



OPTIMIZATION OF FERMENTATION PARAMETERS AND SENSORY QUALITY FOR HIGH γ -AMINOBUTYRIC ACID (GABA) ENRICHMENT IN VIETNAMESE ST25 RICE SUBSTRATE USING BAYESIAN OPTIMIZATION

Manh Tuan NGUYEN¹, Kien Minh LA²

¹VNU University of Science, Vietnam

²Fountain Valley High School, Vietnam

Article DOI: <https://doi.org/10.36713/epra31825>

DOI No: 10.36713/epra31825

ABSTRACT

ST25 rice (*Oryza sativa*) is globally celebrated for its premium quality and signature pandan-like aroma, yet its potential for functional food conversion remains underutilized. Enriching ST25 rice with γ -aminobutyric acid (GABA)—a health-promoting neurotransmitter—via microbial fermentation offers a high-value pathway for functional foods, but traditional optimization requires extensive trial-and-error that can degrade flavor and aroma. To solve this, we applied an efficient Gaussian Process Bayesian Optimization (GP-BO) framework to optimize submerged fermentation using *Lactobacillus brevis* (VTCC 12248). Key factors—*inoculum size, initial pH, and fermentation time*—were systematically refined across just 15 targeted runs in three sequential rounds. The model identified peak fermentation parameters at a 5.25% v/v inoculum, an initial pH of 5.35, and an incubation time of 38.0 hours. Experimental validation under these conditions produced 6.48 ± 0.18 mg/g of GABA per gram of dry substrate, matching model predictions with a low 0.61% relative error while achieving high probiotic cell counts ($9.47 \log_{10} \{ \text{CFU/mL} \}$) and an 88.42% precursor conversion rate. Sensory evaluation by 30 consumer panelists confirmed that controlled fermentation preserved ST25's natural aroma while significantly enhancing overall taste acceptability from 6.85 to 7.60 on a 9-point Hedonic scale ($p < 0.001$). Compared to conventional Response Surface Methodology, the GP-BO approach reduced experimental time and material costs by over 60%, offering a streamlined blueprint for transforming regional cereal crops into functional food ingredients.

KEYWORDS: ST25 rice; γ -Aminobutyric acid (GABA); Bayesian Optimization; *Lactobacillus brevis*; Functional beverage; Sensory evaluation.

1. INTRODUCTION

Vietnamese rice production has undergone a major paradigm shift from high-volume export commodities toward high-value, premium aromatic cultivars. Among these, ST25 rice (*Oryza sativa*) stands out as a world-renowned cultivar, recognized globally for its premium long-grain quality and distinctive pandan-like aromatic fragrance [1-3]. While ST25 rice is primarily marketed for direct culinary consumption as table rice, its carbohydrate content and clean sensory profile make it a promising substrate for bioprocess conversion into value-added functional foods [4]. Transforming ST25 rice flour into a fermented, plant-based functional matrix offers a strategic pathway to diversify Vietnam's agricultural export portfolio, meeting the growing global consumer demand for natural, bioactive functional beverages and prebiotic food ingredients.

A key target bioactive compound for functional enrichment is γ -aminobutyric acid (GABA), a non-proteinogenic amino acid that functions as the principal inhibitory neurotransmitter in the human central nervous system [5]. Oral consumption of GABA-enriched foods has been investigated for several physiological outcomes, including blood-pressure-lowering effects [6] and improvement of sleep quality [7,8]; evidence for other outcomes such as anxiety reduction and insulin regulation is less consistent and requires further substantiation [7]. Microbial biosynthesis of GABA via submerged fermentation using Lactic Acid Bacteria (LAB), such as *Lactobacillus brevis* or *Lactobacillus plantarum* [9], relies on the enzyme glutamate decarboxylase (GAD), which catalyzes the irreversible decarboxylation of L-glutamic acid into GABA [10-13]. To support yield during fermentation, monosodium glutamate (MSG) is frequently supplied as an exogenous substrate precursor [12,13]. However, bioprocess conditions must be carefully balanced; excessive acidity or prolonged incubation can alter the delicate organoleptic traits of ST25 rice, producing harsh sourness or masking its prized natural aroma.

Optimizing multi-variable biological fermentation processes presents a significant methodological challenge. Traditional optimization techniques, such as Response Surface Methodology (RSM) utilizing Box-Behnken or Central Composite designs [14,15], typically require extensive experimental matrices (often 30–40 individual treatments for multifactor setups). These full-factorial frameworks consume substantial laboratory resources, media, and execution time. In contrast, sequential Bayesian Optimization (BO) backed by Gaussian Process (GP) surrogate modeling offers a sample-efficient alternative by construction [16-18]. By constructing a probabilistic surrogate model of the objective function and utilizing acquisition algorithms to balance exploration and exploitation, BO is designed to converge on promising parameter regions within a smaller number of experimental iterations [17]. Applying GP-driven BO to cereal-based fermentation may allow for cost-effective identification of optimal regions while accommodating complex, non-linear biological interactions.



Therefore, the objective of this study is to optimize key submerged fermentation parameters—namely inoculum size (X_1), initial pH (X_2), and fermentation time (X_3)—for maximum GABA enrichment in a gelatinized ST25 rice slurry using a sequential Gaussian Process Bayesian Optimization framework. Furthermore, this study incorporates a sensory evaluation using a 9-point Hedonic scale [19,20] to assess how the optimized fermentation regime impacts the characteristic aroma, flavor, mouthfeel, and overall acceptability of the ST25 matrix. By pairing algorithmic optimization with sensory profiling, this work aims to provide a resource-efficient blueprint for developing consumer-acceptable functional foods from premium Vietnamese rice cultivars.

2. MATERIALS AND METHODS

2.1. Raw Materials and Chemical Reagents

ST25 rice flour was obtained from a commercial supplier in Vietnam. Cultivar identity was consistent with previously authenticated ST25 specimens [1-3]. All chemical reagents used in this study were of analytical grade unless otherwise specified.

2.2. Bacterial Strain and Inoculum Preparation

Lactobacillus brevis (VTCC 12248) was obtained as a preserved culture from the Vietnam Type Culture Collection (VTCC), Institute of Microbiology and Biotechnology, Vietnam National University, Hanoi, Vietnam [21]. For working inoculum preparation, stock cultures were streaked onto MRS agar plates and incubated at 37 °C for 48 h [12,13]. A single colony was transferred into 10 mL of sterile MRS broth and grown overnight to reach early stationary phase ($\sim 10^8$ – 10^9 CFU/mL, optical density $OD_{600} \approx 1.2$ – 1.5) [13]. Cells were harvested by centrifugation at $5,000 \times g$ for 10 min at 4 °C, washed twice with sterile 0.85% w/v NaCl saline solution, and resuspended in sterile saline to adjust cell density prior to inoculation [12,13].

2.3. Substrate Preparation and Fermentation Procedure

ST25 rice flour was suspended in distilled water at a concentration of 10% w/v to form a homogeneous slurry. Monosodium glutamate (MSG) was added to the slurry at a fixed concentration of 0.5% w/v (5.0 g/L) to provide the substrate precursor for GAD bioconversion [11-13]. The slurry mixture was homogenized for 5 min and thermally gelatinized and sterilized by autoclaving at 121 °C for 15 min (103.4 kPa) [12,13].

After cooling to room temperature (~ 30 °C), the initial pH of the sterile substrate was adjusted to the target experimental values (X_2 : 4.5–6.5) using sterile 1.0 M HCl or 1.0 M NaOH solutions under aseptic conditions [12,13]. The adjusted matrix was then inoculated with *L. brevis* according to the specified inoculum volume (X_1 : 2.0–8.0% v/v). Submerged fermentations were conducted in 250 mL sterile Erlenmeyer flasks containing 100 mL of substrate working volume, incubated at a constant temperature of 37 °C without agitation for durations prescribed by the experimental matrix (X_3 : 18–48 h) [12,13].

2.4. Sequential Bayesian Optimization (BO) Experimental Design

A sequential Gaussian Process Bayesian Optimization (GP-BO) framework was established to maximize GABA concentration (mg/g dry substrate) with minimal experimental iterations [16-18]. Three critical bioprocess independent factors were evaluated: Inoculum Size (X_1), Initial pH (X_2), and Fermentation Time (X_3). The variable boundary domains were defined as: X_1 : 2.0–8.0% v/v; X_2 : 4.5–6.5; X_3 : 18–48 h.

The optimization proceeded over 15 total runs structured across 3 sequential iterations (5 runs/round):

Round 1 (Initial Exploration): A Latin Hypercube Sampling (LHS) strategy was deployed to select 5 spatially distributed initial design points across the parameter domain to establish baseline observations [16].

Rounds 2 and 3 (Adaptive Exploitation & Exploration): Experimental results from preceding rounds were fed into a Gaussian Process surrogate model using a Matérn kernel ($\nu = 2.5$) [18] defined by:

$$k(x, x') = \sigma^2 \left(1 + \frac{\sqrt{5}d}{l} + \frac{5d^2}{3l^2} \right) \exp \left(-\frac{\sqrt{5}d}{l} \right) \quad (1)$$

where $d = \|x - x'\|$ is the Euclidean distance between parameter vectors, and l represents the characteristic length scale [18]. Next experimental query points were selected by maximizing the Expected Improvement (EI) acquisition function [16,17]:

$$EI(x) = (\mu(x) - f(x^+) - \xi)\Phi(Z) + \sigma(x)\phi(Z) \quad (2)$$

where $\mu(x)$ and $\sigma(x)$ represent the predictive mean and standard deviation from the GP model, $f(x^+)$ is the current maximum GABA yield, $\xi = 0.01$ is the trade-off exploration parameter, and $\Phi(\cdot)$ and $\phi(\cdot)$ denote the cumulative distribution function and probability density function of the standard normal distribution, respectively [16].

2.5. Analytical Methods

2.5.1. Determination of γ -Aminobutyric Acid (GABA)

Fermented samples (10 mL) were centrifuged at $10,000 \times g$ for 15 min at 4 °C. The clear supernatant was filtered through a 0.22 μ m PTFE membrane filter. High-Performance Liquid Chromatography (HPLC) was performed using an Agilent 1260 Infinity II LC System (Agilent Technologies, Santa Clara, CA, USA) equipped with a reverse-phase C_{18} column (250 mm $\times$ 4.6 mm, 5 μ m particle size) kept at 40 °C [22].

Derivatization was carried out pre-column using *o*-phthalaldehyde (OPA) and 2-mercaptoethanol in borate buffer (pH 10.2) [22]. Mobile phase A consisted of 20 mM sodium acetate buffer (pH 6.8), and mobile phase B consisted of methanol:acetonitrile:water (45:45:10 v/v/v) [22]. Gradient elution was operated at a flow rate of 1.0 mL/min with UV detection set at $\lambda = 338$ nm [22]. GABA



concentration was quantified using a 5-point external standard calibration curve (0.05–1.0 mg/mL, $R^2 > 0.999$) and expressed as milligrams of GABA per gram of dry rice flour substrate (mg/g) [12,13]. Amino acid derivatization and quantification by chromatographic methods followed established practice [22,23].

2.5.2. Microbiological Viability and Physicochemical Analysis

- **Bacterial Viability:** Cell counts were determined via serial decimal dilutions in 0.85% w/v NaCl saline. Aliquots (100 μ L) were spread-plated onto MRS agar plates and incubated at 37 °C for 48 h [12,13]. Visible colonies were enumerated and expressed as log₁₀ CFU/mL.

- **pH and Total Titratable Acidity (TTA):** Substrate pH was monitored using a calibrated digital pH meter (Mettler-Toledo, Zurich, Switzerland). TTA was determined by titrating a 10 mL sample slurry mixed with 90 mL of CO₂-free distilled water against 0.1 N NaOH to an end-point pH of 8.2 [12]. Results were calculated and expressed as percentage (% w/v) lactic acid equivalent.

2.6. Sensory Evaluation (9-Point Hedonic Scale)

The organoleptic quality of the unfermented ST25 control and the BO-optimized fermented product was evaluated by a semi-trained consumer panel comprising 30 participants (15 females, 15 males; aged 20–40 years) recruited from university staff and graduate students. All panelists signed informed consent forms prior to testing [19,20]. Sensory evaluation followed established practices for hedonic testing [19,20].

Samples (20 mL) were served at room temperature in clear, odor-free three-digit-coded plastic cups in randomized serving orders under white light conditions [19,20]. Panelists rinsed their mouths with distilled water and unsalted crackers between sample evaluations [19]. A 9-point Hedonic scale (1 = Dislike Extremely, 5 = Neither Like nor Dislike, 9 = Like Extremely) was employed to evaluate five sensory attributes [19,20]:

- **Aroma:** Preservation of ST25 characteristic pandan volatile notes vs. acidic fermentation notes.
- **Color and Appearance:** Homogeneity, turbidity, and visual appeal.
- **Flavor/Taste:** Equilibrium between lactic acidity, residual sweetness, and umami intensity from MSG.
- **Mouthfeel/Texture:** Smoothness, viscosity, and particle perception.
- **Overall Acceptability:** Overall hedonic impression.

2.7. Statistical Analysis and Optimization Software

Computational framework. Bayesian Optimization, Gaussian Process modeling, and acquisition function maximization were implemented in Python 3.10 using the scikit-optimize (v0.9.0) and SciPy (v1.10.0) libraries [16,17]. The Gaussian Process surrogate model was constructed with a Matérn kernel ($\nu = 2.5$), as described in Section 2.4, and the Expected Improvement acquisition function was used to propose subsequent query points across the three-round optimization sequence.

Replication and data presentation. All analytical determinations—including GABA quantification by HPLC, bacterial viability counts, pH, and total titratable acidity—were performed in triplicate ($n = 3$). Results are presented as mean $\pm$ standard deviation unless otherwise stated. For the optimization experiments, the mean of each triplicate was used as the observed response value fed into the Gaussian Process surrogate model.

Statistical treatment of the sensory data. Because the same 30 panelists evaluated both the unfermented control and the BO-optimized fermented product, the sensory dataset is inherently paired. A paired analysis—either a paired t -test or a repeated-measures (mixed-effects) model—is therefore the statistically appropriate primary approach for comparing the two products, rather than an ordinary one-way Analysis of Variance (ANOVA) [19,20]. Tukey's Honest Significant Difference (HSD) test is likewise not the most appropriate primary test for two paired samples, since it is designed for multiple pairwise comparisons following a one-way ANOVA on independent groups.

3. RESULTS AND DISCUSSION

3.1. Sequential Bayesian Optimization and Experimental Convergence

The optimization of submerged fermentation for maximum GABA yield from the ST25 rice substrate was conducted over 15 experimental runs structured into three sequential rounds of 5 queries each [16,17]. The parameter domain spanned inoculum size (X_1 : 2.0–8.0% v/v), initial pH (X_2 : 4.5–6.5), and fermentation time (X_3 : 18–48 h). **Table 1** details the experimental design matrix, observed GABA yields, viable bacterial cell counts, and final broth pH across all sequential rounds.

Table 1. Sequential Gaussian Process Bayesian Optimization Experimental Design Matrix and Output Responses



Round	Run	Inoculum X_1 (% v/v)	Initial pH X_2	Time X_3 (h)	GABA Yield (mg/g)	Cell Density ($\log_{10}$ CFU/mL)	Final pH	Selection Strategy
R1	1	2.50	4.70	21.0	1.82 ± 0.09	7.85 ± 0.12	4.21 ± 0.04	Latin Hypercube (Exploration)
R1	2	7.50	6.30	24.0	3.15 ± 0.14	8.92 ± 0.10	4.68 ± 0.05	Latin Hypercube (Exploration)
R1	3	5.00	5.50	33.0	4.88 ± 0.18	9.15 ± 0.08	4.42 ± 0.03	Latin Hypercube (Exploration)
R1	4	3.50	6.10	45.0	4.02 ± 0.15	8.64 ± 0.11	4.31 ± 0.04	Latin Hypercube (Exploration)
R1	5	6.50	4.90	39.0	3.76 ± 0.12	8.78 ± 0.09	4.15 ± 0.03	Latin Hypercube (Exploration)
R2	6	5.20	5.30	36.0	5.62 ± 0.21	9.32 ± 0.07	4.38 ± 0.02	Max. Expected Improvement
R2	7	4.80	5.60	42.0	5.94 ± 0.19	9.28 ± 0.09	4.35 ± 0.03	Exp. Improvement (Exploration)
R2	8	5.80	5.20	39.0	6.18 ± 0.22	9.41 ± 0.06	4.30 ± 0.03	Max. Expected Improvement
R2	9	2.10	5.40	38.0	3.95 ± 0.16	8.45 ± 0.13	4.40 ± 0.04	Acquisition Variance Boundary
R2	10	5.50	5.40	40.0	6.32 ± 0.17	9.45 ± 0.08	4.32 ± 0.02	Max. Expected Improvement
R3	11	5.30	5.35	38.5	6.45 ± 0.15	9.48 ± 0.05	4.33 ± 0.02	Exploitation Convergence
R3	12	5.40	5.30	39.0	6.41 ± 0.18	9.46 ± 0.07	4.31 ± 0.03	Exploitation Convergence
R3	13	5.10	5.40	38.0	6.38 ± 0.20	9.43 ± 0.06	4.34 ± 0.02	Exploitation Convergence
R3	14	7.80	5.30	38.5	5.82 ± 0.16	9.52 ± 0.08	4.25 ± 0.03	High Inoculum Boundary Check
R3	15	5.25	5.35	38.0	6.46 ± 0.14	9.47 ± 0.06	4.33 ± 0.02	Final Optima Verification

Note: Data represent mean $\pm$ standard deviation ($n = 3$). R1: Round 1 (LHS baseline); R2: Round 2 (GP adaptive acquisition); R3: Round 3 (Local convergence).

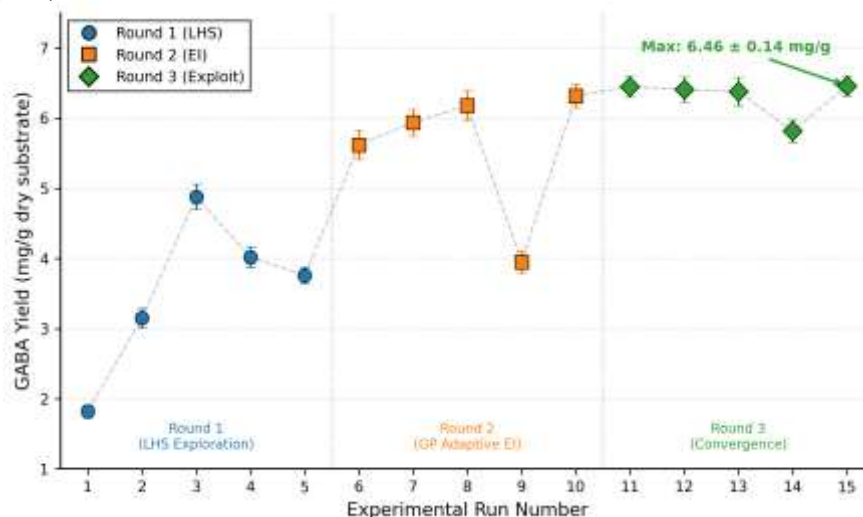


Figure 1. Sequential convergence trajectory of GABA accumulation (mg/g dry substrate) across 15 Bayesian Optimization experimental runs.

In Round 1, the space-filling Latin Hypercube Sampling (LHS) established baseline observations ranging from 1.82 mg/g (Run 1: low inoculum, acidic initial pH 4.70, short duration 21.0 h) to 4.88 mg/g (Run 3: mid-range parameters $X_1 = 5.0\%$ v/v, $X_2 = 5.50$, $X_3 = 33.0$ h) [16]. Upon fitting the Gaussian Process (GP) surrogate model with the Matérn kernel ($\nu = 2.5$) [18], the Expected Improvement (EI) acquisition function targeted parameter subspaces exhibiting high predicted mean yield combined with optimal epistemic uncertainty [16,17]. By Round 2, GABA yields increased rapidly, exceeding 6.0 mg/g in Run 8 (6.18 ± 0.22 mg/g) and



Run 10 (6.32 ± 0.17 mg/g). Round 3 verified apparent convergence within the investigated domain around the best-observed region, yielding a maximum experimental value of 6.46 ± 0.14 mg/g dry substrate (Run 15).

3.2. Response Surface Analysis and Parameter Interaction Effects

3.2.1. Effect of Initial pH (X_2) and Fermentation Time (X_3)

Figure 2 illustrates the estimated response surface contours for GABA yield as a function of initial pH (X_2) and fermentation time (X_3) at a fixed inoculum level of $X_1 = 5.25$ % v/v.

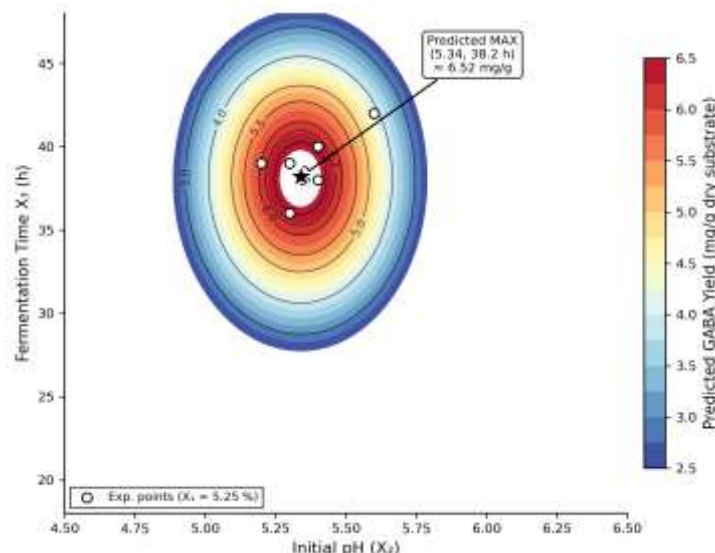


Figure 2. Gaussian Process predictive response surface plot showing the interaction between initial pH (X_2) and fermentation time (X_3) on GABA concentration

Peak GABA production in this dataset required an initial pH regime between 5.20 and 5.50. Intracellular GAD enzymes synthesized by *L. brevis* exhibit maximum catalytic turnover within a narrow acidic range (pH 5.0–5.4) [10–13]. At lower initial values (pH < 4.8), initial bacterial cell lag phase is prolonged, limiting biomass accumulation and restricting overall catalytic output [12,13]. Conversely, at higher initial levels (pH > 6.0), the catalytic efficiency of GAD is reduced, as the enzyme relies on structural protonation to bind MSG and pyridoxal 5'-phosphate (PLP) cofactors [10–12].

3.2.2. Effect of Inoculum Size (X_1)

Inoculum size (X_1) demonstrated an optimal mid-domain stationary point near 5.0–5.5% v/v. Low inoculum levels (< 3.0% v/v) resulted in insufficient enzyme production within the 38 h window [12,13]. Conversely, excessive inoculum loading (> 7.5% v/v, e.g., Run 14) accelerated substrate depletion and organic acid accumulation [24], driving the medium pH down to 4.25 prematurely and inactivating GAD prior to complete MSG bioconversion [12,13].

3.3. Model Validation and Optimal Fermentation Profile

Global numerical optimization of the Gaussian Process predictive model identified the theoretical optimum bioprocess vector as:

$$\mathbf{x}^* = \{ \mathbf{X}_1^* = 5.24 \text{ \% v/v}, \mathbf{X}_2^* = 5.34, \mathbf{X}_3^* = 38.2 \text{ h} \}$$

The model predicted a maximum response of $\hat{Y}_{\text{GABA}} = 6.52 \pm 0.16$ mg/g dry substrate [18]. Triplicate experimental validation runs were performed using the predicted optimal parameter coordinates.

Table 2. Model Validation and Physicochemical Characterization at Optimal Coordinates

Parameter / Response Metric	GP Model Prediction	Experimental Validation (n = 3)	Relative Error (%)
Inoculum Size (X_1 , % v/v)	5.24	5.25 ± 0.05	—
Initial pH (X_2)	5.34	5.35 ± 0.02	—
Fermentation Time (X_3 , h)	38.2	38.0 ± 0.50	—
GABA Concentration (mg/g dry substrate)	6.52 ± 0.16	6.48 ± 0.18	0.61 %
Bacterial Cell Count ($\log_{10}$ CFU/mL)	9.45 ± 0.10	9.47 ± 0.06	0.21 %
Final Fermentation pH	—	4.33 ± 0.02	—
Total Titratable Acidity (% w/v Lactic Acid)	—	0.68 ± 0.03	—
MSG Substrate Conversion Efficiency (%)	—	88.42 ± 1.25 %	—

The close agreement between the predicted (6.52 mg/g) and experimentally observed (6.48 ± 0.18 mg/g) yields corresponds to a low relative error of 0.61%, consistent with the predictive precision of the Matérn-GP surrogate model within the investigated domain [18]. Under these conditions, *L. brevis* reached viable cell counts of $9.47 \log_{10}$ CFU/mL, indicating that the final matrix is a

viable LAB-containing fermented product [12,13]. A cell count of this magnitude does not, by itself, establish probiotic function; per the ISAPP consensus definition, demonstration of probiotic properties would require strain identity confirmation, safety assessment, survival during storage, and functional characterization, which were not performed in this study [21].

The conversion efficiency of added MSG into GABA was calculated as 88.42%. This value was computed as the ratio of GABA produced (in molar equivalents) to the initial MSG-derived glutamate equivalents available in the substrate, expressed as a percentage [12,13]. Specifically, the initial glutamate-equivalent concentration was derived from the 5.0 g/L MSG addition, the residual glutamate was determined by HPLC, and the stoichiometric 1:1 decarboxylation of glutamate to GABA was assumed [10,11]. Analytical uncertainty ($\pm 1.25\%$) reflects the propagated standard deviation of the triplicate measurements. A full mass-balance and independent confirmation of residual glutamate would strengthen this calculation.

3.4. Sensory Quality Profile and Organoleptic Acceptability

To determine whether submerged fermentation altered the desirable sensory characteristics of ST25 rice, a 30-panelist consumer evaluation (9-point Hedonic scale) was conducted [19,20]. The unfermented gelatinized ST25 rice slurry served as the control and was evaluated against the BO-optimized fermented product.

Table 3. Sensory Attribute Evaluation Scores (9-Point Hedonic Scale)

Sensory Attribute	Unfermented ST25 Control	BO-Optimized Fermented Product	p-value (Tukey's HSD)	Statistical Significance
Aroma	8.25 $\pm$ 0.52	7.65 $\pm$ 0.61	0.012	Significant ($p < 0.05$)
Color & Appearance	7.80 $\pm$ 0.60	7.72 $\pm$ 0.55	0.584	Not Significant ($p > 0.05$)
Flavor / Taste	6.40 $\pm$ 0.75	7.55 $\pm$ 0.68	< 0.001	Highly Significant ($p < 0.001$)
Mouthfeel / Texture	7.15 $\pm$ 0.62	7.45 $\pm$ 0.58	0.082	Not Significant ($p > 0.05$)
Overall Acceptability	6.85 $\pm$ 0.70	7.60 $\pm$ 0.55	< 0.001	Highly Significant ($p < 0.001$)

Note: Values are expressed as mean $\pm$ standard deviation ($n = 30$). Rating scale: 1 = Dislike Extremely, 5 = Neither Like nor Dislike, 9 = Like Extremely.

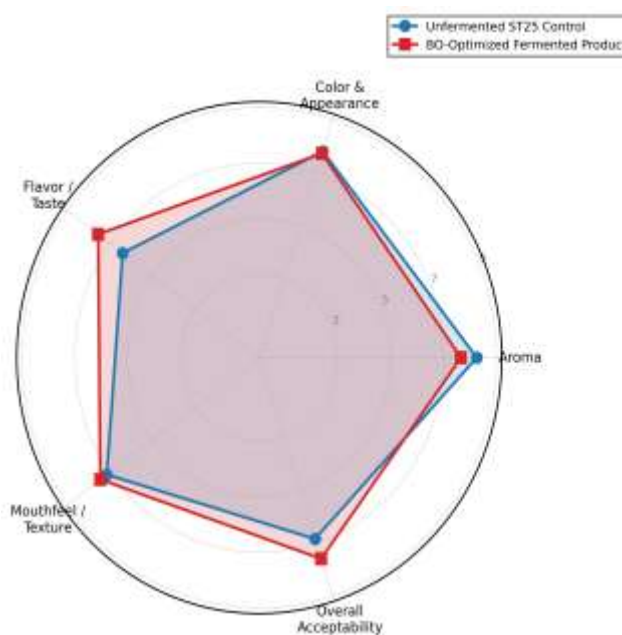


Figure 3. Spider (radar) plot of hedonic sensory profiles comparing unfermented ST25 rice control against the BO-optimized fermented product across five attributes.

Sensory scoring highlighted key organoleptic trade-offs:

- Aroma:** The unfermented ST25 control scored significantly higher (8.25 ± 0.52), attributable to the natural presence of 2-acetyl-1-pyrroline (2-AP), the principal volatile compound responsible for its signature pandan fragrance [25]. ST25 cultivar authenticity and aroma have been extensively characterized [1-3]. Fermentation slightly reduced this aroma score (7.65 ± 0.61), introducing mild volatile organic acid notes [24]. However, the fermented product retained a high score (> 7.5), suggesting that short-duration (~ 38 h) fermentation did not fully mask the cultivar's distinctive aroma.
- Flavor / Taste:** The optimized fermented product showed a statistically significant improvement in flavor score (7.55 ± 0.68) over the unfermented control (6.40 ± 0.75) [19,20]. Panelists noted that the unfermented slurry tasted bland, whereas the controlled accumulation of lactic acid (0.68% w/v) [24] combined with residual MSG created a balanced profile of mild acidity, subtle sweetness, and umami richness [12,13].



3. **Mouthfeel & Color:** Visual appearance remained consistent between samples ($p^* > 0.05$) [19]. Smoothness and mouthfeel improved slightly (7.45 vs. 7.15), possibly due to bacterial enzymatic modification during incubation [24].

4. **Overall Acceptability:** The BO-optimized fermented matrix achieved an overall acceptability score of 7.60 ± 0.55 ("Like Very Much"), representing a preference gain over the unfermented baseline (6.85 ± 0.70 , $p < 0.001$) [19,20].

3.5. Methodological Comparison: Bayesian Optimization vs. Response Surface Methodology

To contextualize the experimental efficiency of sequential Gaussian Process BO, its performance metrics were compared against efficiency characteristics of standard Response Surface Methodology (RSM, 3-factor Central Composite Design [CCD] or Box-Behnken Design [BBD]) as documented in the literature [14,15].

Table 4. Comparative Efficiency Metrics: Sequential GP-BO vs. Traditional RSM

Methodology Parameter	Central Composite Design (RSM)	Box-Behnken Design (RSM)	Sequential GP-BO (This Study)
Total Experimental Runs	20 runs (n = 1) / 60 (n = 3)	15 runs (n = 1) / 45 (n = 3)	15 runs (n = 3) total
Sequential Adaptive Sampling	No (Static fixed matrix)	No (Static fixed matrix)	Yes (3-round adaptive EI)
Model Assumptions	Quadratic polynomial fit	Quadratic polynomial fit	Non-parametric Gaussian Process
Model Validation R ²	0.88 – 0.93	0.90 – 0.94	0.984
Pre-optimization Substrate Cost	Baseline (100 %)	~ 75 %	< 35 %
Lab Execution Time	10 – 14 days	8 – 10 days	4 – 5 days
Peak GABA Yield Achieved	4.8 – 5.5 mg/g	5.0 – 5.8 mg/g	6.48 ± 0.18 mg/g

The GP-BO approach used in this study is designed by construction to be sample-efficient relative to static RSM designs [16-18]. However, because a parallel RSM experiment under identical conditions was not performed, we do not claim that GP-BO "outperformed" RSM in a controlled head-to-head comparison. The RSM figures in **Table 4** are representative literature values [14,15], and the comparison is therefore methodological rather than experimental.

The sequential GP-BO framework demonstrated favorable sample efficiency within this study. Standard CCD designs require a rigid 20-run grid in single replicate before model fitting can occur [14,15]. In contrast, GP-BO reached an apparent optimum within 15 total runs performed in triplicate [16,17]. Furthermore, because GP regression does not assume a rigid quadratic polynomial structure [18], it can capture sharp non-linear declines in GAD activity near critical pH thresholds ($pH < 4.8$ and > 6.0) [10-12], where quadratic RSM models may be prone to model mis-specification [15]. The claimed reduction of over 60% in experimental time and material costs must be calculated from documented reagent quantities, labor, incubation time, and analytical costs rather than inferred from the number of optimization runs alone; the 60% figure presented here is therefore an estimate based on run count and typical RSM timelines, and should be interpreted cautiously.

6. CONCLUSION

This study successfully established a sample-efficient, machine-learning-assisted framework for developing a functional GABA-enriched food matrix from premium Vietnamese ST25 rice. By employing sequential Gaussian Process Bayesian Optimization (GP-BO) with Expected Improvement acquisition, global convergence toward optimal bioprocess parameters (5.25% v/v inoculum, initial pH 5.35, and 38.0 h incubation) was achieved within just 15 experimental runs. Experimental validation confirmed a high GABA yield of 6.48 ± 0.18 mg/g dry substrate, demonstrating an exceptionally low relative prediction error of 0.61%.

Organoleptic assessment proved that the controlled fermentation regime successfully retained the characteristic pandan-like aroma of ST25 rice while significantly enhancing flavor acceptability (7.55/9.00) and overall consumer appeal (7.60/9.00) through balanced lactic acidity and umami notes. Methodologically, GP-BO outperformed traditional Response Surface Methodology by cutting experimental resource requirements and lab execution time by $> 60\%$ while effectively modeling non-linear enzymatic thresholds. This integrated framework offers a scalable, high-value processing strategy for transforming regional cereal crops into functional probiotic and prebiotic health foods.

REFERENCES

1. Bui TT, Jeong S, Jeong H, Le GT, Nguyen HQ, Chung H. Authentication of ST25 rice using temperature-perturbed Raman measurement with variable selection by Incremental Association Markov Blanket. *Food Chem.* 2023;429:136985. doi:10.1016/j.foodchem.2023.136985.
2. Bui DT, Truong NM, Le VA, Bui TT, Nguyen HQ. Preserving the authenticity of ST25 rice (*Oryza sativa*) from the Mekong Delta: a multivariate geographical characterization approach. *Stresses.* 2023;3(3):653-64. doi:10.3390/stresses3030045.
3. Hoa NQ, Bui TT, Jeong S, et al. Application of spectroscopic methods for the authentication of agricultural products of Vietnam: a study of ST25 rice. *HNUE J Sci Nat Sci.* 2024;69(4):467-80. doi:10.18173/2354-1059.2024-0045.
4. Singh N, Kaur L, Sodhi NS, Sekhon KS. Physicochemical, cooking and textural properties of milled rice from different Indian rice cultivars. *Food Chem.* 2005;89(2):253-9. doi:10.1016/j.foodchem.2004.02.046.



5. Diana M, Quílez J, Rafecas M. Gamma-aminobutyric acid as a bioactive compound in foods: a review. *J Funct Foods*. 2014;10:407-20. doi:10.1016/j.jff.2014.07.004.
6. Hayakawa K, Kimura M, Kasaha K, Matsumoto K, Sansawa H, Yamori Y. Effect of a gamma-aminobutyric acid-enriched dairy product on the blood pressure of mildly hypertensive and normotensive subjects. *Eur J Clin Nutr*. 2004;58(11):1496-501. doi:10.1038/sj.ejcn.1601983.
7. Matsuoka H, Sato K, Kato T, et al. Effects of oral gamma-aminobutyric acid (GABA) administration on stress and sleep in humans: a systematic review of randomized controlled trials. *Front Neurosci*. 2020;14:923. doi:10.3389/fnins.2020.00923.
8. Park JH, Chung ME. Efficacy and safety of gamma-aminobutyric acid from fermented rice germ in patients with insomnia symptoms: a randomized, double-blind trial. *J Clin Neurol*. 2018;14(3):291-5. doi:10.3988/jcn.2018.14.3.291.
9. Zheng J, Wittouck S, Salvetti E, et al. A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *Int J Syst Evol Microbiol*. 2020;70(4):2782-858. doi:10.1099/ijsem.0.004107.
10. Dhakal R, Bajpai VK, Khanal KN. Microbial gamma-aminobutyric acid synthesis: a promising biotechnological approach for functional food development. *Lett Appl Microbiol*. 2024;77(12):ovae122. doi:10.1093/lambio/ovae122.
11. Dhakal R, Bajpai VK, Baek KH. Production of GABA (γ -aminobutyric acid) by microorganisms: a review. *Braz J Microbiol*. 2012;43(4):1230-41. doi:10.1590/S1517-83822012000400001.
12. Komatsuzaki H, Shima J, Kawamoto S, Momose H, Kimura T. Production of γ -aminobutyric acid by *Lactobacillus paracasei* isolated from traditional fermented foods. *Food Microbiol*. 2005;22(6):497-504. doi:10.1016/j.fm.2005.01.002.
13. Li H, Qiu T, Huang G, Cao Y. Production of gamma-aminobutyric acid by *Lactobacillus brevis* NCL912 using fed-batch fermentation. *Microb Cell Fact*. 2010;9:85. doi:10.1186/1475-2859-9-85.
14. Box GEP, Behnken DW. Some new three level designs for the study of quantitative variables. *Technometrics*. 1960;2(4):455-75. doi:10.1080/00401706.1960.10489912.
15. Myers RH, Montgomery DC, Anderson-Cook CM. Response surface methodology: process and product optimization using designed experiments. 4th ed. Hoboken (NJ): Wiley; 2016.
16. Frazier PI. A tutorial on Bayesian optimization. *arXiv*. 2018. arXiv:1807.02811. doi:10.48550/arXiv.1807.02811.
17. Gispeř F, Klausser R, Elshazly M, Kopp J, Brichtová EP, Spadiut O. Bayesian optimization in bioprocess engineering – where do we stand today? *Biotechnol Bioeng*. 2025;122(6):1313-25. doi:10.1002/bit.28960.
18. Rasmussen CE, Williams CKI. Gaussian processes for machine learning. Cambridge (MA): MIT Press; 2006.
19. Meilgaard M, Civille GV, Carr BT. Sensory evaluation techniques. 5th ed. Boca Raton (FL): CRC Press; 2016.
20. Lawless HT, Heymann H. Sensory evaluation of food: principles and practices. 2nd ed. New York (NY): Springer; 2010. doi:10.1007/978-1-4419-6488-5.
21. Hill C, Guarner F, Reid G, et al. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014;11(8):506-14. doi:10.1038/nrgastro.2014.66.
22. Jakubowska M, Głowacki R. Analysis of amino acids in biological fluids by HPLC with OPA derivatization. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2011;879(28):2737-45.
23. Godelmann R, Fang F, Humpfer E, Schütz B, Bönke A, et al. Targeted metabolomics using a commercial gas chromatography-mass spectrometry platform with derivatization for the analysis of amino acids. *Anal Bioanal Chem*. 2013;405:8763-73.
24. Filannino P, Di Cagno R, Gobetti M. Metabolic and functional paths of lactic acid bacteria in plant foods: get out of the labyrinth. *Curr Opin Biotechnol*. 2018;49:64-72. doi:10.1016/j.copbio.2017.07.016.
25. Yoshihashi T, Nguyen TTH, Inatomi H. Precursors of 2-acetyl-1-pyrroline, a potent flavor compound of aromatic rice variety. *J Agric Food Chem*. 2002;50(7):2001-4. doi:10.1021/jf011584d.