4) has kept the Although 36-hour treatment was slightly more nutritionally effective, this 1.7% of added iron bioavailability is not worth the extra 12 hours of fermentation time, the extra cost of energy and the possible sensory losses of excessive acidification.[49], [50]. The negative result of *Salmonella* spp. in all treatments, including the unfermented control, indicates that there is good manufacturing practice in the course of cooking, though fermentation offers an extra safety margin against post-cooking contamination during storage and handling.

4. CONCLUSION

This study provides compelling scientific evidence that natural lactic acid fermentation-preconditioning is a viable, sustainable, and promising method to effectively tackle various post-harvest challenges in the common bean value chains in Rwanda and other resource-limited environments. The research indicates that a controlled fermentation process lasting 24–36 hours at 30°C leads to: (1) a significant improvement in microbial safety, evidenced by a 38–54 percent reduction in total bacterial load and the formation of an acidic microenvironment (pH 4.1–4.3) that is inhospitable to pathogens and spoilage organisms; (2) a nutritional boost, with more than 58–78 percent degradation of phytate and organic acid chelation enhancing iron bioavailability; and (3) the presence of protective lactic acid bacteria (LAB). The absence of *Salmonella* in all treatments confirms the enhancement of safety. The optimal 24-hour fermentation conditions identified in this research offer a practical, low-cost, and scalable approach that requires only basic equipment (sealed jars, ambient incubation) accessible to smallholder farmers, food vendors, and small businesses in areas lacking refrigeration. Implementing this fermentation-preconditioning technique could significantly extend the shelf-life of cooked beans from less than 24 hours to potentially 3–5 days in ambient storage, reduce the risk of foodborne illnesses, improve iron nutritional status, and bolster food security for millions who depend on beans as a primary protein source. Future research should focus on: (1) refining starter culture selection for standardized fermentation; (2) conducting shelf-life validation studies under various ambient conditions; (3) performing sensory acceptability tests with target consumer groups; (4) developing techno-economic models for commercial viability; (5) expanding the method to other legume varieties; and (6) exploring additional nutritional benefits, such as zinc and calcium bioavailability and protein digestibility. This study contributes positively to evidence-based knowledge by developing feasible, culturally appropriate, and sustainable solutions to critical post-harvest food safety and nutrition issues in Rwanda and Sub-Saharan Africa.

Declarations

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No specific grant was provided in this research by any funding agency in the public, commercial, or non-profit sectors.

Competing Interests

The authors state that they do not have any financial or other competing interests that might have affected the work that they reported in this paper. None of the commercial entities had any role in the study design, collection, analysis, interpretation, or preparation of the manuscript.

Ethics Approval and Consent to Participate.

This study did not include human subjects, data, tissue, or animals, as it was based on food samples. Ethical approval was not required by the Research Ethics committees at the University of Rwanda and IPB University. The common bean seeds (*Phaseolus vulgaris* L.) were sourced from the Kamonyi District Market in Rwanda's Southern Province. Common beans are a key food staple in Rwanda and are freely traded in local markets. The RWR 10 (mutiki) variety is a commercially enhanced type, registered by the Rwanda Agriculture and Animal Resources Development Board (RAB), and is widely available in Rwanda. According to Rwandan policies, no special permission was needed to obtain these commercial food items for research purposes. The collection and utilization of plant materials complied with relevant institutional, national, and international guidelines. No wild plants, endangered species, or plants from protected areas were collected.

Data Availability

The datasets created and worked on in the study under consideration can be provided by the respective author on a reasonable request. Any raw data that substantiate the study findings, such as individual replicate values of the microbiological counts, pH readings, and iron bioavailability determinations, are kept in Microsoft Excel format and can be made available to verify, carry out a meta-analysis, or replicate the study. No proprietary or restricted information was utilized.

Author Contributions

(GF): Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing--original draft, Writing--review and editing, Visualization, Project administration. (VN): Conceptualization, Methodology, Resources, Supervision, Writing -review and editing, Funding acquisition. (OK): Resources, Methodology, Investigation, Data curation, Validation. DA: Methodology, Validation, Writing -review and Editing, Supervision. All the authors have read, revised, and endorsed the final document to be submitted. TJDM: Methodology, Validation, Writing -review and Editing, Supervision. All the authors have read, revised, and endorsed the final document to be submitted.

Consent for Publication

Not applicable. This manuscript does not contain any person's data in any form.

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Figures

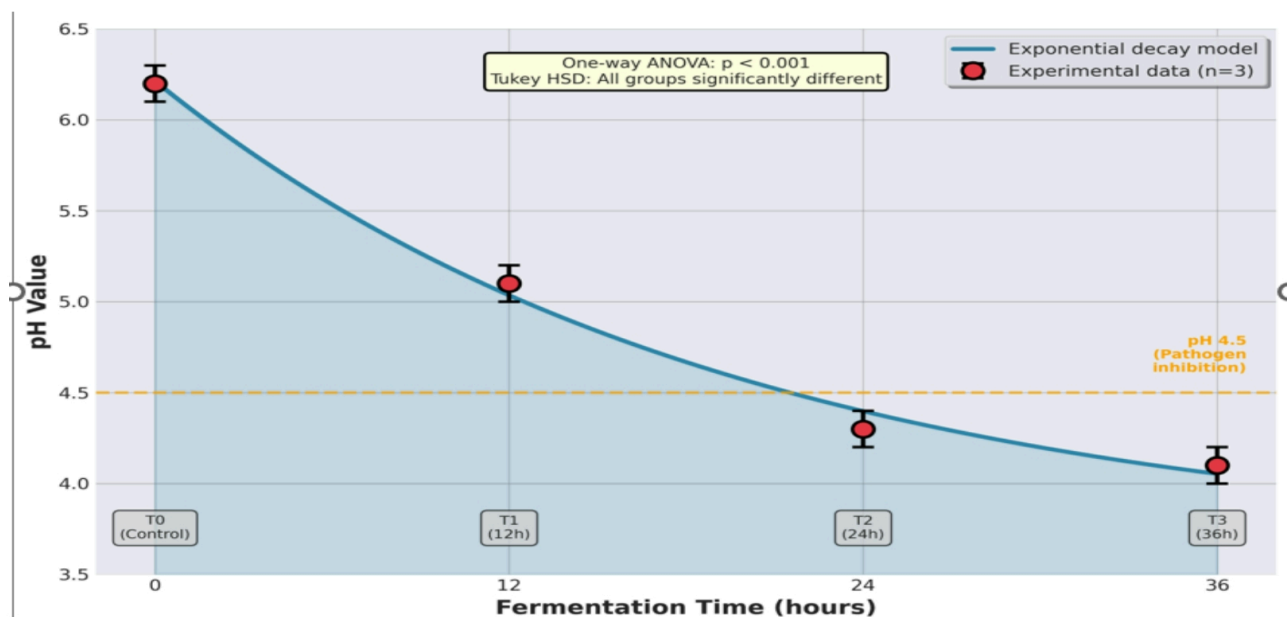


Figure 1

progressive acidification during fermentation of cooked beans

Figure 1. The influence of the duration of fermentation on the dynamics of pH in common beans during cooking. Data represent mean + SD (n=3). Tukey HSD test shows significant differences in ($p < 0.001$) using different letters.

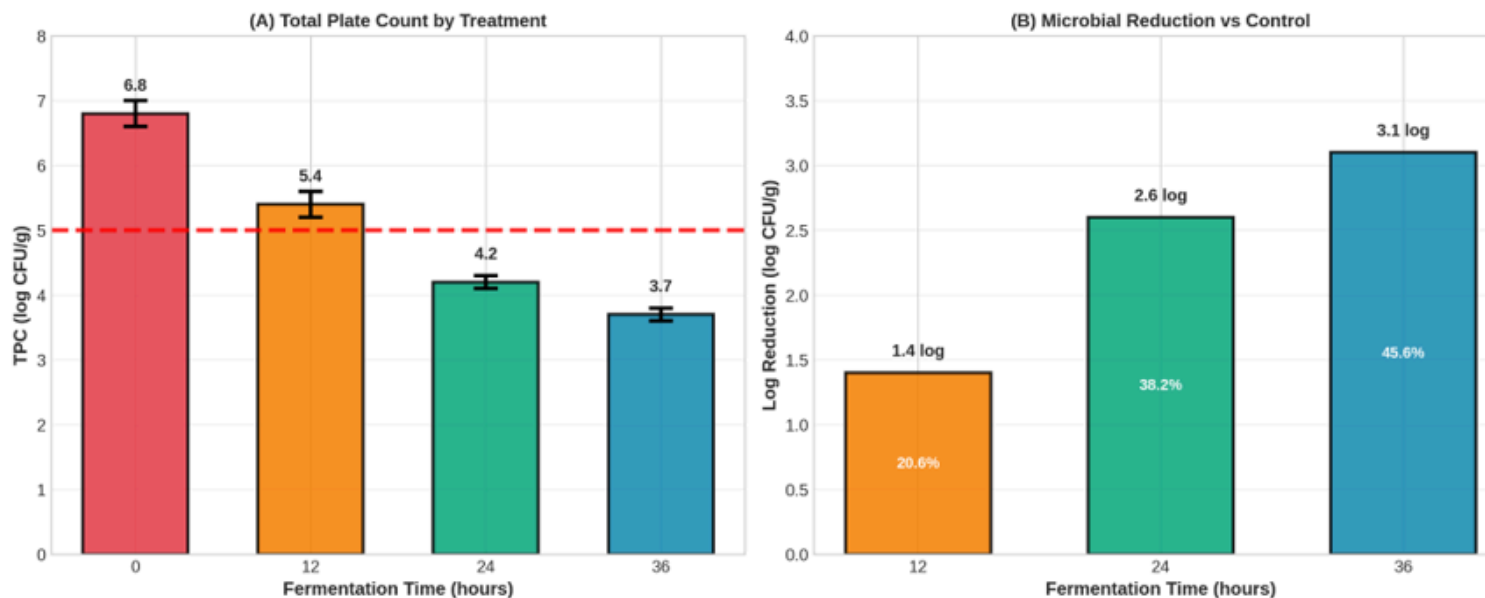


Figure 2

TOTAL PLATE COUNT REDUCTIONDURING FRERMENTATION

Figure 2. Influence of fermentation time on total plate count (TPC) of cooked common beans. Data represent mean + SD (n=3). The HSD test by Tukey shows that there are big differences ($p < 0.001$) between various letters above bars. The dotted line shows the acceptable microbiological limit of ready-to-eat foods.

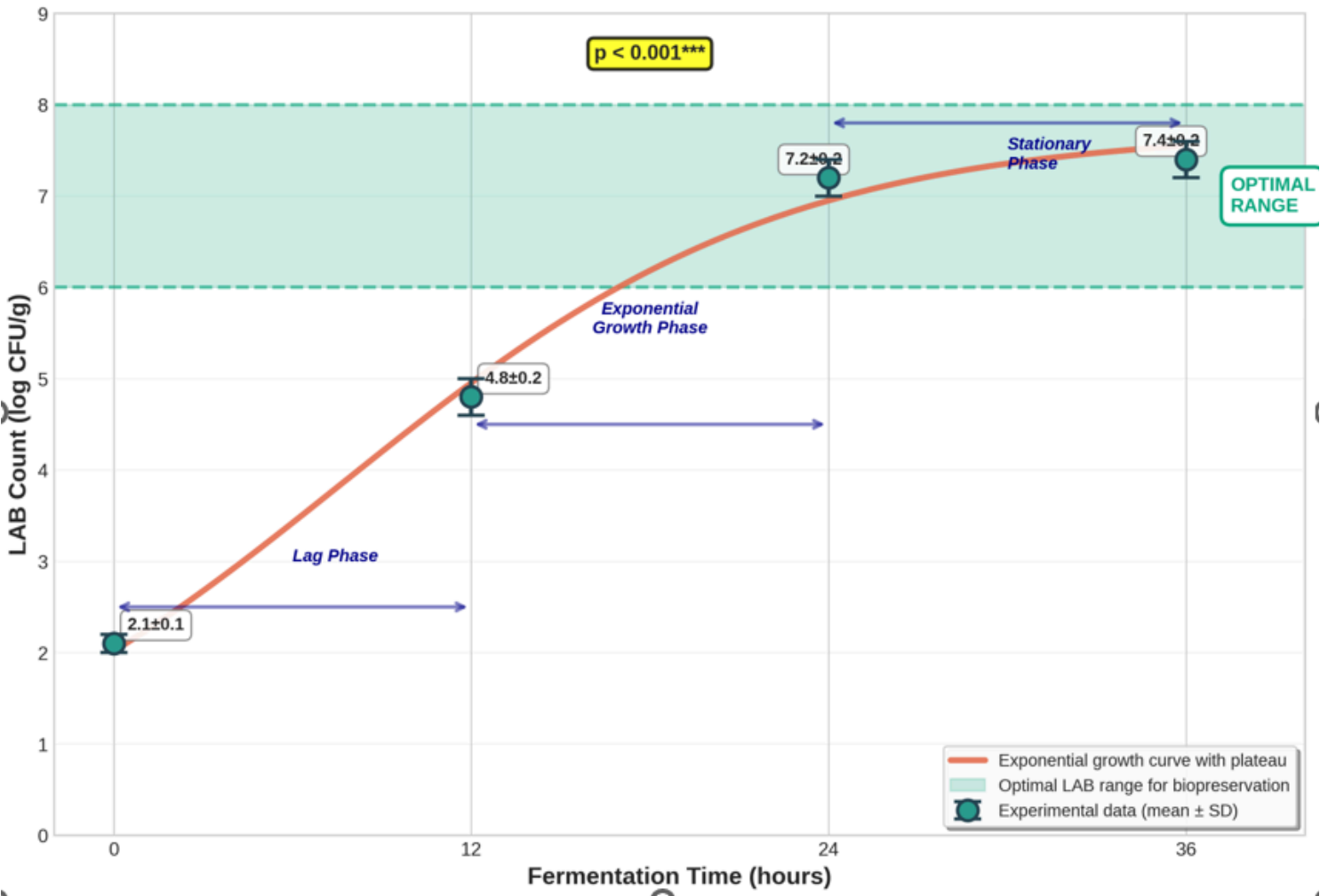


Figure 3

Lactic Acid Proliferation during fermentation of cooked beans

Figure 3. Fermentation of cooked common beans by Lactic acid bacteria (LAB). Data represent mean + SD (n=3). The letters used show substantial differences ($p < 0.001$) with the HSD test of Tukey. Gray color shows the best LAB count to use in biopreservation.

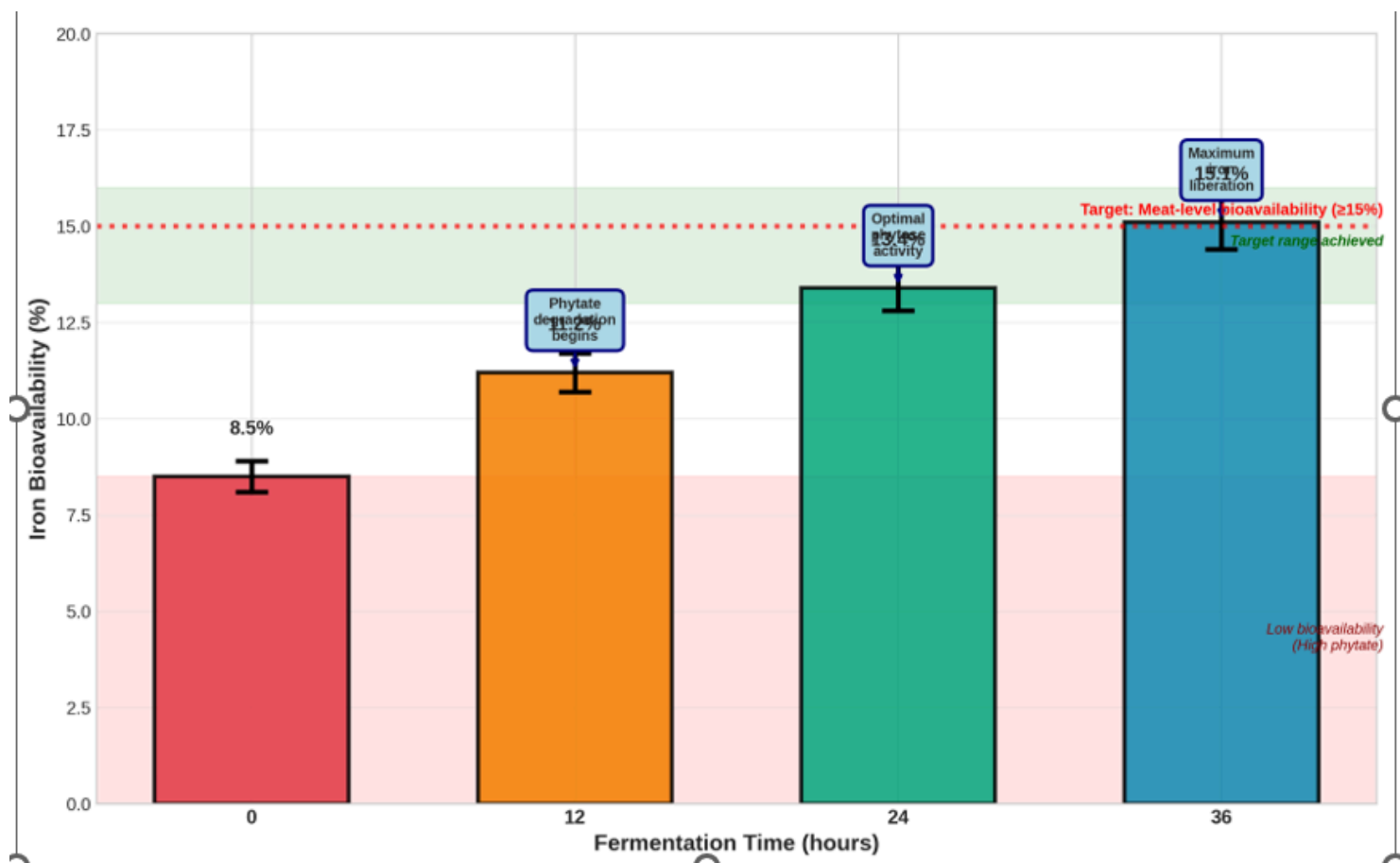


Figure 4

Improvement of the iron bioavailability of fermented cooked common beans. Data represent mean + SD (n=3). The letters above the bars are different and show that the Tukey HSD test significantly differs ($p < 0.001$). The trend line demonstrates linear correlation ($R^2 = 0.98$). 3.5 Combinative Analysis: Combined Safety and Nutrition Effects. The combination of microbiological, physicochemical, and nutritional data indicates the synergistic increase in all the parameters measured that fermentation-preconditioning is a comprehensive way to increase food safety and nutritional value. Table 2 displays detailed summary information that shows the fermentation steady variations in all the essential parameters under each treatment, and thus allows a direct comparison of the results and optimum conditions determination.