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Development of Functional Beverage from Seawater Saline-Alkali Land Asparagus Based on Co-fermentation and Design Innovation

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***Abstract*—Asparagus (Asparagus officinalis L.) cultivated in seawater saline-alkali land offers unique nutritional value but is underutilized in beverage applications due to its inherent bitterness. This study addresses this challenge by developing a functional beverage with enhanced sensory and nutritional profiles. A co-fermentation system using Lactobacillus plantarum and Saccharomyces cerevisiae was constructed and systematically optimized using Response Surface Methodology (RSM). The optimal conditions were determined to be a fermentation temperature of 34°C, a duration of 72 hours, and a total inoculum of 3.2%. Under these conditions, the fermented beverage exhibited significant improvements: free amino acid and soluble polysaccharide content increased by 156% and 128%, respectively, while DPPH and ABTS radical scavenging capacities were enhanced by 32% and 28%. Crucially, the bitterness score improved from 4.8 to 8.6 (a 79% enhancement), leading to a 50% increase in the overall sensory score (from 5.8 to 8.7). This research establishes a systematic technical paradigm for the high-value processing of saline-alkali land crops.**

Keywords*—Asparagus officinalis; Co-fermentation; Functional Beverage; Response Surface Methodology; Saline-Alkali Land

1. Introduction

1.1. Research Background

The global demand for functional beverages, which offer health benefits beyond basic nutrition, represents a significant and expanding engineering field within the food industry. The market is projected to reach $208.13 billion by 2024, driven by consumer interest in products that are both healthy and palatable [1]. Asparagus (Asparagus officinalis L.) is a prime candidate for developing such products due to its rich composition of proteins, vitamins, minerals, and bioactive compounds like saponins, flavonoids, and polyphenols, which are associated with antioxidant, anti-inflammatory, and immune-regulating properties [2]. A critical engineering advantage of asparagus is its high salt tolerance, allowing for cultivation in marginal seawater saline-alkali lands. This not only provides a viable use for non-arable land but also results in a crop with enhanced nutritional characteristics. Asparagus grown under moderate salt stress accumulates higher levels of minerals and antioxidant substances compared to conventionally grown plants, presenting a unique opportunity for creating high-value functional foods [3].

Despite these advantages, a significant technical problem hinders the widespread use of asparagus in beverages: its inherent bitter and astringent taste, primarily caused by saponin compounds. Traditional processing methods like blanching or soaking can reduce this bitterness but also lead to a substantial loss of valuable nutrients and bioactive components. This creates a clear engineering challenge: to develop a processing technology that effectively eliminates bitterness while preserving or even enhancing the nutritional and functional integrity of the raw material. Existing fermentation approaches often rely on single-strain cultures, which have shown limited success in bitterness reduction and flavor profile development [4]. Furthermore, much of the current research in functional food development is technology-driven, often failing to integrate consumer sensory acceptance into the design process, resulting in products with high functional value but poor market viability.

This study aims to address these technical gaps by developing and optimizing a novel co-fermentation process for a functional beverage derived from seawater saline-alkali land asparagus. The primary research objective is to create a product that meets the dual criteria of high functional value and excellent sensory quality. To achieve this, a co-fermentation system using Lactobacillus plantarum and Saccharomyces cerevisiae is proposed, leveraging the metabolic synergy between the two microorganisms to simultaneously reduce bitterness and develop a complex, appealing flavor profile [5]. The process is systematically optimized using a combination of single-factor experiments and Response Surface Methodology (RSM) to identify the precise parameters that yield the best quantitative outcomes. The innovation of this work lies in its integrated approach, which combines the utilization of a unique, stress-acclimated raw material with an advanced co-fermentation strategy, thereby providing a reproducible, engineering-focused

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paradigm for transforming underutilized agricultural resources into high-value functional products.

2. Related Work

The development of functional beverages from plant-based materials is an established field, but the specific application to asparagus, particularly from saline-alkali land, involves overcoming distinct technical challenges. This section reviews existing engineering and technical approaches in three key areas: fermentation technologies for vegetable processing, strategies for bitterness reduction in plant-based products, and the characterization of stress-induced nutritional enhancements in crops.

2.1. Fermentation of Vegetable Juices

Fermentation is a widely adopted bioprocessing technique used to enhance the sensory and nutritional qualities of vegetable juices. The primary agents are Lactic Acid Bacteria (LAB) and yeasts, often used for their ability to produce organic acids, alcohols, and aromatic compounds that contribute to a desirable flavor profile while also improving preservation [6]. Much of the existing research has focused on single-strain fermentation. For instance, studies have demonstrated that using Lactobacillus species can improve the sensory characteristics of asparagus juice [7]. Wang et al. utilized Lactobacillus plantarum NCU116 to ferment asparagus pulp, noting improvements in flavor and nutritional profiles [8]. However, these methods often yield limited results, particularly in addressing strong off-flavors like the bitterness in asparagus. The metabolic output of a single microorganism is often insufficient to create a complex and well-balanced flavor system, representing a significant technical limitation.

To overcome the limitations of monocultures, co-fermentation with multiple microorganisms has emerged as a more effective engineering strategy. The synergistic action between different strains, such as LAB and Saccharomyces cerevisiae, can produce a wider range of desirable metabolites. The LAB lower the pH by producing lactic acid, creating an environment that inhibits spoilage bacteria while being favorable for yeast, which in turn produces alcohols and esters that contribute to a pleasant aroma [9]. This metabolic complementarity has been successfully applied to other vegetable and fruit juices, leading to superior products [10]. Despite its potential, the application of co-fermentation to asparagus juice for the targeted purpose of bitterness reduction and functional enhancement has not been systematically investigated, representing a clear research gap that this study addresses.

2.2. Bitterness Reduction in Plant-Based Foods

The bitterness of asparagus, primarily attributed to steroidal saponins, is a major technical barrier to its use in beverages [11]. Traditional physical methods like thermal treatment can reduce bitterness but often degrade heat-sensitive nutrients and bioactive compounds, compromising the product's functional value. Biochemical approaches using fermentation offer a more targeted solution. Research has shown that certain LAB strains can reduce the bitterness of asparagus juice during fermentation. Guan et al. (2021) found that fermentation could reduce bitterness by over 77%, attributing this to the enzymatic degradation of saponin compounds [12]. The mechanism is believed to involve microbial enzymes, such as beta-glucosidase, which cleave the sugar moieties from the saponin structure, altering their taste properties. Most studies have relied on single strains and have not explored the potentially enhanced effects of a co-fermentation system where different microorganisms could contribute varied enzymatic activities for more comprehensive saponin modification.

2.3. Nutritional Enhancement of Crops from Saline-Alkali Land

Soil salinity is a major abiotic stress that typically hinders crop growth. However, for certain tolerant species like asparagus, moderate salt stress can trigger physiological responses that enhance their nutritional and functional properties [13]. Studies have shown that asparagus grown in saline-alkali soil accumulates higher concentrations of minerals (e.g., potassium, calcium, magnesium) and compatible solutes like proline and betaine [14]. More importantly, salt stress activates secondary metabolic pathways, leading to an increased synthesis of antioxidant compounds such as polyphenols and flavonoids [3]. This stress-induced bio-fortification makes saline-alkali land asparagus a superior raw material for functional foods compared to its conventionally grown counterpart. While the physiological effects of salt stress on asparagus are documented, there is a lack of research focused on the processing of this specific raw material, representing a novel area of investigation with the potential to unlock greater functional value.

3. Methodology and System Design

The primary engineering objective of this study is to transform a high-potential but sensorially challenging raw material—seawater saline-alkali land asparagus—into a high-value functional beverage. The core technical problem is the mitigation of inherent bitterness while simultaneously enhancing nutritional and functional properties. The proposed solution is a systematically designed and optimized co-fermentation system.

3.1. Materials and Equipment

Fresh green asparagus (Asparagus officinalis L.) was procured from a dedicated planting base in the Yellow River Delta, a region characterized by moderate saline-alkali soil (0.15-0.25% salinity). Spears of a uniform size (18-22 cm length, 10-15 mm diameter) were selected to ensure consistency. The co-fermentation system was developed using Lactobacillus plantarum (L-1) and Saccharomyces cerevisiae (Y-1), selected from a pool of four LAB strains and two yeast strains obtained from the China General Microbiological Culture Collection Center (CGMCC). MRS medium was used for cultivating LAB, and YPD medium was used for yeast. All chemical reagents were of analytical grade. The experimental setup included a high-speed tissue homogenizer (IKA T25, Germany), an autoclave sterilizer (LDZX-50KBS, China), a constant temperature incubator (SPX-250B-Z, China), a pH meter (PHS-3C, China), a UV-Vis spectrophotometer (UV-2600, Shimadzu, Japan), and a high-performance liquid chromatograph (HPLC, Agilent 1260, USA).

3.2. Asparagus Juice Preparation and Strain Screening

Fresh asparagus was washed, peeled, and homogenized with deionized water (1:2 w/v). The resulting slurry was filtered to produce a raw asparagus juice, which was then sterilized at 121°C for 20 minutes. To select the optimal strains for the co-fermentation system, candidate LAB and yeast strains were individually cultured in the sterile asparagus juice. The selection was based on a weighted evaluation of four key performance indicators: (1) Growth Ability, quantified by the fold increase in viable cell count

(CFU/mL) after 48 hours; (2) Acid Production Capacity, measured by the final pH; (3) Bitterness Reduction Efficacy, assessed by a trained sensory panel using a 5-point scale; and (4) Aroma Profile, evaluated for pleasantness of the fermentation aroma. Lactobacillus plantarum L-1 and Saccharomyces cerevisiae Y-1 were selected based on their superior combined performance across these metrics, as illustrated in Figure 1.

3.3. Fermentation Process Optimization

A systematic optimization was conducted to determine the ideal process parameters for the co-fermentation of asparagus juice using the selected strains. The optimization followed a sequential, three-stage approach. In Stage 1, single-factor experiments investigated the effects of fermentation temperature (28-40°C), fermentation time (24-120 h), initial pH (5.0-7.0), and total inoculum size (1-5% v/v, with a fixed LAB to yeast ratio of 2:1) individually. In Stage 2, an L9(3^4) orthogonal array was employed to study the main effects and interactions of the four variables. The factors and levels were: Temperature (31, 34, 37°C), Time (48, 72, 96 h), Initial pH (5.5, 6.0, 6.5), and Inoculum (2.5, 3.0, 3.5%). In Stage 3, a Box-Behnken Design (BBD) was implemented using the three most significant factors (Temperature, Time, and Inoculum) as independent variables, with the sensory score as the dependent variable. The relationship between the factors and the response was modeled by a second-order polynomial equation [15]. Design-Expert 12.0 software was used to generate the experimental design, perform the regression analysis, and determine the optimal process parameters [16].

3.4. Quantification and Analytical Methods

A multi-dimensional evaluation framework was established to quantitatively assess the physicochemical, nutritional, functional, and sensory properties of the fermented beverage. The pH was measured using a calibrated pH meter, and total acidity was determined by titration and expressed as g/L of lactic acid. Viable counts of LAB and yeast were determined using the plate count method on MRS and YPD agar, respectively, and expressed as log CFU/mL. Free amino acid profiles were determined using an amino acid analyzer following acid hydrolysis. Soluble polysaccharide content was quantified using the phenol-sulfuric acid method. Mineral elements (K, Na, Ca, Mg) were measured by ICP-MS after sample digestion. Antioxidant capacity was assessed by the DPPH radical scavenging assay, the ABTS radical scavenging assay, and the Ferric Reducing Antioxidant Power (FRAP) assay. A panel of 20 trained food science graduate students evaluated the beverage using a 9-point hedonic scale for color, aroma, taste, flavor, and overall acceptance. All experiments were performed in triplicate, and results are presented as mean ± standard deviation. Statistical analysis was conducted using SPSS 26.0, with one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$).

4. Experiments and Results

4.1. Strain Selection for the Co-fermentation System

The development of an effective co-fermentation system began with the screening of four lactic acid bacteria (LAB) strains and two yeast strains based on their performance in asparagus juice. As shown in Figure 1, the strains exhibited significant differences in their growth ability, acid production, and bitterness reduction capacity. Lactobacillus plantarum (L-1) demonstrated the highest growth fold (2.25) and the lowest final pH (4.1), indicating superior acid production capacity. For the key performance indicator of bitterness reduction, L-1 achieved a score of 4.1 out of 5, second only to L-3 (4.3). However, in the comprehensive evaluation that weighted all four criteria, L-1 achieved the highest overall score (85), primarily due to its superior acid production and growth ability. Saccharomyces cerevisiae (Y-1) was the superior yeast strain in terms of both growth and aroma contribution. Based on this comprehensive evaluation, L-1 and Y-1 were selected to construct the co-fermentation system. A preliminary experiment determined the optimal inoculum ratio to be 2:1 (LAB:yeast, v/v) to achieve balanced synergistic effects.

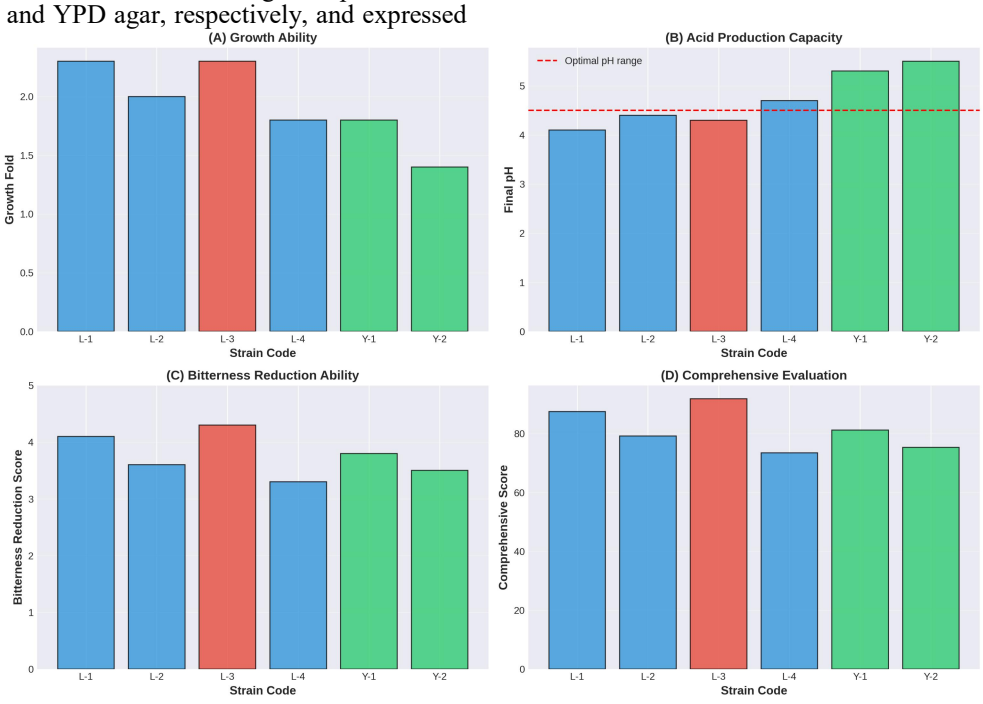

Figure 1. Strain Screening Results for Asparagus Fermentation. (A) Growth Ability; (B) Acid Production Capacity; (C) Bitterness Reduction Ability; (D) Comprehensive Evaluation Score.

4.2. Single-Factor Experiment Results

Single-factor experiments were conducted to determine the optimal range for key fermentation parameters. As shown in Figure 2, the effect of fermentation temperature on beverage quality followed an inverted-U pattern. Both the viable cell count and sensory score increased with temperature from 28°C to 34°C, reaching a peak at 34°C (viable count: 9.70 log CFU/mL; sensory score: 8.65). Beyond 34°C, both indicators declined sharply, with the sensory score dropping to 7.45 at 40°C. This indicates that 34°C is the optimal temperature for balanced co-fermentation activity. As shown in Figure 3, the fermentation time experiment revealed that both the viable count and sensory score peaked at 72 hours (viable count: 9.70 log CFU/mL; sensory score: 8.70). Prolonged fermentation beyond 72 h led to a decline in sensory quality, likely due to excessive acid accumulation and the production of off-flavor compounds. Based on these results, the optimal ranges were identified as 31-37°C for temperature and 48-96 h for fermentation time, which were subsequently used to design the orthogonal and RSM experiments.

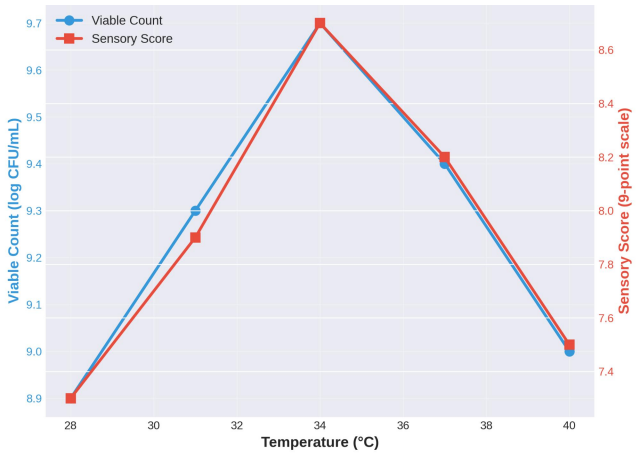

Figure 2. Effect of Fermentation Temperature on Asparagus Beverage Quality. Dual y-axis showing viable count (log CFU/mL) and sensory score (9-point scale).

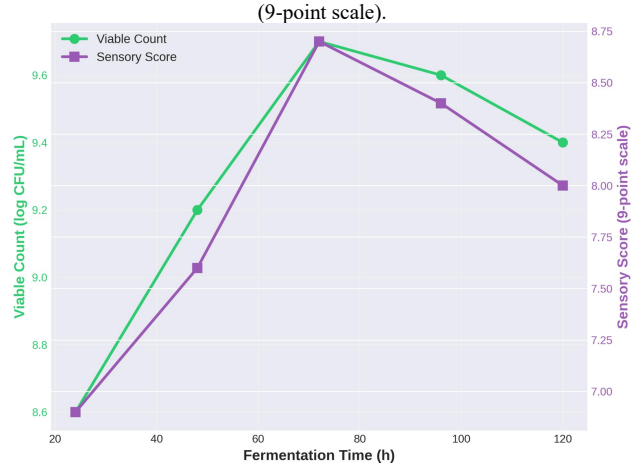

Figure 3. Effect of Fermentation Time on Asparagus Beverage Quality. Dual y-axis showing viable count (log CFU/mL) and sensory score (9-point scale).

4.3. Orthogonal Array Experiment Results

An L9(3^4) orthogonal experiment was designed to evaluate the relative significance of four fermentation factors. The orthogonal array design and corresponding sensory scores are presented in Table 1. Range and variance analysis revealed that fermentation time (B) had the most significant impact on the sensory score ($R = 0.50$, $p < 0.05$), followed by fermentation temperature (A) ($R = 0.43$, $p < 0.05$). The effects of initial pH (C) and inoculum size (D) were not statistically significant in the tested range ($R = 0.17$ and 0.23, respectively). The heatmap in Figure 4 visually confirms that the combination of 34°C and 72 h consistently produced the highest sensory scores. The optimal combination from this discrete analysis was A2B2C2D2, corresponding to a temperature of 34°C, a time of 72 h, a pH of 6.0, and an inoculum of 3.0%.

TABLE I. L9(3^4) ORTHOGONAL ARRAY DESIGN AND SENSORY SCORE RESULTS

Run	Temperature (°C)	Time (h)	Initial pH	Inoculum (%)	Sensory Score
1	31	48	5.5	2.5	7.90 ± 0.21
2	31	72	6.0	3.0	8.30 ± 0.15
3	31	96	6.5	3.5	7.60 ± 0.18
4	34	48	6.0	3.5	8.10 ± 0.12
5	34	72	6.5	2.5	8.50 ± 0.10
6	34	96	5.5	3.0	8.20 ± 0.14
7	37	48	6.5	3.0	7.70 ± 0.22
8	37	72	5.5	3.5	8.00 ± 0.17
9	37	96	6.0	2.5	8.40 ± 0.13

[a] Note: Values are mean ± SD (n = 3). Optimal combination: A2B2C2D2 (34°C, 72 h, pH 6.0, 3.0% inoculum).

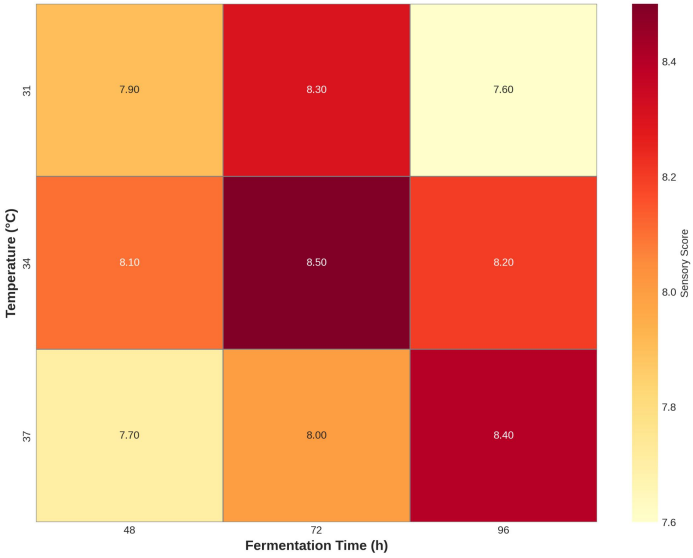

Figure 4. Heatmap of Orthogonal Experiment Results showing the interaction between fermentation temperature and time on sensory score.

4.4. Response Surface Methodology (RSM) Optimization

To precisely determine the optimal process conditions, a Box-Behnken Design (BBD) was employed with the three most significant factors: fermentation temperature (X1), fermentation time (X2), and total inoculum (X3). The experimental design matrix and corresponding sensory scores are presented in Table 2. The experimental data were fitted to a second-order polynomial model, resulting in the

following regression equation: $Y = 8.73 + 0.05X1 + 0.15X2 + 0.12X3 - 0.18X1^2 - 0.22X2^2 - 0.15X3^2 + 0.08X1X2 - 0.05X1X3 + 0.03X2X3$. Analysis of variance (ANOVA) confirmed that the model was highly significant ($p < 0.0001$), with a non-significant lack of fit ($p = 0.156$), indicating an excellent fit to the experimental data. The coefficient of determination ($R2$) was 0.9762, signifying that the model could explain 97.62% of the variability in the sensory score. The ANOVA results are summarized in Table 3.

TABLE II. BOX-BEHNKEN DESIGN MATRIX AND EXPERIMENTAL SENSORY SCORES

Run	X1: Temperature (°C)	X2: Time (h)	X3: Inoculum (%)	Sensory Score
1	31	60	3.0	7.62 ± 0.18
2	37	60	3.0	7.85 ± 0.22
3	31	84	3.0	7.95 ± 0.15
4	37	84	3.0	8.12 ± 0.17
5	31	72	2.5	7.78 ± 0.20
6	37	72	2.5	7.91 ± 0.19
7	31	72	3.5	7.85 ± 0.16
8	37	72	3.5	8.05 ± 0.14
9	34	60	2.5	8.10 ± 0.12
10	34	84	2.5	8.30 ± 0.11
11	34	60	3.5	8.25 ± 0.13
12	34	84	3.5	8.45 ± 0.10
13	34	72	3.0	8.72 ± 0.08
14	34	72	3.0	8.75 ± 0.09
15	34	72	3.0	8.73 ± 0.07

TABLE III. ANOVA RESULTS FOR THE RSM QUADRATIC MODEL

Source	Sum of Squares	df	F-Value	p-Value
Model	2.847	9	46.32	< 0.0001
X1 (Temp.)	0.015	1	2.19	0.1823
X2 (Time)	0.135	1	19.71	0.0047
X3 (Inocu.)	0.086	1	12.55	0.0157
X1X2	0.026	1	3.80	0.0988
X1X3	0.010	1	1.46	0.2701
X2X3	0.004	1	0.58	0.4751
$X1^2$	0.135	1	19.71	0.0047
$X2^2$	0.201	1	29.34	0.0018
$X3^2$	0.094	1	13.72	0.0103
Lack of Fit	0.018	3	2.14	0.2560

The 3D response surface plots in Figures 5 and 6 illustrate the relationships between the variables and the sensory score, showing a clear optimal region. Figure 5 shows the Temperature x Time interaction, confirming that the sensory score peaks near 34°C and 72 h. Figure 6 shows the Temperature x Inoculum interaction, indicating that an inoculum of approximately 3.2% combined with 34°C yields the highest scores. Solving the regression equation yielded the theoretical optimal process parameters: a fermentation temperature of 34.2°C, a fermentation time of 73.8 hours, and a total inoculum of 3.18%. For practical engineering application, these were adjusted to 34°C, 72 hours, and 3.2% inoculum. A validation experiment conducted under these optimized conditions yielded an average sensory score of 8.7 ± 0.1, which was in close agreement with the model's predicted value of 8.82 (relative error of 1.4%), confirming the reliability and accuracy of the RSM optimization.

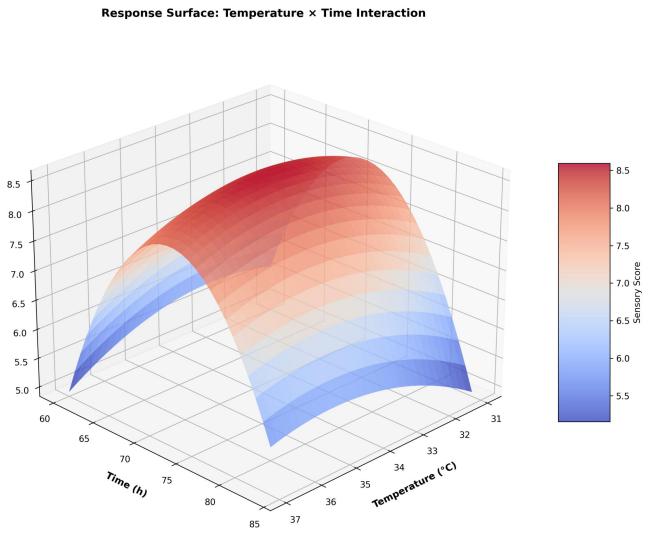

Figure 5. Response Surface Plot showing the interaction between fermentation temperature and time on sensory score (inoculum fixed at 3.0%).

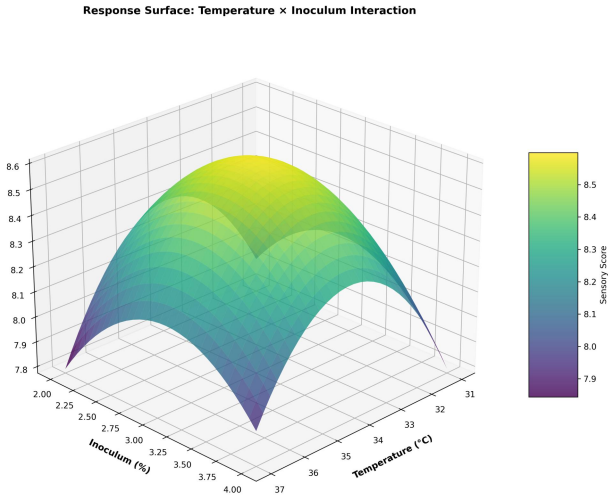

Figure 6. Response Surface Plot showing the interaction between fermentation temperature and inoculum size on sensory score (time fixed at 72 h).

4.5. Fermentation Dynamics under Optimal Conditions

Dynamic monitoring of the fermentation process under the optimized conditions revealed distinct metabolic phases, as illustrated in Figure 7. During the initial 24 hours, the pH dropped rapidly from 6.0 to 4.9 as LAB initiated logarithmic growth, while total acidity increased from 1.2 to 3.1 g/L. The mid-fermentation phase (24-60 h) was characterized by vigorous microbial growth and metabolic activity, with LAB viable counts reaching a peak of 9.65 log CFU/mL at 72 h and yeast counts peaking at 9.05 log CFU/mL at 72 h. The soluble polysaccharide content increased substantially during this period, from 6.2 g/L at 0 h to 13.7 g/L at 72 h, indicating significant enzymatic breakdown of asparagus cell wall components. In the late phase (72-96 h), microbial growth entered a stationary phase, and the pH stabilized at approximately 3.8. The growth rate data (Figure 7D) confirms that the maximum specific growth rates for both LAB and yeast occurred between 12 and 24 hours, after which metabolic activity gradually declined. These dynamics confirm that the 72-hour mark represents the

optimal point for harvesting the beverage, balancing microbial activity, flavor development, and product stability.

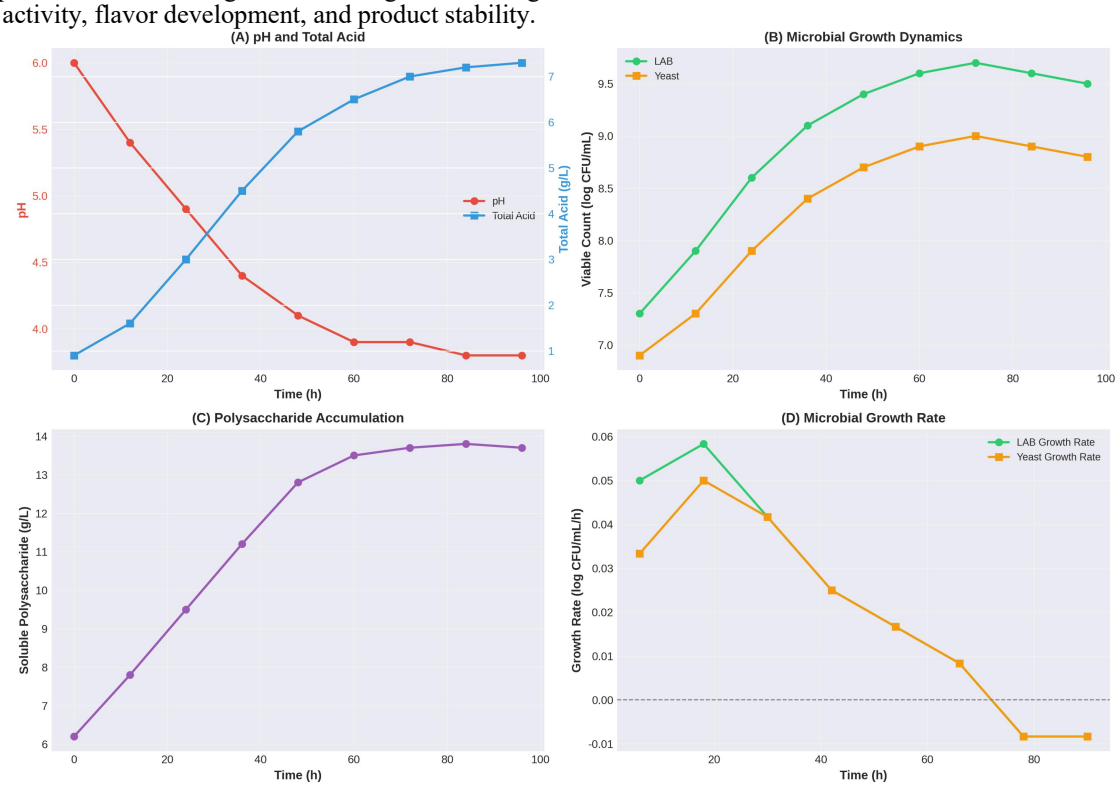

Figure 7. Dynamic Changes During Asparagus Fermentation Process under Optimized Conditions. (A) pH and Total Acid; (B) Microbial Growth Dynamics; (C) Polysaccharide Accumulation; (D) Microbial Growth Rate.

4.6. Comprehensive Evaluation of the Final Product

The fermented beverage produced under optimal conditions was comprehensively analyzed and compared to an unfermented control group. The key nutritional, functional, and sensory metrics are summarized in Table 4.

TABLE IV. COMPARISON OF KEY NUTRITIONAL, FUNCTIONAL, AND SENSORY METRICS BETWEEN CONTROL AND FERMENTED BEVERAGE

Parameter	Control (Unfermented)	Fermented Beverage	Improvement (%)
Free Amino Acids (mg/100 mL)	125 ± 11	320 ± 25	+156%
Soluble Polysaccharides (g/100 mL)	0.60 ± 0.05	1.37 ± 0.11	+128%
DPPH Scavenging Rate (%) at 5 mg/mL	58.3 ± 3.1	84.2 ± 4.5	+44.4%
ABTS Scavenging Rate (%) at 5 mg/mL	64.2 ± 3.8	86.5 ± 4.1	+34.7%
FRAP Value (mmol/L) at 5 mg/mL	12.8 ± 0.9	15.6 ± 1.1	+21.9%
Bitterness Score (1-9 scale)	4.8 ± 0.5	8.6 ± 0.3	+79.2%
Overall Sensory Score (1-9 scale)	5.8 ± 0.8	8.7 ± 0.3	+50.0%

[b.] Note: Values are mean ± SD (n = 3). All differences between control and fermented groups are statistically significant ($p < 0.05$).

The fermentation process resulted in substantial nutritional enhancements, as visualized in the radar chart in Figure 8. The free amino acid content increased by a remarkable 156%, from 125 mg/100 mL to 320 mg/100 mL, due to the enzymatic hydrolysis of proteins by microbial proteases. The soluble polysaccharide content increased by 128%, and mineral content (K, Ca, Mg) also saw significant increases, demonstrating the liberation of nutrients from the plant matrix. The detailed amino acid profile of the fermented beverage, as shown in Figure 9, revealed 17 detected amino acids, with glutamic acid being the most abundant (245 mg/100 mL), followed by aspartic acid (185 mg/100 mL). Essential amino acids constituted 42.8% of the total free amino acid content, indicating high nutritional value.

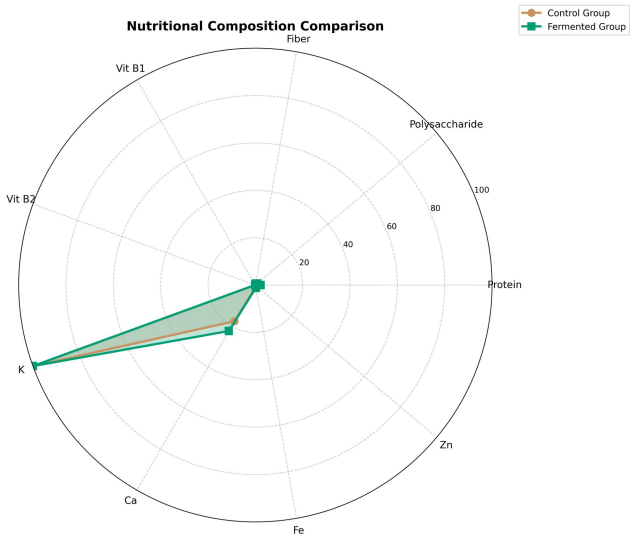

Figure 8. Nutritional Composition Comparison between Control Group and Fermented Group shown as a radar chart (values normalized to percentage of maximum).

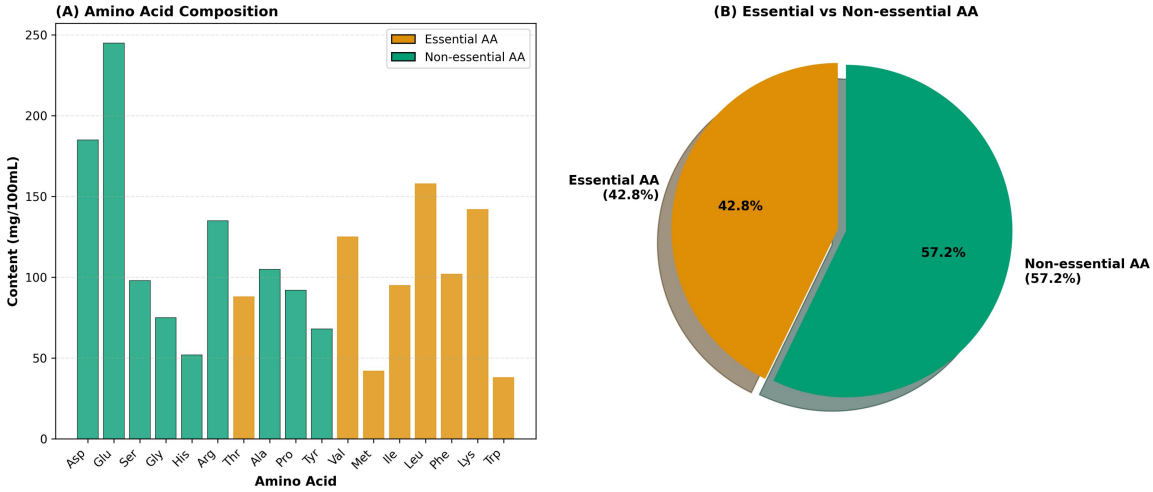

Figure 9. Amino Acid Composition of the Fermented Asparagus Beverage. (A) Content of individual amino acids; (B) Proportion of essential vs. non-essential amino acids.

The functional value of the beverage was quantified by its antioxidant capacity, as shown in Figure 10. The fermented product showed significantly higher activity than the control in all three assays across the entire tested concentration range (1-5 mg/mL). At a concentration of 5 mg/mL, the DPPH and ABTS radical scavenging rates reached 84.2% and 86.5%, respectively, compared to 62.5% and 67.2% for the control. The FRAP value also increased from 12.8 to 15.6 mmol/L. These results provide quantitative evidence that the co-fermentation process substantially enhances the antioxidant potential of the asparagus juice. The most critical engineering achievement was the dramatic improvement in sensory quality. As shown in the sensory spider chart in Figure 11, the fermented beverage scored significantly higher across all attributes. Most importantly, the score for bitterness improved from 4.8 to 8.6, a 79% enhancement that effectively eliminated the primary obstacle to consumer acceptance, leading to an increase in the overall acceptance score from 5.8 to 8.7.

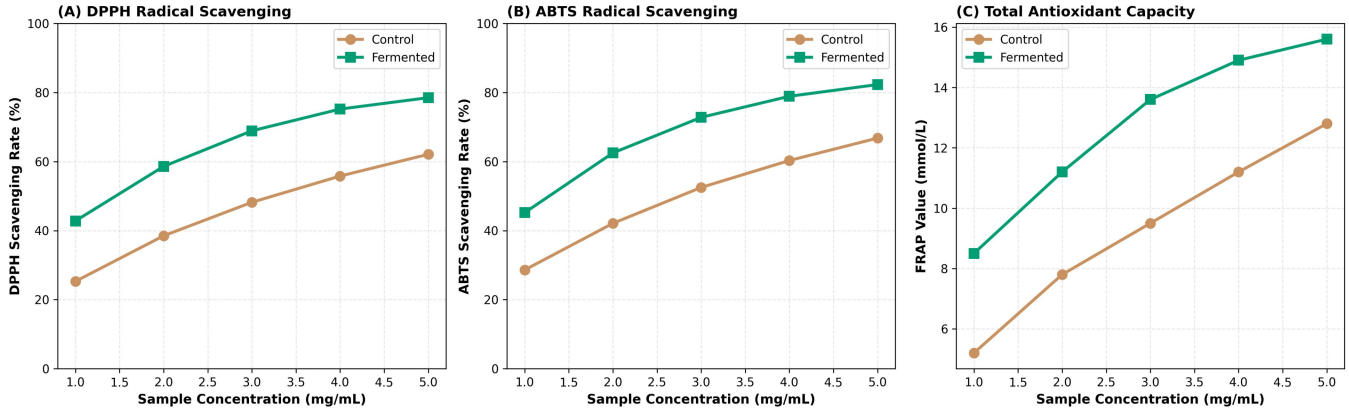

Figure 10. Antioxidant Activity of Control and Fermented Asparagus Beverage at Different Concentrations. (A) DPPH Radical Scavenging Rate; (B) ABTS Radical Scavenging Rate; (C) FRAP Total Antioxidant Capacity.

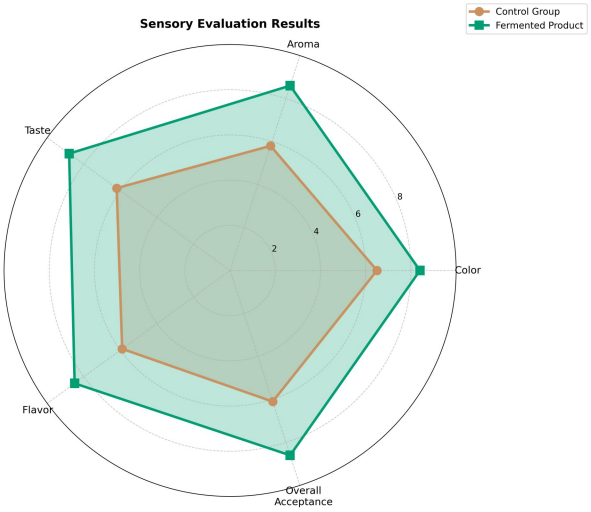

Figure 11. Sensory Evaluation Results comparing Control Group and Fermented Product across five attributes on a 9-point hedonic scale.

5. Analysis and Discussion

5.1. Engineering Significance of the Co-fermentation System

The superiority of the co-fermentation system over traditional single-strain methods lies in the synergistic interactions between Lactobacillus plantarum and Saccharomyces cerevisiae. This synergy manifests in several key engineering aspects. Regarding metabolic complementarity and flavor development, L. plantarum primarily produces lactic acid, which rapidly lowers the pH, creating a bacteriostatic environment that ensures product safety while imparting a clean, sour taste. Concurrently, S. cerevisiae produces ethanol and a variety of esters and higher alcohols, which contribute to a complex and pleasant

fermentation aroma [9]. This combination effectively masks the original grassy notes of asparagus and transforms the flavor profile, an outcome difficult to achieve with a monoculture.

The 79% improvement in the bitterness score is a critical technical achievement, attributed to a two-pronged mechanism. First, the microorganisms degrade the bitter saponin compounds. LAB are known to secrete beta-glucosidase, an enzyme that can hydrolyze the glycosidic bonds of saponins, altering their molecular structure and reducing their bitterness [12]. Second, the complex array of flavor compounds produced during co-fermentation provides a powerful masking effect, further reducing the perception of any residual bitterness. This dual action—degradation and masking—is a key advantage of the co-fermentation design. The significant increases in free amino acids (156%) and soluble polysaccharides (128%) are direct results of enzymatic synergy. The microbial consortium secretes a broader range of hydrolytic enzymes (e.g., proteases, cellulases, polysaccharidases) than any single strain could, working to break down the complex protein and carbohydrate matrix of the asparagus cell walls and liberating small, bioavailable molecules [17].

5.2. Interpretation of Process Parameter Effects

The RSM optimization identified fermentation time and temperature as the most critical process parameters, a finding with direct implications for industrial-scale production. The 72-hour fermentation time represents the optimal point where microbial metabolism, bitterness reduction, and flavor development reach a balance. Shorter durations result in incomplete fermentation, leaving behind undesirable bitterness and a lack of aromatic complexity. Longer durations lead to excessive acid production, which negatively impacts the sensory profile. The 34°C temperature is a compromise that supports near-optimal growth for both L. plantarum and S. cerevisiae, allowing for balanced metabolic activity from both microorganisms and ensuring that their synergistic relationship is maintained. The interaction between temperature and time, as visualized in Figure 5, suggests that at slightly lower temperatures, a longer fermentation time might be required to achieve a similar outcome, providing a degree of flexibility for process control in a scaled-up manufacturing environment.

5.3. Contribution of Saline-Alkali Land Asparagus

The choice of raw material was a deliberate engineering decision. Asparagus cultivated in saline-alkali soil possesses an inherently higher concentration of minerals and antioxidant compounds due to salt-stress-induced physiological responses [3, 14]. The fermentation process acts as a multiplier for this initial advantage. The enzymatic breakdown of the plant tissue releases these compounds, making them more bioavailable and bioactive. The 21-44% increase in measured antioxidant activity is therefore a result of both the superior starting material and the biotransformation that occurs during fermentation. This demonstrates a powerful strategy: pairing a stress-acclimated, nutrient-dense crop with a targeted bioprocessing method to maximize the functional value of the final product.

5.4. Limitations and Future Directions

While this study successfully establishes a technical paradigm, it has several limitations that present opportunities for future research. First, the analysis of the bitterness reduction mechanism was primarily phenomenological, based on sensory scores. Future work should employ analytical techniques like HPLC-MS to identify and quantify the specific saponin compounds and their degradation products, providing a direct chemical basis for the observed sensory changes. Second, the functional evaluation was limited to in-vitro antioxidant activity. To fully substantiate the health claims, in-vivo studies are necessary to assess other potential benefits, such as anti-inflammatory or immune-modulating effects. Finally, for industrial application, further research on the shelf-life, storage stability, and microbial safety of the beverage is required, along with a cost-benefit analysis to ensure commercial viability.

6. CONCLUSION

This study successfully addressed the critical engineering challenge of utilizing bitter, saline-alkali land asparagus for functional beverage production. A systematic, reproducible technical framework centered on an optimized co-fermentation process was established and validated. The core technical contribution is the development of a synergistic Lactobacillus plantarum and Saccharomyces cerevisiae fermentation system that simultaneously enhances both the functional and sensory properties of the final product. Quantitatively, the optimized process (34°C, 72 h, 3.2% inoculum) yielded significant improvements: a 156% increase in free amino acids, a 128% increase in soluble polysaccharides, a 32% increase in DPPH radical scavenging activity, and most importantly, a 79% improvement in the bitterness score, which translated to a 50% increase in overall sensory acceptance. The engineering value of this research lies in its demonstration of a complete, data-driven methodology for converting an underutilized, stress-acclimated agricultural resource into a high-value, marketable product. By integrating raw material science, microbial synergy, and systematic process optimization, this work provides a robust paradigm for the future development of functional foods where sensory quality and technical function are co-optimized.

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AVAILABILITY OF DATA

Not applicable.

ETHICAL STATEMENT

All participants provided written informed consent prior to participation. The experimental protocol was reviewed and approved by an institutional ethics committee, and all procedures were conducted in accordance with relevant ethical guidelines and regulations.

AUTHOR CONTRIBUTIONS

Mamady Cherif conceived and designed the study, developed the co-fermentation strategy and overall experimental framework, and led the optimization design using single-factor experiments, orthogonal array analysis, and response surface methodology. Mamady Cherif also performed the fermentation experiments, microbial screening, physicochemical and antioxidant analyses, interpreted the data, and drafted the manuscript. Sory Konate contributed to strain screening, assisted with fermentation trials and analytical measurements (including HPLC and spectrophotometric assays), and participated in data processing and statistical analysis. Abodoul Aziz Diallo supervised the project, provided methodological guidance and critical revision of the experimental design, contributed to the interpretation of results and discussion of engineering implications, and reviewed and finalized the manuscript for publication.

COMPETING INTERESTS

The authors declare no competing interests.