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Research Article

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Assessing the Efficacy of Lactic Acid Bacteria as a Preservative in Zobo Drinks

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Abstract

Zobo, a traditional Nigerian beverage made from the petals of the Hibiscus sabdariffa plant, is known for its refreshing taste and health benefits. However, its high susceptibility to microbial spoilage poses a significant challenge for preservation. This study investigates the efficacy of lactic acid bacteria (LAB) as a natural preservative in zobo drinks, evaluating their impact on microbial stability, sensory properties, and nutritional content. Through a series of fermentation experiments, we assessed the viability of LAB, changes in pH, and sensory attributes over a storage period of 21 days. Our findings demonstrate that LAB not only enhance the shelf life of zobo drinks but also improve their flavor profile and nutritional value.

Keywords: Zobo, Hibiscus Sabdariffa, Lactic Acid Bacteria, Microbial Preservation, Sensory Evaluation

1. Introduction

Zobo drinks, derived from the calyces of Hibiscus sabdariffa, are rich in antioxidants, vitamins, and minerals. Despite their popularity, the beverage's natural sweetness and moisture content make it an ideal substrate for spoilage microorganisms, leading to short shelf life and potential food safety issues. Traditional preservation methods often rely on chemical additives, which may not align with the growing consumer preference for natural ingredients.

Lactic acid bacteria (LAB) have gained recognition for their ability to produce organic acids and antimicrobial compounds that inhibit spoilage organisms. This study aims to assess the potential of LAB as a natural preservative in zobo drinks by investigating their effects on microbial stability, sensory attributes, and nutritional content.

2. Materials and Methods

2.1 Sample Preparation

Fresh Hibiscus sabdariffa petals were procured from local markets

in Anyigba, Kogi State, Nigeria to ensure freshness and quality. The petals were thoroughly washed under running water to remove any surface contaminants and dirt. Subsequently, they were boiled in distilled water at a ratio of 1:5 (w/v) for 15 minutes, following the method outlined by [1,2]. The extract was then cooled to room temperature. After cooling, the extract was sweetened with granulated sugar (10% w/v) to enhance palatability, as described by Okafor and Ibeh.

The prepared extract was divided into four treatment groups:
Control (no LAB)
• LAB strain A (Lactobacillus plantarum)
• LAB strain B (Lactobacillus rhamnosus)
• LAB strain C (Lactobacillus casei). All organisms were reconfirmed at the Laboratory of the department of Microbiology, Prince Abubakar Audu University, Anyigba, Kogi State

Each treatment group was inoculated with a concentration of 10^7 CFU/mL of the respective LAB strains, using methods adapted from [3]. The inoculated samples were then stored in sterile containers at 4°C for the duration of the study.

2.2 Microbial Analysis

Microbial analysis was performed at 0, 7, 14, and 21 days to monitor microbial counts in each treatment group. Total viable counts (TVC) were determined using the spread plate technique, as outlined in the Standard Methods for the Examination of Water and Wastewater [4].
• Preparation of Dilutions: Serial dilutions of each sample were prepared up to 10^-6 in sterile peptone water.
• Inoculation: 100 µL of each dilution was spread onto De Man, Rogosa, and Sharpe (MRS) agar for LAB counts and Potato Dextrose Agar (PDA) for yeast and mold counts.
• Incubation: The plates were incubated anaerobically at 37°C for 48 hours for LAB and aerobically at 25°C for 5 days for yeast and molds [5].
• Colony-forming units (CFU) were counted, and results were expressed as CFU/mL.

2.3 Ph Measurement

The pH of each treatment was measured using a calibrated pH meter at 0, 7, 14, and 21 days. Prior to measurement, the pH meter was calibrated using standard buffer solutions (pH 4.0 and 7.0) following the procedure outlined by the manufacturer (pH Meter Manual, [Year]). The sample pH was measured directly by immersing the electrode in the zobo extract and recording the values [6].

2.4 Sensory Evaluation

A sensory evaluation was conducted with a panel of 20 trained judges to assess the sensory properties of the drinks, including taste, aroma, color, and overall acceptability. The evaluation used a 5-point hedonic scale, where 1 = dislike extremely and 5 = like extremely. Judges were selected based on prior experience in sensory evaluation and trained according to the guidelines set by the American Society for Testing and Materials. The samples were presented in a randomized order to minimize bias, and the evaluation took place under controlled lighting and temperature conditions.

2.5 Nutritional Analysis

Nutritional components, specifically vitamin C, total phenolic content, and antioxidant activity, were assessed using standard spectrophotometric methods.

• Vitamin C Determination: Vitamin C was quantified using the 2,6-dichlorophenolindophenol (DCPIP) method as described by Clydesdale [7].
• Total Phenolic Content: The total phenolic content was measured using the Folin-Ciocalteu method [8]. Results were expressed as mg of gallic acid equivalents (GAE) per 100 mL of sample.
• Antioxidant Activity: Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as outlined by [9]. The percentage of DPPH scavenging was calculated based on the absorbance readings before and after the reaction.

3. Results

3.1 Microbial Stability

The control sample exhibited significant microbial growth by day 7, with TVC exceeding 10^7 CFU/mL. In contrast, LAB-inoculated samples showed a steady decline in spoilage organisms, maintaining counts below 10^3 CFU/mL throughout the storage period. LAB strain A demonstrated the most effective preservation, maintaining a stable population of LAB and inhibiting spoilage.

Treatment	Day 0	Day 7	Day 14	Day 21
Control	<10^3	>10^7	>10^7	>10^7
LAB Strain A (L. plantarum)	<10^3	<10^3	<10^3	<10^3
|LAB Strain B (L. rhamnosus)	<10^3	<10^3	<10^3	<10^3
LAB Strain C (L. casei)	<10^3	<10^3	<10^3	<10^3

Table 1: Microbial Counts (CFU/mL) Over Storage Period

3.2 Ph Changes

The initial pH of zobo drinks ranged from 3.8 to 4.0. LAB fermentation resulted in a decrease in pH over the storage period, with strain A reaching a final pH of 3.2, indicating increased acidity due to lactic acid production.

Treatment	Initial pH	Day 7	Day 14	Day 21
Control	3.8	4.1	4.3	4.5
LAB Strain A	3.8	3.6	3.4	3.2
LAB Strain B (L. rhamnosus)	3.8	3.7	3.5	3.3
|LAB Strain C (L. casei)	3.8	3.7	3.5	3.4

Table 2: Ph Changes Over Storage Period

3.3 Sensory Evaluation

All LAB-inoculated samples received higher acceptability scores compared to the control. Notably, strain A was favored for its balanced flavor and aroma, while the control was marked down for off-flavors associated with spoilage.

Treatment	Taste (1-5)	Aroma (1-5)	Color (1-5)	Overall Acceptability (1-5)
Control	2.0	2.1	3.0	2.2
LAB Strain A (L. plantarum)	4.5	4.6	4.4	4.6
LAB Strain B (L. rhamnosus)	4.0	4.1	4.0	4.2
LAB Strain C (L. casei)	3.8	3.9	4.1	4.0

Table 3: Sensory Evaluation Scores

3.4 Nutritional Analysis

LAB-inoculated zobo drinks showed a significant increase in total phenolic content and antioxidant activity compared to the control. The vitamin C content remained stable across all samples, indicating that LAB fermentation does not adversely affect this nutrient.

Nutritional Component	Control	LAB Strain A	LAB Strain B	LAB Strain C
Vitamin C (mg/100mL)	10	10	10	10
Total Phenolic Content (mg GAE/100mL)	30	50	45	40
Antioxidant Activity (% DPPH Scavenging)	25	65	60	55

Table 4: Nutritional Analysis Results

4. Discussion

The results of this study underscore the significant potential of lactic acid bacteria (LAB) as natural preservatives in zobo drinks, highlighting their role in microbial stability, pH regulation, sensory enhancement, and nutritional value.

4.1 Microbial Counts and Stability

As shown in Table 1, the control sample exhibited rapid microbial proliferation, surpassing 10^7 CFU/mL by Day 7, indicating a severe spoilage risk. In contrast, the LAB-inoculated samples maintained microbial counts below 10^3 CFU/mL throughout the study, with LAB Strain A (Lactobacillus plantarum) demonstrating the most effective preservation. This finding aligns with previous research indicating that LAB can effectively inhibit spoilage organisms through the production of organic acids and bacteriocins [10]. The ability of LAB to suppress pathogenic and spoilage bacteria has been documented in various food matrices, reinforcing the idea that these bacteria can enhance the safety and longevity of perishable products [11].

4.2 Ph Changes

Table 2 highlights the pH dynamics, with LAB fermentation leading to a significant drop in pH over the storage period, particularly in the LAB Strain A treatment. This reduction is crucial as lower pH values inhibit the growth of spoilage organisms and pathogens, making the environment less hospitable to microbial contamination [12]. The initial pH of zobo drinks (around 3.8) provided an optimal setting for LAB growth, resulting in an increased acidity that is consistent with findings from studies on fermented beverages. The correlation between pH reduction and microbial stability further supports the application of LAB in food preservation.

4.3 Sensory Evaluation

In terms of sensory attributes (Table 3), LAB-inoculated samples received significantly higher acceptability scores across all evaluated parameters compared to the control. LAB Strain A was particularly favored, with scores reflecting its balanced taste and aroma. This result is consistent with the findings of, who reported that fermentation enhances the flavor profile of beverages, likely due

to the metabolic activities of LAB that produce flavor compounds. The sensory improvement associated with LAB fermentation not only enhances consumer appeal but also underscores the functional benefits of using these microorganisms in food products.

4.4 Nutritional Analysis

Table 4 indicates that while vitamin C content remained stable across treatments, LAB fermentation resulted in a notable increase in total phenolic content and antioxidant activity in zobo drinks. The LAB Strain A led to the highest total phenolic content (50 mg GAE/100mL) and antioxidant activity (65% DPPH scavenging), suggesting that LAB fermentation may enhance the nutritional profile of zobo drinks. This is in line with research indicating that LAB can increase the bioavailability of antioxidants through their metabolic processes . The observed stability of vitamin C is particularly significant, as it ensures that the nutritional quality of the beverage is maintained throughout the storage period.

4.5 Implications of Findings

The implications of these findings are profound for both food science and public health. Utilizing LAB as natural preservatives could reduce reliance on synthetic additives, aligning with consumer trends favoring natural and healthy food options. Furthermore, the enhanced sensory properties and nutritional value of zobo drinks present an opportunity for market expansion, potentially attracting health-conscious consumers. Additionally, the ability of LAB to effectively inhibit spoilage microorganisms enhances food safety, addressing significant concerns regarding foodborne illnesses linked to improperly stored beverages.

The results presented in Table: Antioxidant Activity of Hibiscus sabdariffa Extracts illustrate the varying efficacy of different treatments in scavenging free radicals, as indicated by the DPPH scavenging activity percentages. The control sample, which did not contain any lactic acid bacteria (LAB), exhibited a scavenging activity of only 25%. This relatively low antioxidant capacity aligns with findings by Nascimento, which noted that unfermented Hibiscus sabdariffa extracts generally possess lower antioxidant properties compared to fermented variants [13].

In contrast, the extracts inoculated with LAB strains demonstrated significantly higher DPPH scavenging activities, with *Lactobacillus plantarum* showing the highest at 65%. This finding supports the hypothesis that LAB fermentation enhances the antioxidant capacity of Hibiscus sabdariffa beverages by promoting the production of bioactive compounds [14]. The observed activities for L. rhamnosus (60%) and L. casei (55%) further emphasize the role of LAB in improving the health benefits of fermented products, corroborating studies that highlight the synergistic effects of probiotics and plant extracts [15].

The marked reduction in absorbance after the DPPH reaction further illustrates the efficacy of these LAB strains. Lower absorbance values indicate higher scavenging activity, which corresponds to the effective neutralization of DPPH radicals by the antioxidants present in the LAB-fermented extracts. This is consistent with the methodology described by Brand-Williams, which emphasizes absorbance changes as a reliable measure of antioxidant activity.

Overall, the enhanced antioxidant activity in LAB-inoculated samples signifies the potential health benefits of consuming fermented Hibiscus sabdariffa drinks, supporting their use as functional beverages. Future studies could explore the specific mechanisms by which LAB enhance antioxidant properties, paving the way for developing health-promoting food products.

5. Conclusion

In conclusion, the study effectively demonstrates that lactic acid bacteria can serve as a natural and effective means of preserving zobo drinks, offering microbial stability, enhanced sensory properties, and improved nutritional value. Future research should delve deeper into optimizing LAB strains and fermentation conditions to maximize these benefits, further solidifying the role of LAB in food preservation strategies.

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