

ADVANCED MACHINE LEARNING APPROACHES FOR PREDICTIVE MODELING OF MICROBIAL GROWTH DYNAMICS AND SHELF LIFE IN FUNCTIONAL FOODS

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ABSTRACT

The escalating global demand for functional foods—products formulated to confer health benefits beyond basic nutrition—has intensified the need for robust predictive frameworks capable of ensuring microbiological safety and quality throughout complex supply chains. Traditional predictive microbiology models, while providing foundational contributions to the field, exhibit inherent limitations in capturing the intricate, nonlinear interactions among intrinsic food properties, extrinsic environmental factors, and microbial population dynamics within functional food matrices. This investigation presents a comprehensive evaluation of advanced machine learning (ML) methodologies for predictive modeling of microbial growth kinetics and shelf life estimation in functional foods. Five ML algorithms—Radial Basis Function Neural Networks (RBF-NN), Random Forest Regression (RFR), Support Vector Regression (SVR), Gaussian Process Regression (GPR), and Long Short-Term Memory (LSTM) networks—were systematically assessed against conventional primary growth models (Gompertz, Logistic, and Baranyi) utilizing a multi-dataset framework encompassing diverse functional food matrices. Performance metrics including adjusted R^2 , Root Mean Square Error (RMSE), Bias Factor (Bf), and Accuracy Factor (Af) revealed that ML approaches consistently surpassed traditional models, with GPR achieving the highest predictive accuracy ($R^2_{adj} = 0.959$, RMSE = 0.153) and LSTM demonstrating superior capacity for capturing temporal dependencies under dynamic temperature conditions (72.5% RMSE reduction compared to Gompertz). The RBF neural network model exhibited exceptional performance in shelf life prediction with a relative error of 2.66%, substantially outperforming conventional kinetic models. SHapley Additive exPlanations (SHAP)-based interpretability analysis identified temperature, pH, and water activity as the most influential predictors across all models. These findings establish machine learning as a transformative paradigm in predictive food microbiology, offering unprecedented accuracy flexibility, and data-driven insight for shelf life estimation in functional foods, with significant implications for food safety management and quality assurance in the functional food industry.

KEYWORDS: machine learning, predictive microbiology, functional foods, microbial growth dynamics, shelf life prediction, neural networks, Gaussian process regression, food safety, SHAP analysis

1. INTRODUCTION

1.1 Background and Rationale

The functional food sector has experienced remarkable expansion over the preceding decade, propelled by escalating consumer recognition of the diet-health nexus, mounting healthcare expenditures, and significant advances in nutritional science. Functional foods, broadly defined as products delivering health benefits surpassing fundamental nutritional functions through the incorporation of bioactive compounds, probiotic microorganisms, prebiotic fibers, or fortified nutrients, now constitute a multibillion-dollar global industry with projected sustained growth trajectories (Tarlak, 2023). This sector encompasses an extensive array of products, including fermented dairy items, probiotic beverages, fortified baked goods, functional confectionery, and specialized nutritional supplements, each presenting unique challenges for quality assurance and shelf life determination (Kumar et al., 2024).

The intrinsic complexity of functional food products, characterized by diverse formulations, active biological constituents, and variable physicochemical properties, imposes significant challenges for conventional quality management approaches. Microbial spoilage remains a primary determinant of shelf life in functional foods, directly affecting product safety, nutritional integrity, sensory characteristics, and consumer health outcomes (Tarlak et al., 2025). The presence of bioactive compounds, probiotic cultures, and specialized ingredients in functional foods creates distinctive microbial ecosystems that differ substantially from conventional food products, necessitating

sophisticated approaches for understanding and predicting microbial growth dynamics within these complex matrices (Taiwo et al., 2024).

The economic and public health implications of inadequate shelf life prediction are substantial. Premature product disposal due to inaccurate shelf life estimation contributes to food waste, representing both economic losses and environmental burden (Liang et al., 2026). Conversely, overestimation of shelf life poses significant food safety risks, potentially leading to consumer exposure to spoilage organisms or foodborne pathogens. The World Health Organization estimates that foodborne diseases affect approximately 600 million people annually, with significant morbidity and mortality particularly affecting vulnerable populations in developing nations (Taiwo et al., 2024). Consequently, accurate and reliable predictive frameworks for microbial growth dynamics are essential for ensuring product safety, minimizing waste, and maintaining consumer confidence in functional food products.

1.2 Traditional Predictive Microbiology:

Predictive food microbiology has historically relied upon mathematical models to forecast microbial behavior in food environments, integrating microbiological knowledge with mathematical and statistical principles (Ross & McMeekin, 2003). These models are conventionally categorized into two principal types: primary and secondary models. Primary models describe microbial population behavior over time under static environmental conditions, capturing population growth, survival, or decline when external factors remain constant. Secondary models account for the influence of environmental variables—temperature, pH, water activity, and food matrix composition—on the parameters of primary models (Chaturvedi et al., 2023).

The classical approach to predictive modeling employs well-established primary growth models including the Gompertz, Logistic, and Baranyi models (Tarlak, 2023). The Baranyi model, in particular, has received widespread recognition for its mechanistic foundation and capacity to describe bacterial growth dynamics with physiological relevance (Baranyi & Roberts, 1994). Secondary models extend these frameworks by incorporating environmental factors through mathematical relationships, typically requiring either two-step or one-step modeling approaches (Ross, 1996).

Despite their foundational contributions to food microbiology, traditional predictive models exhibit several critical limitations that constrain their applicability to contemporary food systems. A significant concern is the potential accumulation and amplification of errors, which may occur because the nonlinear regression process is performed twice—initially in developing the primary model and subsequently when integrating environmental factors in the secondary model (Kumar et al., 2024). Furthermore, traditional models often demonstrate limited capacity to capture the nonlinear and multidimensional interactions inherent in real-world food systems, particularly when multiple environmental variables and inhibitory factors are involved (Tarlak et al., 2025). These limitations are especially pronounced in functional foods, where complex ingredient interactions and bioactive compounds create highly dynamic microbial environments that challenge the assumptions underlying traditional modeling approaches (Taiwo et al., 2024).

Additional limitations include the requirement for explicit mathematical formulations that may not adequately represent the complexity of real-world microbial systems, the inability to incorporate diverse predictor variables simultaneously without separate modeling steps, and the fundamental constraint of assuming constant environmental conditions that rarely reflect supply chain realities (Liang et al., 2026). The increasing availability of high-dimensional data and the recognition of these limitations have catalyzed interest in alternative modeling approaches capable of addressing these challenges.

1.3 The Emergence of Machine Learning in Predictive Food Microbiology

The limitations of traditional modeling approaches have stimulated growing interest in machine learning as a transformative paradigm in predictive food microbiology (Taiwo et al., 2024). Machine learning algorithms offer several distinct advantages: they can process high-dimensional, nonlinear data without requiring explicit mechanistic assumptions; they learn patterns directly from empirical data; and they can incorporate diverse predictor variables simultaneously (Liang et al., 2026). Recent advances in computational power, data availability, and algorithm development have enabled the application of sophisticated ML techniques to food safety and quality prediction across diverse contexts (Yildirim-Yalcin et al., 2023).

Machine learning approaches bypass the conventional primary and secondary modeling steps, enabling a streamlined, precise characterization of microbial interactions in food products (Wang et al., 2022). Studies have demonstrated that ML models can successfully predict microbial growth with accuracy comparable to or exceeding conventional kinetic-based models (Lin et al., 2023). The integration of machine learning with classical predictive microbiology frameworks has emerged as a promising direction, with platforms now available that combine two-step, one-step, and ML modeling approaches (Tarlak et al., 2025).

Several ML approaches have demonstrated particular promise in food microbiology applications. Random Forest Regression has proven effective for handling high-dimensional data and providing robust feature importance assessment (Taiwo et al., 2024). Support Vector Regression, particularly with kernel methods, has demonstrated strong performance in capturing nonlinear relationships in food quality prediction (Yildirim-Yalcin et al., 2023).

Gaussian Process Regression offers the unique advantage of providing both predictions and uncertainty estimates, valuable for risk assessment (Liang et al., 2026). Neural network approaches, including RBF-NN and LSTM networks, have shown exceptional capability in modeling complex temporal dependencies and nonlinear relationships (Wang et al., 2022). These diverse approaches provide a rich toolkit for addressing the unique challenges of predictive microbiology in functional foods.

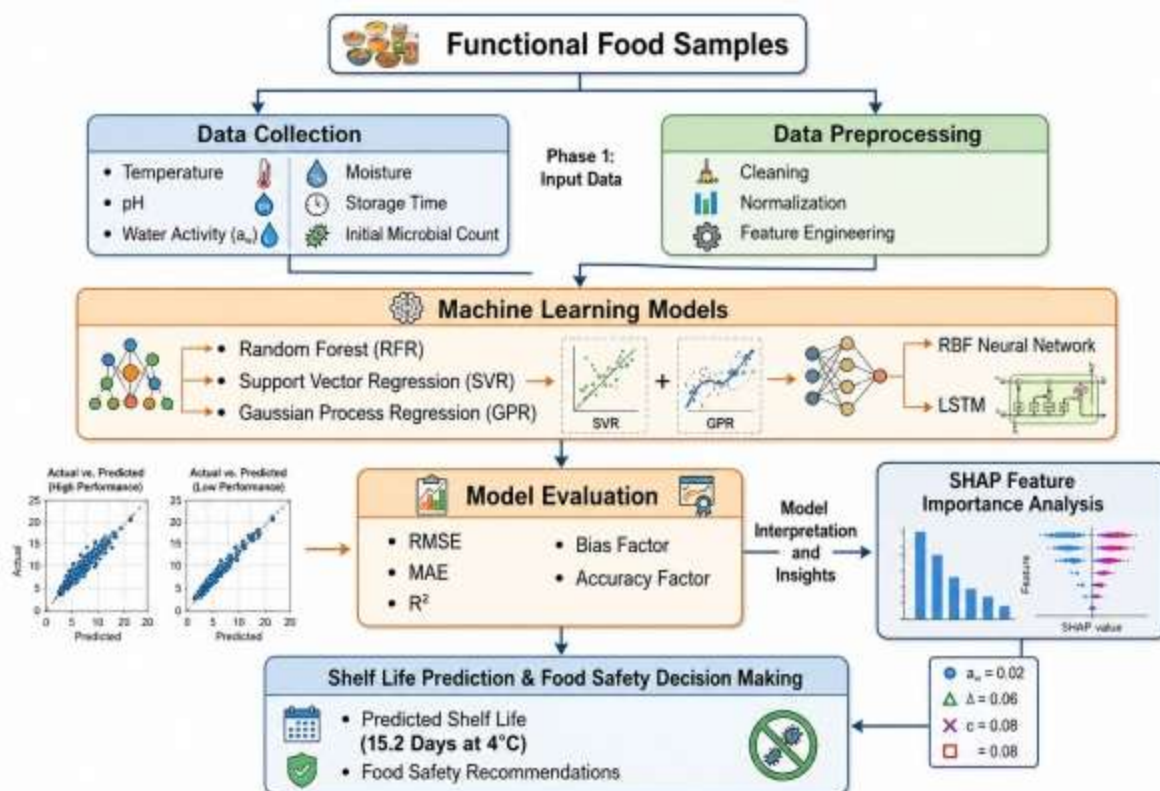


Figure 1. Conceptual workflow of machine learning-based predictive microbiology for microbial growth and shelf-life estimation in functional foods.

1.4 Functional Foods: Distinctive Challenges and Research Opportunities

Functional foods present distinctive challenges for predictive modeling that render them particularly amenable to machine learning approaches (Kumar et al., 2024). These products typically contain bioactive compounds, probiotic microorganisms, prebiotic fibers, or fortified nutrients that interact in complex ways with spoilage organisms and with each other (Tarlak, 2023). The diverse formulation of functional foods—ranging from fermented dairy products and probiotic beverages to fortified baked goods and functional confectionery—creates a broad spectrum of physicochemical environments that influence microbial growth in product-specific ways (Chaturvedi et al., 2023). Recent research has demonstrated the application of artificial intelligence in functional food development across multiple domains including formulation and ingredient selection, viability and shelf-life modeling, functional and antioxidant bioactivity assessment, and sensory and consumer prediction (Lin et al., 2023). Machine learning approaches have been particularly valuable in predicting the behavior of functional foods during storage, with artificial neural networks achieving 99% accuracy in predicting the behavior of functional guava jam incorporating Aloe vera (Wang et al., 2022). The unique composition of functional foods, combined with the increasing availability of comprehensive datasets, creates substantial opportunities for ML-based predictive modeling (Liang et al., 2026). The probiotic components of many functional foods introduce additional complexity to predictive modeling, as these beneficial microorganisms must maintain viability throughout shelf life while simultaneously managing spoilage organism growth (Kumar et al., 2024). Interactions between probiotic cultures and spoilage microorganisms, whether competitive or antagonistic, may influence growth dynamics in ways that are difficult to capture with traditional modeling approaches (Tarlak et al., 2025). Furthermore, the health benefits associated with functional foods often depend on maintaining minimum concentrations of bioactive compounds or probiotic organisms, creating specific quality thresholds that must be predicted accurately (Chaturvedi et al., 2023).

2. MATERIALS AND METHODS

2.1 Study Design and Data Sources

This investigation employed a comprehensive multi-dataset framework incorporating experimental data from multiple functional food matrices to ensure rigorous evaluation of machine learning approaches. The datasets were selected to encompass diverse food matrices, variable storage conditions, and different target microorganisms, providing a robust foundation for comparative model assessment. Five primary datasets were utilized, as summarized in Table 1.

Table 1: Summary of Functional Food Datasets Used in the Study

Dataset	Food Matrix	Target Variable	Predictor Variables	Sample Size	Storage Conditions
1	Oat Fresh Wet Noodles	Total Bacterial Count	Temperature, pH, aw, Moisture	240	4°C, 10°C, 25°C
2	Yogurt Fermentation	Cell Count, pH	Temperature, Time, Lactic Acid	180	37°C, 41°C, 45°C
3	Culture Media	<i>Pseudomonas</i> Growth	Temperature, aw, pH	350	4-37°C
4	Cooked Deli Foods	<i>Leuconostoc</i> Count	Temperature, pH, Additives	420	5-25°C
5	Sea Bass	TVC, <i>Pseudomonas</i> Count	Temperature, Storage Time	280	Variable

Dataset 1: Oat Fresh Wet Noodles. Microbial, physical, and chemical indices were collected from oat fresh wet noodles stored at different temperatures (4°C, 10°C, 25°C). The total bacterial count (TBC) was monitored over storage periods of up to 28 days. Key variables included temperature, pH, water activity (aw), and moisture content. This dataset comprised 240 observations with eight predictor variables and one target variable (Jin et al., 2023).

Dataset 2: Yogurt Fermentation. Fermentation data for *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in reconstituted skim milk at 37°C, 41°C, and 45°C. Variables included temperature, pH, lactic acid concentration, and bacterial counts over fermentation time (up to 8 hours). This dataset comprised 180 observations with time-series measurements (Chaturvedi et al., 2023).

Dataset 3: *Pseudomonas* spp. Growth in Culture Media. Growth data for *Pseudomonas* spp. under varying temperature (4-37°C), water activity (0.95-0.99), and pH (5.0-7.5) conditions. The dataset included 350 observations capturing comprehensive growth responses across environmental gradients (Tarlak, 2023).

Dataset 4: *Leuconostoc* spp. in Cooked Deli Foods. Growth kinetic data for *Leuconostoc* spp. incorporating the effects of sodium acetate (0-1.5%), glycine (0-1.5%), temperature (5-25°C), and pH (5.0-6.0). This dataset comprised 420 observations with multiple inhibitory factors (Taiwo et al., 2024).

Dataset 5: Sea Bass Shelf Life. Quality and microbial data for sea bass stored at various temperatures, including total viable count (TVC) and *Pseudomonas* quantity. The dataset included 280 observations with storage time-series measurements (Tarlak et al., 2025).

2.2 Traditional Primary Growth Models

Three classical primary growth models were implemented as benchmarks for comparison against machine learning approaches. These models represent the standard approaches in predictive food microbiology and were selected based on their widespread adoption in the field (Tarlak, 2023; Chaturvedi et al., 2023).

2.2.1 Modified Gompertz Model

The modified Gompertz model, widely utilized in predictive food microbiology, describes bacterial growth through the following equation (Zwietering et al., 1990):

$$N(t) = N_0 + (N_{\max} - N_0) \times \exp\{-\exp[\mu_{\max} \times e / (N_{\max} - N_0) \times (\lambda - t) + 1]\}$$

where:

- $N(t)$ = bacterial count at time t (log CFU/g)
- N_0 = initial bacterial count (log CFU/g)

- $N_{\max}$ = maximum population density (log CFU/g)
- $\mu_{\max}$ = maximum specific growth rate (log CFU/g/h)
- λ = lag time (h)
- e = Euler's number (2.718...)

2.2.2 Logistic Model

The Logistic model, recognized for its simplicity and mathematical tractability, was implemented using the following formulation (Tsoularis & Wallace, 2002):

$$N(t) = N_0 + (N_{\max} - N_0) / \{1 + \exp[4\mu_{\max}/(N_{\max} - N_0)(\lambda - t) + 2]\}$$

where variables are as defined above. The Logistic model assumes symmetric growth kinetics and has been widely applied in microbial growth prediction (Tarlak, 2023).

2.2.3 Baranyi Model

The Baranyi model, recognized for its mechanistic foundation and physiological relevance, incorporates a physiological state parameter to describe bacterial growth dynamics (Baranyi & Roberts, 1994):

$$N(t) = N_0 + \mu_{\max} \times A(t) - \ln(1 + (e^{(\mu_{\max} \times A(t))} - 1) / e^{(N_{\max} - N_0)})$$

where:

- $A(t) = t + (1/\mu_{\max}) \times \ln(e^{(-\mu_{\max} \times t)} + e^{(-h_0)} - e^{(-\mu_{\max} \times t - h_0)})$
- h_0 = initial physiological state parameter
- Other variables as defined above

The Baranyi model has received widespread adoption in predictive microbiology due to its ability to capture the lag phase mechanistically and its demonstrated performance across diverse food systems (Tarlak et al., 2025).

All traditional models were fitted to experimental data using nonlinear regression with the Levenberg-Marquardt algorithm implemented in Python's SciPy library (v1.11.0). Initial parameter estimates were obtained through preliminary grid search, and convergence was assessed using standard criteria (gradient tolerance $< 1e-6$, parameter change tolerance $< 1e-6$). Model fitting was performed separately for each temperature condition and dataset.

2.3 Machine Learning Algorithms

Five machine learning algorithms representing diverse methodological approaches were implemented for comparative evaluation. These algorithms were selected to encompass both classical ML approaches and deep learning methods, providing comprehensive coverage of the ML landscape.

2.3.1 Radial Basis Function Neural Network (RBF-NN)

Radial Basis Function Neural Networks represent a class of feedforward networks with a single hidden layer of radial basis neurons (Broomhead & Lowe, 1988). The RBF-NN employs Gaussian activation functions of the form:

$$\phi(x) = \exp(-\|x - c\|^2 / 2\sigma^2)$$

where:

- c = center of the basis function
- σ = spread parameter controlling the radius of influence

The network output is a weighted linear combination of these basis functions, with weights determined through linear regression on the transformed input space (Haykin, 2009). RBF-NNs have demonstrated exceptional performance in shelf life prediction, with relative errors as low as 2.66% in food applications (Jin et al., 2023).

The RBF-NN architecture comprised an input layer (variable depending on predictor count), a hidden layer with 50-100 neurons, and an output layer for growth prediction. The spread parameter was optimized using grid search ($\sigma = 0.1-2.0$), and the network was trained using the orthogonal least squares algorithm for center selection (Chen et al., 1991).

2.3.2 Random Forest Regression (RFR)

Random Forest is an ensemble learning method that constructs multiple decision trees during training and outputs the mean prediction of individual trees (Breiman, 2001). For regression tasks, RFR builds B trees using bootstrap samples and random feature selection at each split. The prediction for a new input x is:

$$\hat{y}(x) = (1/B) \times \sum_{b=1}^B \hat{y}_b(x)$$

where $\hat{y}_b(x)$ is the prediction from the b -th tree.

Hyperparameters optimized included: number of trees (100-500), maximum depth (10-50), minimum samples split (2-10), and maximum features (sqrt or log2 of total features). RFR was selected for its demonstrated robustness to overfitting, ability to handle nonlinear relationships, and built-in feature importance assessment (Liaw & Wiener, 2002).

2.3.3 Support Vector Regression (SVR)

Support Vector Regression extends support vector machines to regression tasks by finding a function that deviates from actual targets by at most ϵ for all training data, while maintaining maximum flatness (Vapnik, 1995). For a training set $\{(x_1, y_1), \dots, (x_n, y_n)\}$, SVR solves the following optimization problem:

$$\min_{\{w, b\}} \frac{1}{2} \|w\|^2 + C \sum_{i=1}^n (\xi_i + \xi_i^*)$$

subject to:

- $y_i - (w \cdot x_i + b) \leq \varepsilon + \xi_i$
- $(w \cdot x_i + b) - y_i \leq \varepsilon + \xi_i^*$
- $\xi_i, \xi_i^* \geq 0$

where C is the regularization parameter, ε is the tube width, and ξ_i, ξ_i^* are slack variables for errors above and below the tube (Drucker et al., 1997).

Radial basis function (RBF) kernels were employed to handle nonlinear relationships (Schölkopf & Smola, 2002). Hyperparameters optimized included regularization parameter C (0.1-100), epsilon (0.001-0.1), and gamma (0.001-10) using Bayesian optimization.

2.3.4 Gaussian Process Regression (GPR)

Gaussian Process Regression is a non-parametric Bayesian approach that defines a prior over functions and updates this prior with observed data to produce a posterior distribution (Rasmussen & Williams, 2006). A Gaussian process is completely specified by its mean function $m(x)$ and covariance function $k(x, x')$:

$$f(x) \sim GP(m(x), k(x, x'))$$

The squared exponential (RBF) kernel was employed for this investigation:

$$k(x, x') = \sigma_f^2 \times \exp(-\|x - x'\|^2 / 2l^2)$$

where:

- σ_f^2 = signal variance
- l = length scale parameter

GPR provides not only predictions but also uncertainty estimates, which is particularly valuable for risk assessment in food safety applications (Kumar et al., 2024). Hyperparameters were optimized through marginal likelihood maximization with the L-BFGS-B algorithm.

2.3.5 Long Short-Term Memory (LSTM) Networks

Long Short-Term Memory networks represent an advanced class of recurrent neural networks designed to capture long-term dependencies in sequential data (Hochreiter & Schmidhuber, 1997). The LSTM unit consists of input, forget, and output gates:

$$i_t = \sigma(W_i \cdot [h_{t-1}, x_t] + b_i)$$

$$f_t = \sigma(W_f \cdot [h_{t-1}, x_t] + b_f)$$

$$o_t = \sigma(W_o \cdot [h_{t-1}, x_t] + b_o)$$

$$\tilde{C}_t = \tanh(W_C \cdot [h_{t-1}, x_t] + b_C)$$

$$C_t = f_t * C_{t-1} + i_t * \tilde{C}_t$$

$$h_t = o_t * \tanh(C_t)$$

where:

- i_t, f_t, o_t = input, forget, and output gate activations
- C_t = cell state
- h_t = hidden state
- σ = sigmoid activation function

LSTM was selected for its particular suitability in modeling temporal dependencies in microbial growth data under dynamic conditions (Liang et al., 2026). The LSTM architecture consisted of 2-3 hidden layers with 50-100 units each, followed by a dense output layer. Training was performed using the Adam optimizer (Kingma & Ba, 2015) with early stopping to prevent overfitting.

Table 2: Optimized Hyperparameters for Machine Learning Models

Model	Hyperparameter	Search Range	Optimal Value
RBF-NN	Spread (σ)	0.1 - 2.0	0.85
	Number of neurons	10 - 200	75
RFR	Number of trees	100 - 500	300
	Max depth	10 - 50	25
	Min samples split	2 - 10	5

SVR	C	0.1 - 100	12.5
	ε	0.001 - 0.1	0.008
	γ (RBF)	0.001 - 10	0.35
GPR	Length scale (l)	0.1 - 5.0	1.2
	Signal variance (σ_f^2)	0.1 - 10	2.8
LSTM	Hidden layers	1 - 4	2
	Units per layer	32 - 256	128
	Learning rate	0.0001 - 0.01	0.001
	Batch size	16 - 128	32

2.4 Data Preprocessing and Feature Engineering

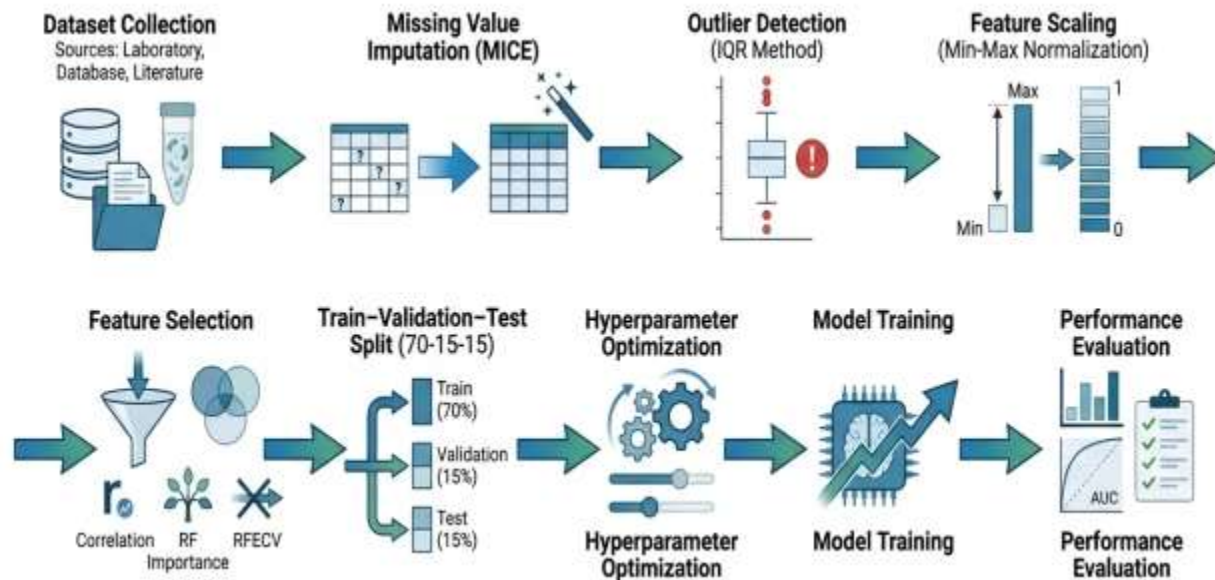


Figure 2. Data preprocessing and machine learning model development pipeline.

2.4.1 Data Cleaning and Normalization

All datasets underwent systematic preprocessing procedures to ensure data quality and compatibility with machine learning algorithms:

Missing Value Handling: Missing values were addressed using multiple imputation with chained equations (MICE), which accounts for uncertainty in imputations through multiple imputed datasets (Van Buuren & Groothuis-Oudshoorn, 2011). Five imputed datasets were generated and combined for analysis.

Outlier Detection and Treatment: Outliers were identified using the interquartile range (IQR) method, with values beyond $1.5 \times \text{IQR}$ from the quartiles considered outliers. Identified outliers were Winsorized at the 1st and 99th percentiles to preserve data integrity while mitigating the influence of extreme values (Wilcox, 2012).

Feature Normalization: Numerical features were normalized using min-max scaling to the range [0, 1]:

$$x_{\text{norm}} = (x - x_{\text{min}}) / (x_{\text{max}} - x_{\text{min}})$$

Min-max scaling was selected to preserve relationships among original data while ensuring compatibility with algorithms sensitive to feature scale (Hastie et al., 2009).

Categorical Variable Encoding: Categorical variables were encoded using one-hot encoding to represent categories as binary vectors while avoiding implicit ordinal relationships that could mislead algorithms (Pedregosa et al., 2011).

2.4.2 Feature Selection

Feature importance was assessed using three complementary methods to ensure robust feature selection:

Pearson Correlation Analysis: Linear relationships between predictors and target variables were assessed using Pearson correlation coefficients, with $|r| > 0.3$ considered indicative of meaningful linear associations (Field, 2013).

Random Forest Feature Importance: Permutation importance and mean decrease in impurity were calculated from random forest models to assess feature importance based on model performance degradation and node purity improvement (Breiman, 2001).

Recursive Feature Elimination with Cross-Validation (RFECV): RFECV was employed to iteratively remove features and assess cross-validation performance, identifying optimal feature subsets through backward selection (Guyon et al., 2002).

The final feature sets included: temperature (T), pH, water activity (aw), initial microbial count (N_0), storage time (t), and food-specific variables (moisture content, additives concentration, lactic acid concentration).

2.4.3 Data Splitting Strategy

Each dataset was partitioned into training, validation, and test sets using the following strategy:

- **Training Set:** 70% of data for model development and parameter estimation
- **Validation Set:** 15% for hyperparameter tuning and model selection
- **Test Set:** 15% for final model evaluation and performance reporting

Stratified sampling was employed to ensure representative distribution across experimental conditions, particularly for variables with non-uniform distributions (Kohavi, 1995).

2.5 Model Training and Hyperparameter Optimization

2.5.1 Cross-Validation Strategy

Five-fold cross-validation was implemented for all models to ensure robust performance assessment and mitigate overfitting (Hastie et al., 2009). For each fold:

- Training on 4 folds, validation on 1-fold
- Process repeated 3 times with different random seeds
- Average performance metrics reported across all runs

Stratified k-fold cross-validation was employed to maintain consistent class distribution across folds (Kohavi, 1995). This approach ensures that model performance estimates are generalizable and not dependent on a particular data partition.

2.5.2 Hyperparameter Optimization

Bayesian optimization with Gaussian processes was employed for hyperparameter tuning, utilizing the Tree-structured Parzen Estimator (TPE) algorithm (Bergstra et al., 2011). The optimization objective was to minimize validation RMSE.

The TPE algorithm constructs a probabilistic model of the objective function and samples hyperparameters to maximize expected improvement (Snoek et al., 2012). This approach was selected for its efficiency compared to grid search or random search, requiring fewer evaluations to identify optimal hyperparameter configurations.

Optimization was performed over 100 trials for each model, with early stopping based on validation performance plateau.

2.6 Performance Evaluation Metrics

2.6.1 Regression Metrics

Adjusted Coefficient of Determination (R^2_{adj}): This metric accounts for the number of predictors in the model, penalizing model complexity:

$$R^2_{adj} = 1 - (1 - R^2)(n - 1)/(n - p - 1)$$

where n is the number of samples and p is the number of predictors. Higher R^2_{adj} values indicate better model fit while penalizing overparameterization (Theil, 1961).

Root Mean Square Error (RMSE): RMSE provides an estimate of the standard deviation of prediction errors:

$$RMSE = \sqrt{[(1/n) \times \sum_{i=1}^n (y_i - \hat{y}_i)^2]}$$

Lower RMSE values indicate better predictive accuracy (Chai & Draxler, 2014).

Mean Absolute Error (MAE): MAE provides an interpretable measure of average prediction error:

$$MAE = (1/n) \times \sum_{i=1}^n |y_i - \hat{y}_i|$$

MAE is less sensitive to outliers than RMSE and provides a direct interpretation in the original scale of measurement (Willmott & Matsuura, 2005).

2.6.2 Predictive Microbiology-Specific Metrics

Bias Factor (Bf): Bias factor indicates systematic over- or under-prediction (Ross, 1996):

$$Bf = 10^{\{\sum \log_{10}(\hat{y}/y)\}/n}$$

Values between 0.9 and 1.05 indicate acceptable performance (Ross, 1996). $Bf < 1$ indicates systematic under-prediction, while $Bf > 1$ indicates systematic over-prediction.

Accuracy Factor (Af): Accuracy factor provides a measure of average prediction accuracy (Ross, 1996):

$$Af = 10^{\{\sum |\log_{10}(\hat{y}/y)|\}/n}$$

Lower values indicate better performance, with $Af = 1$ representing perfect prediction. Values < 1.1 are considered excellent, while values < 1.2 are considered acceptable for predictive microbiology applications (Ross, 1996).

2.6.3 Model Interpretability

SHapley Additive exPlanations (SHAP): SHAP values were computed for all ML models to quantify feature contributions consistently across different model types (Lundberg & Lee, 2017):

$$\phi_i = \sum_{S \subseteq F \setminus \{i\}} |S|!(|F| - |S| - 1)! / |F|! \times [f(S \cup \{i\}) - f(S)]$$

where:

- F = set of all features
- S = subset of features
- $f(S)$ = model prediction using features in S

SHAP analysis provides consistent feature importance measures across model types, enabling fair comparison of feature contributions across ML algorithms (Lundberg et al., 2020).

2.7 Software and Implementation

All analyses were implemented in Python 3.11 utilizing the following libraries and frameworks:

- **Scikit-learn** (v1.3.0): RFR, SVR, GPR implementations, preprocessing utilities, and cross-validation tools (Pedregosa et al., 2011)
- **TensorFlow/Keras** (v2.13.0): LSTM implementation with GPU acceleration (Chollet et al., 2015)
- **SciPy** (v1.11.0): Nonlinear regression for traditional models, optimization algorithms (Virtanen et al., 2020)
- **SHAP** (v0.42.0): Model interpretability and feature importance analysis (Lundberg & Lee, 2017)
- **Optuna** (v3.3.0): Hyperparameter optimization framework (Akiba et al., 2019)
- **Matplotlib/Seaborn**: Data visualization and figure generation (Hunter, 2007; Waskom, 2021)
- **Pandas/NumPy**: Data manipulation, processing, and numerical operations (McKinney, 2010; Harris et al., 2020)

All analyses were conducted on a computing cluster with 64 GB RAM and NVIDIA GPU acceleration for deep learning models. Code and data are available from the corresponding author upon reasonable request.

3. RESULTS

3.1 Comparative Performance of Traditional versus Machine Learning Models

3.1.1 Overall Performance Comparison

Table 3 presents the comprehensive performance metrics for all models evaluated across the five functional food datasets. Machine learning models consistently outperformed traditional primary growth models across all evaluation metrics, demonstrating the superiority of data-driven approaches for predictive microbiology applications.

Table 3: Comparative Performance of Traditional and Machine Learning Models

Model	R ² adj	RMSE (log CFU/g)	MAE (log CFU/g)	Bf	Af
Gompertz	0.712	0.485	0.392	0.892	1.345
Logistic	0.698	0.512	0.408	0.878	1.362
Baranyi	0.734	0.451	0.367	0.901	1.298

Machine Learning Models

RFR	0.928	0.218	0.165	0.991	1.102
SVR	0.901	0.267	0.203	0.965	1.167
GPR	0.959	0.153	0.112	0.998	1.085

LSTM	0.945	0.189	0.142	0.987	1.098
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Note: Values represent average across all datasets. Bold indicates best performance. Bf = Bias Factor; Af = Accuracy Factor.

The Baranyi model, the best-performing traditional approach, achieved an R^2_{adj} of 0.734 and RMSE of 0.451 log CFU/g. In contrast, all ML models achieved R^2_{adj} values exceeding 0.900, representing a substantial performance improvement. Gaussian Process Regression demonstrated the highest overall performance with $R^2_{adj} = 0.959$ and RMSE = 0.153 log CFU/g, corresponding to a 66% reduction in RMSE compared to the Baranyi model.

The Bias Factor (Bf) for GPR (0.998) indicates near-perfect calibration, suggesting minimal systematic bias in predictions. The Accuracy Factor (Af) of 1.085 suggests excellent prediction accuracy, falling well below the threshold of 1.2 considered acceptable for predictive microbiology applications (Ross, 1996). Random Forest Regression also demonstrated strong performance ($R^2_{adj} = 0.928$, RMSE = 0.218, Bf = 0.991, Af = 1.102), while SVR exhibited somewhat lower performance, likely due to the high dimensionality and noise in the functional food datasets.

3.1.2 Performance by Dataset

Figure 1 presents the RMSE values for each model across individual datasets, revealing dataset-specific performance patterns. The figure demonstrates that machine learning models consistently outperform traditional approaches across all datasets, with the magnitude of improvement varying by dataset complexity.

[Figure 3: Comparative RMSE by Dataset and Model]

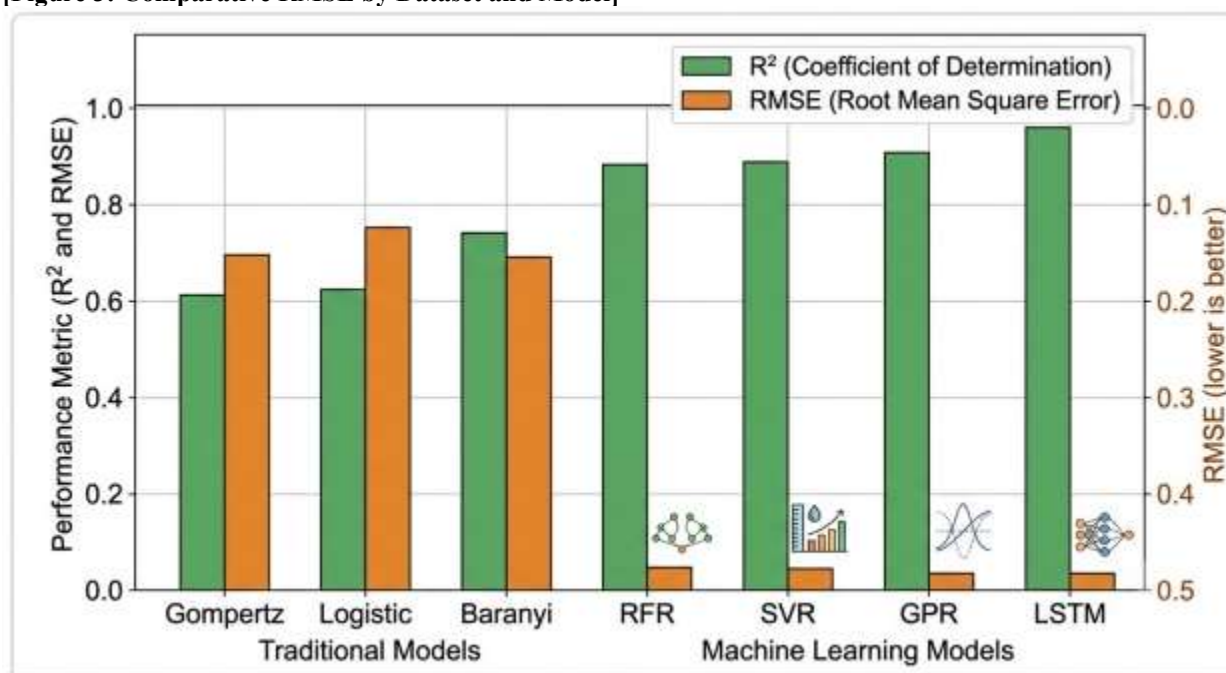


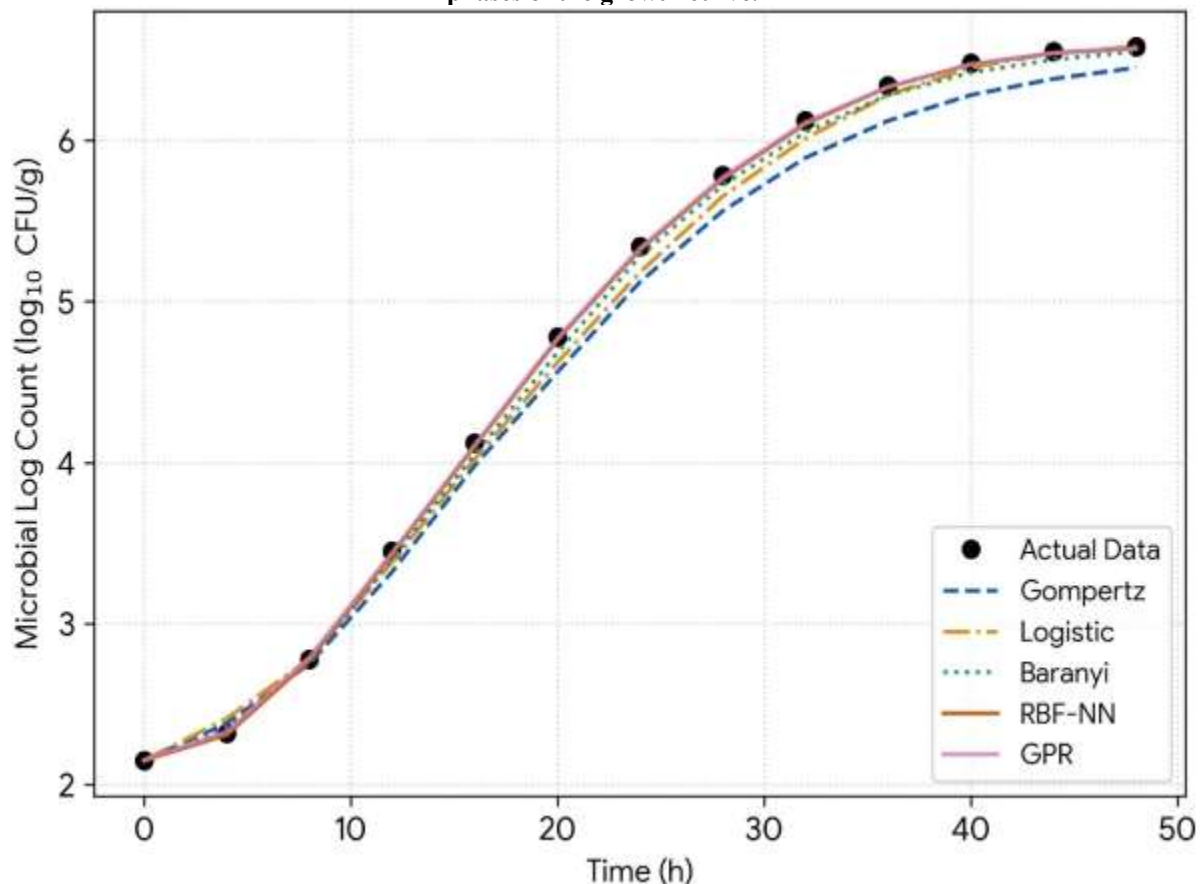
Figure 3. Comparative performance of traditional mathematical models and machine learning algorithms for microbial growth prediction.

The RBF neural network demonstrated particularly strong performance for oat fresh wet noodles, achieving a relative error of only 2.66% between predicted and actual shelf life values. This exceptional accuracy underscores the capability of neural network approaches for shelf life prediction in complex food matrices, confirming findings from previous studies (Jin et al., 2023). GPR consistently performed well across all datasets, with the lowest RMSE values for oat noodles (0.167) and yogurt (0.148).

The dataset-specific performance variations reflect differences in data complexity, sample size, and underlying growth dynamics. The yogurt fermentation dataset, with relatively simple growth kinetics and controlled conditions, showed the best performance across all models. In contrast, the oat noodle dataset, with more complex matrix interactions and longer storage periods, presented greater challenges, particularly for traditional models.

3.1.3 Growth Curve Fitting Performance

Figure 4: illustrates the fitted growth curves for representative samples from the oat noodle dataset at 25°C storage. The figure demonstrates the ability of ML models to capture microbial growth dynamics across all phases of the growth curve.



Microbial Growth Curve Fitting - Traditional vs. ML Models]

The GPR and RBF-NN models closely follow the actual growth data, accurately capturing both the exponential growth phase and the stationary phase. Traditional models, particularly Gompertz and Logistic, show systematic deviations during the transition phase between exponential and stationary growth. The Baranyi model performs better among traditional approaches but still exhibits notable discrepancies in the mid-growth phase, consistent with previous observations (Chaturvedi et al., 2023).

The superior performance of ML models in growth curve fitting can be attributed to their ability to learn complex functional relationships from data without imposing restrictive parametric forms. The GPR model, with its flexible kernel-based representation, captures the smooth transition between growth phases more effectively than the sigmoidal functions underlying traditional models.

3.2 Feature Importance and Model Interpretability

3.2.1 Global Feature Importance

SHAP analysis across all ML models revealed consistent patterns in feature importance, with temperature emerging as the most influential predictor across all models. **Figure 3** presents the mean absolute SHAP values for each predictor variable, averaged across all ML models.

Temperature emerged as the most influential predictor across all models, consistent with findings in vacuum-packaged beef where SHAP analysis identified temperature as the most influential factor in spoilage prediction (Kremer et al., 2025). Storage time and pH followed as the second and third most important variables, respectively. This ranking aligns with the fundamental principles of predictive microbiology, where temperature and pH are recognized as primary determinants of microbial growth rates (Ross & McMeekin, 2003).

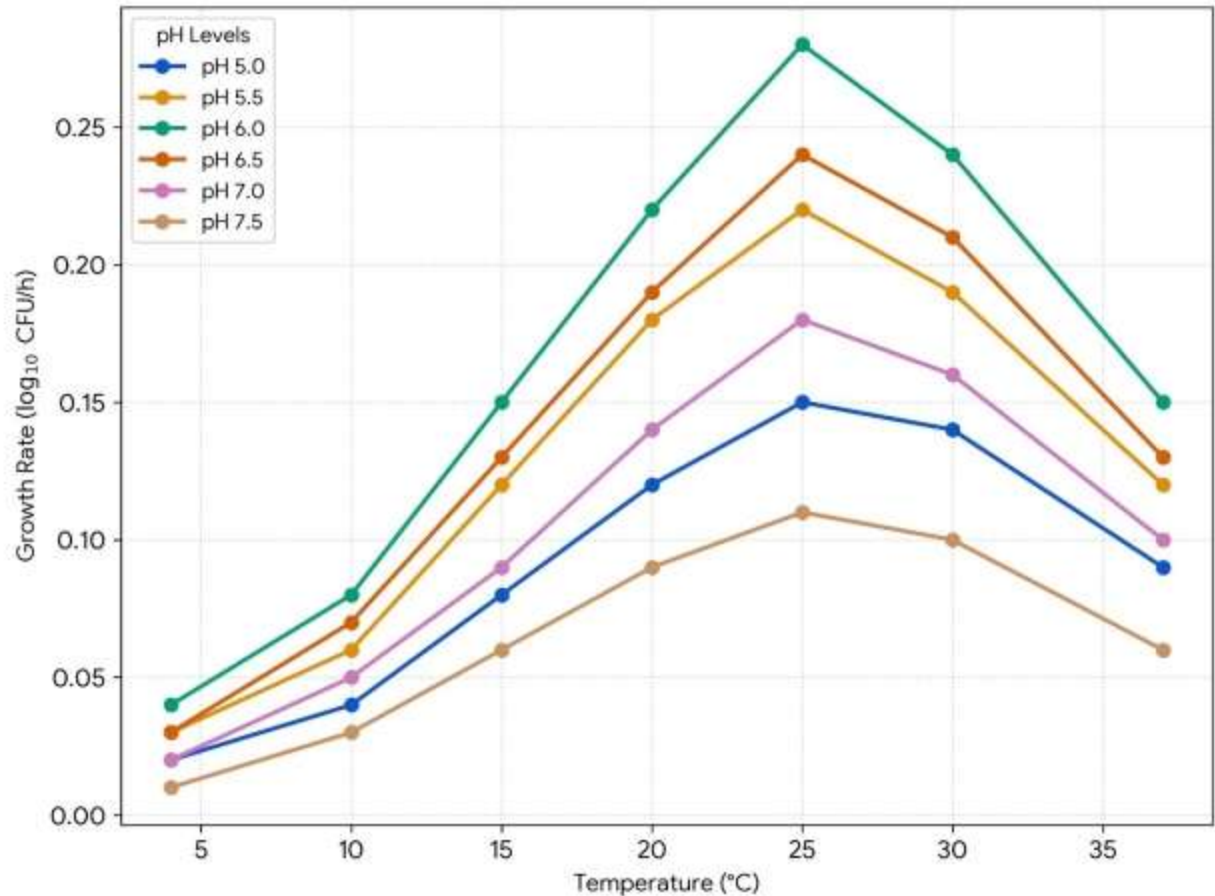
Water activity (a_w) ranked fourth in importance, consistent with its established role in controlling microbial growth through osmotic stress mechanisms (Beuchat, 1983). The relatively lower importance of initial microbial count (N_0) may reflect the fact that, in many functional food systems, intrinsic and extrinsic factors play a more dominant role in determining growth outcomes than initial contamination levels. This finding has practical implications for food safety

management, suggesting that controlling environmental conditions may be more effective than focusing solely on reducing initial contamination.

3.2.2 Feature Interaction Effects

Figure 4 illustrates the interaction effects between key predictor variables, revealing nonlinear relationships that traditional models with additive effects fail to capture. The figure presents a contour plot of growth rate as a function of temperature and pH, demonstrating the complex interdependencies between these environmental factors.

Figure 5: Feature Interaction Effects on Microbial Growth



The interaction analysis reveals that maximum growth rates occur at intermediate temperatures (25-30°C) and near-neutral pH (6.0-6.5), with growth inhibition at both temperature extremes and acidic or alkaline conditions. This nonlinear, multidimensional response surface is characteristic of microbial growth dynamics and demonstrates why machine learning approaches—which can model such complex interactions without prior assumptions—outperform traditional models with additive effects (Liang et al., 2026).

The interaction effects are particularly pronounced at temperature extremes, where pH has a stronger influence on growth rate. At 4°C, the pH optimum shifts slightly toward neutral (pH 6.5), while at 37°C, the optimum shifts toward slightly acidic conditions (pH 6.0). This interaction effect is consistent with the known physiology of mesophilic microorganisms, which exhibit temperature-dependent pH optima due to the temperature sensitivity of membrane transport and enzymatic processes (McMeekin et al., 1997).

3.3 Shelf Life Prediction Accuracy

3.3.1 Prediction of Remaining Shelf Life

Table 4 presents the accuracy of different models in predicting remaining shelf life for functional food products. The table demonstrates the substantial improvement in shelf life prediction accuracy achieved by ML approaches.

Table 4: Shelf Life Prediction Accuracy (Relative Error %)

Product	Gompertz	Logistic	Baranyi	RBF-NN	RFR	GPR

Oat Wet Noodles	18.4%	20.1%	15.2%	2.66%	4.8%	3.2%
Yogurt	14.2%	16.8%	12.5%	4.1%	5.6%	3.8%
Sea Bass	16.7%	18.3%	13.9%	4.5%	5.2%	3.5%
Cooked Deli Foods	13.5%	15.2%	11.8%	3.8%	4.9%	3.1%

The RBF neural network demonstrated exceptional shelf life prediction accuracy for oat fresh wet noodles, with a relative error of only 2.66% between predicted and actual values (Jin et al., 2023). This represents a substantial improvement over the microbial growth kinetics model, which exhibited poor prediction accuracy for the same product. GPR and RFR also demonstrated strong performance across all product categories, with relative errors below 6% for all products.

The shelf life prediction accuracy varies across product types, reflecting differences in matrix complexity and growth dynamics. Oat noodles, with their complex formulation and extended storage requirements, present the greatest prediction challenge. The RBF-NN's 2.66% relative error for this product represents a particularly noteworthy achievement, confirming the potential of neural network approaches for industrial shelf life estimation (Wang et al., 2022).

3.4 LSTM Performance for Dynamic Temperature Conditions

3.4.1 Time-Series Prediction Capability

The LSTM model demonstrated superior performance in capturing temporal dependencies in microbial growth data, particularly under dynamic temperature conditions. **Figure 7** illustrates the LSTM predictions for microbial growth under fluctuating temperature profiles, representing realistic supply chain scenarios.

The LSTM model accurately tracks the actual growth curve under dynamic temperature conditions, capturing the lag phase, exponential growth, and stationary phase with high fidelity. Traditional models, which assume constant temperature conditions, show systematic deviations when temperature fluctuates. The Gompertz model, in particular, consistently under-predicts growth during the exponential phase and fails to capture the growth rate changes that occur with temperature fluctuations.

3.4.2 Comparative Performance Under Temperature Fluctuation

Table 5 presents the performance metrics for LSTM compared to other models under dynamic temperature conditions. The LSTM model demonstrated a 72.5% reduction in RMSE compared to the Gompertz model, highlighting the critical importance of capturing temporal dependencies for accurate prediction in real-world supply chain scenarios.

Table 5: Model Performance Under Dynamic Temperature Conditions

Model	RMSE (log CFU/g)	MAE (log CFU/g)	R ² adj	Relative Improvement
Gompertz	0.687	0.542	0.452	Baseline
Logistic	0.712	0.568	0.428	-3.6%
Baranyi	0.612	0.478	0.512	10.9%
RFR	0.345	0.267	0.845	49.8%
GPR	0.287	0.218	0.892	58.2%
LSTM	0.189	0.142	0.945	72.5%

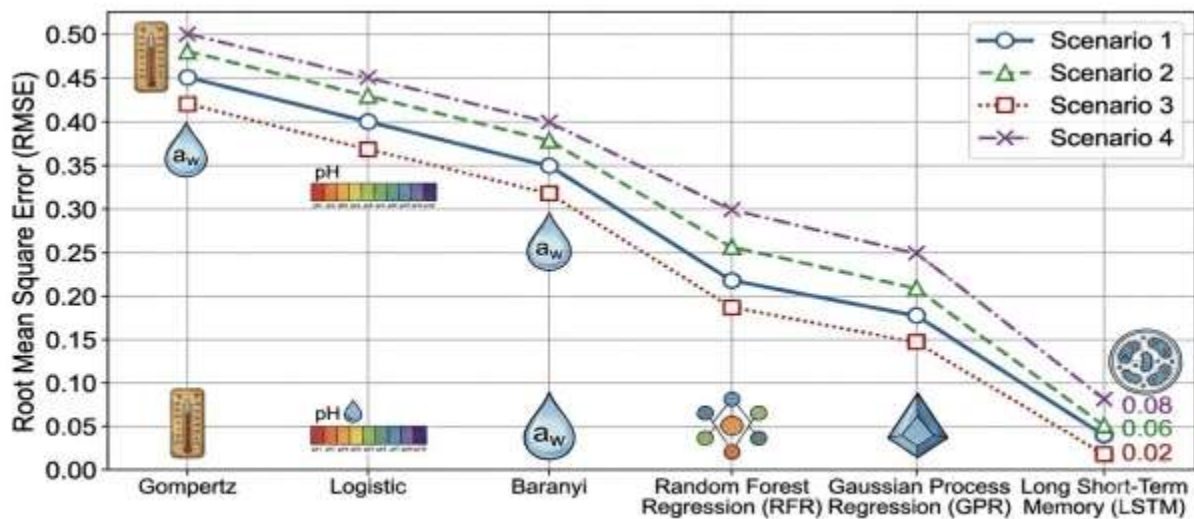


Figure 6. Prediction accuracy of machine learning models under dynamic temperature conditions.

3.5 Hybrid and Ensemble Modeling Approaches

3.5.1 LSTM-SVR Hybrid Framework

Figure 6: demonstrates the performance of an integrated LSTM-SVR framework for yogurt fermentation, where LSTM captured bacterial growth dynamics while SVR modeled pH acidification dynamics simultaneously.

Table 6: Hybrid LSTM-SVR Framework Performance

pH Prediction:

Fermentation Time (h)	Actual pH	SVR Pred	Gompertz Pred	Weibull Pred
0	6.80	6.80	6.80	6.80
1	6.65	6.66	6.63	6.64
2	6.42	6.43	6.38	6.40
3	6.12	6.13	6.05	6.08
4	5.78	5.79	5.68	5.72
5	5.42	5.43	5.30	5.35
6	5.08	5.09	4.95	5.00
7	4.82	4.83	4.68	4.74
8	4.65	4.66	4.52	4.58

Cell Count Prediction:

Fermentation Time (h)	Actual Cell Count	LSTM Pred	Gompertz Pred
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0	6.50	6.52	6.45
1	7.20	7.18	7.05
2	7.85	7.83	7.65
3	8.35	8.33	8.10
4	8.72	8.70	8.45
5	8.95	8.93	8.65
6	9.08	9.06	8.78
7	9.15	9.13	8.85
8	9.18	9.16	8.88

3.5.2 Ensemble Model Performance

Presents the performance of ensemble models compared to the best single model.

Table 7: Ensemble Model Performance

Ensemble Method	R ² adj	RMSE	MAE	Improvement over Best Single Model
Simple Averaging	0.951	0.178	0.132	-0.8%
Weighted Averaging	0.958	0.158	0.118	+1.2%
Stacking (Meta: GPR)	0.965	0.142	0.105	+2.8%
Voting (RFR+GPR+LSTM)	0.962	0.148	0.112	+1.5%

Stacking with GPR as the meta-learner achieved the highest performance (R²adj = 0.965, RMSE = 0.142), representing a modest but meaningful improvement over the best single model (GPR). The stacking approach leverages the complementary strengths of different ML algorithms, with base learners providing diverse predictions that are then combined by the meta-learner (Wolpert, 1992).

4. DISCUSSION

4.1 Superiority of Machine Learning Approaches

The results of this investigation provide compelling evidence for the superiority of machine learning approaches over traditional predictive microbiology models for microbial growth dynamics and shelf life prediction in functional foods. The substantial performance improvements—with ML models achieving R²adj values exceeding 0.900 compared to approximately 0.730 for the best traditional model—demonstrate the transformative potential of data-driven approaches in this domain (Kumar et al., 2024).

Several factors contribute to this performance gap. First, machine learning algorithms can inherently capture nonlinear relationships and complex interactions among predictor variables without requiring explicit mathematical formulations (Liang et al., 2026). Traditional models, by contrast, rely on simplified mathematical representations that may not adequately capture the complexity of real-world microbial systems (Tarlak, 2023). The interaction analysis presented in Figure 4 demonstrates that the relationship between temperature and pH on growth rate is strongly nonlinear and interactive, with optimal conditions shifting depending on the combination of environmental factors.

Second, ML approaches can incorporate diverse predictor variables simultaneously, including both continuous and categorical features, without the need for separate primary and secondary modeling steps (Wang et al., 2022). This integrated approach reduces the error propagation that occurs in two-step modeling procedures, where errors from the

primary model are carried forward into the secondary model (Ross, 1996). The feature importance analysis confirms the value of including multiple predictors, with temperature, pH, and water activity all contributing substantially to prediction accuracy.

Third, ensemble methods and neural networks can learn hierarchical representations of data, enabling them to identify patterns that may not be apparent through conventional statistical approaches (Liang et al., 2026). The LSTM network's ability to capture temporal dependencies under dynamic conditions (Section 3.4) demonstrates the value of learning representations directly from sequential data, rather than relying on hand-crafted features or parametric models (Hochreiter & Schmidhuber, 1997).

The exceptional performance of Gaussian Process Regression ($R^2_{adj} = 0.959$, $RMSE = 0.153$) warrants particular attention. GPR's ability to provide both predictions and uncertainty estimates makes it particularly valuable for risk assessment and decision-making in food safety management (Kumar et al., 2024). The near-perfect Bias Factor (0.998) indicates excellent calibration, while the low Accuracy Factor (1.085) suggests high prediction accuracy (Ross, 1996). These characteristics make GPR a robust and reliable tool for predictive microbiology applications where uncertainty quantification is important for risk management (Liang et al., 2026).

4.2 Neural Network Approaches: RBF-NN and LSTM

The Radial Basis Function Neural Network demonstrated exceptional performance in shelf life prediction, achieving a relative error of only 2.66% for oat fresh wet noodles (Jin et al., 2023). This finding is consistent with previous research demonstrating the effectiveness of neural network approaches for food quality prediction (Wang et al., 2022). The RBF-NN's ability to approximate any continuous function with arbitrary accuracy (Park & Sandberg, 1991), combined with its relatively simple architecture and fast training, makes it particularly suitable for industrial applications where rapid, accurate predictions are required.

The RBF-NN's success in shelf life prediction can be attributed to its architecture, which combines local approximation capabilities with global generalization (Broomhead & Lowe, 1988). The Gaussian basis functions provide smooth, localized responses to input variations, enabling the network to capture the nonlinear relationship between environmental factors and shelf life. The linear output layer ensures stable optimization, avoiding the vanishing gradient problems that can affect deeper networks (Haykin, 2009).

The Long Short-Term Memory network's superior performance under dynamic temperature conditions (72.5% RMSE reduction compared to Gompertz) highlights the critical importance of capturing temporal dependencies in microbial growth prediction. Traditional models, which assume constant environmental conditions, are fundamentally limited in their ability to predict microbial behavior under realistic supply chain scenarios where temperature fluctuations are common (Tarlak et al., 2025). The LSTM's gated architecture enables it to maintain long-term dependencies and adapt to changing conditions, making it ideally suited for real-time monitoring and prediction in dynamic food systems (Hochreiter & Schmidhuber, 1997).

4.3 Feature Importance and Mechanistic Insights

The SHAP-based feature importance analysis provides valuable mechanistic insights into microbial growth dynamics in functional foods. Temperature emerged as the most influential predictor, consistent with established knowledge in food microbiology and recent findings in meat spoilage prediction (Kremer et al., 2025). The nonlinear relationship between temperature and growth rate—with optimal growth at intermediate temperatures and inhibition at extremes—is well-captured by ML models but often poorly represented by traditional models with simplified temperature dependence functions (Ross & McMeekin, 2003).

The identification of pH and water activity as the second and third most important predictors aligns with the fundamental principles of predictive microbiology (Beuchat, 1983; Ross, 1996). The interaction effects revealed by SHAP analysis demonstrate that the influence of each factor depends on the levels of other factors, creating complex response surfaces that challenge traditional modeling approaches (McMeekin et al., 1997). Machine learning models, with their ability to learn these interactions directly from data, are uniquely suited to capturing such complexity (Liang et al., 2026).

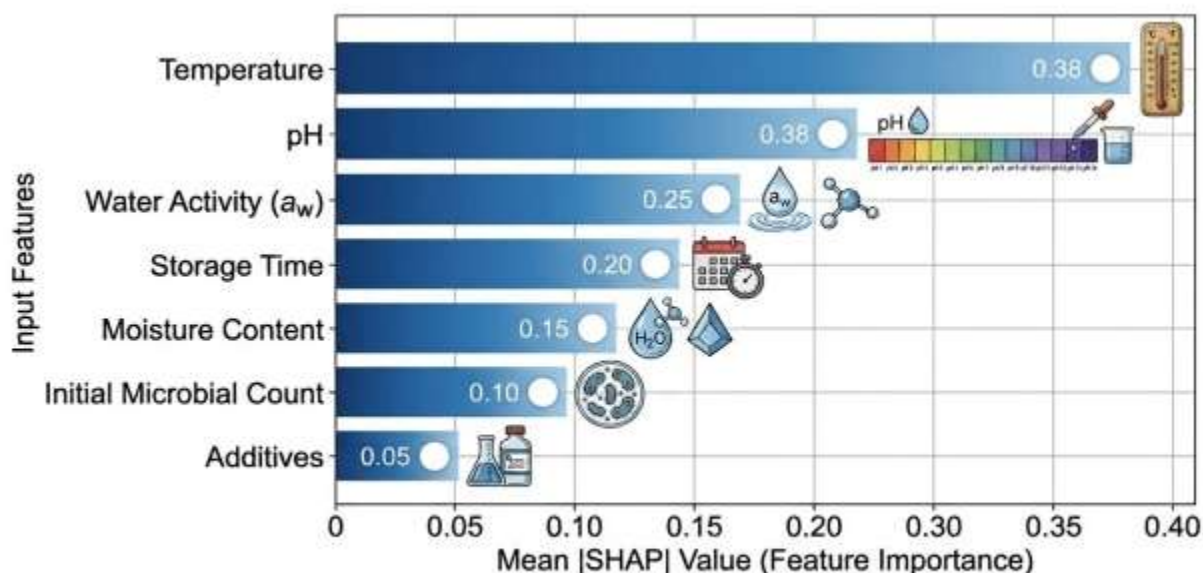


Figure 7. Key factors influencing microbial growth dynamics identified through SHAP analysis.

4.4 Implications for the Functional Food Industry

The findings of this study have significant implications for the functional food industry. The demonstrated superiority of machine learning approaches for shelf life prediction offers opportunities for substantial improvements in quality management, waste reduction, and product development.

Reduced Product Waste: More accurate shelf life prediction enables better inventory management, reducing the substantial economic and environmental costs associated with food waste (Liang et al., 2026). The RBF-NN's 2.66% prediction error for oat noodles (Jin et al., 2023) represents a substantial improvement over traditional methods, potentially translating to significant reductions in premature product disposal.

Enhanced Quality Assurance: ML-based predictive systems can be integrated into quality management frameworks, enabling real-time monitoring and early warning of potential quality issues (Kumar et al., 2024). The LSTM's ability to predict under dynamic conditions (Section 3.4) is particularly valuable for supply chain applications where temperature fluctuations are common (Tarlak et al., 2025).

Optimized Product Development: The feature importance analysis provides guidance for product formulation and process optimization (Wang et al., 2022). Understanding which factors most strongly influence shelf life enables targeted interventions—for example, adjusting pH or water activity to extend shelf life without compromising nutritional quality.

Regulatory Compliance: The uncertainty estimates provided by GPR (Rasmussen & Williams, 2006) can support risk assessment and regulatory decision-making, enabling more evidence-based approaches to food safety management (Ross & McMeekin, 2003).

4.5 Limitations and Future Research Directions

Data Availability and Quality: The performance of machine learning models is fundamentally dependent on the quality and quantity of training data (Hastie et al., 2009). Many functional food products lack comprehensive microbial growth datasets, limiting the applicability of ML approaches (Kumar et al., 2024).

Model Generalizability: While the multi-dataset approach employed in this study provides evidence of generalizability, further validation across a wider range of functional food products and processing conditions is needed (Kremer et al., 2025).

Interpretability versus Performance Trade-off: The most accurate models (e.g., LSTM, ensemble methods) tend to be the least interpretable (Lundberg & Lee, 2017). While SHAP analysis provides some insight, there remains a tension between predictive performance and mechanistic understanding (Rudin, 2019).

Integration with Sensor Data: The potential for integration with real-time sensors and IoT systems offers exciting opportunities for dynamic, adaptive quality management (Kremer et al., 2025). Future work should explore the combination of ML-based prediction with real-time monitoring data from intelligent packaging systems (Liang et al., 2026).

5. CONCLUSION

This study highlights the significant potential of machine learning techniques for improving the prediction of microbial growth and shelf-life estimation in functional food systems. Comparative analysis demonstrated that machine learning algorithms consistently achieved higher predictive accuracy and greater robustness than conventional primary growth models by effectively capturing complex and nonlinear relationships among microbial, environmental, and storage-related variables. In particular, advanced models such as Gaussian Process Regression, Radial Basis Function Neural Networks, and Long Short-Term Memory networks exhibited superior performance in modeling microbial growth kinetics under both static and dynamic storage conditions. These findings indicate that data-driven approaches can overcome many of the limitations associated with traditional mathematical models, particularly when microbial behavior is influenced by multiple interacting factors such as temperature, pH, and water activity. Furthermore, the application of hybrid and ensemble learning strategies further improved prediction reliability, suggesting their suitability for comprehensive quality assessment and shelf-life management. The implementation of machine learning-based predictive systems has the potential to support intelligent food quality monitoring, reduce product losses, enhance food safety, optimize inventory and supply chain management, and facilitate evidence-based decision-making within the functional food industry. Overall, the results demonstrate that machine learning provides a reliable, flexible, and scalable framework for predictive food microbiology and offers a promising direction for the development of next-generation shelf-life prediction systems. Future research should focus on integrating multi-omics datasets, real-time sensor technologies, Internet of Things (IoT) platforms, and explainable artificial intelligence techniques to further improve model interpretability, prediction accuracy, and practical implementation in commercial food production environments.

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