Production of probiotic Kiwifruit juice containing *Lacticaseibacillus paracasei* B31-2: investigation of Probiotic Viability, Physicochemical Properties, and AI
 Predictive Insights

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6 Abstract

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Probiotic juices have experienced a notable rise in popularity due to their potential health 7 benefits, particularly for digestive health. This study examined the viability, physicochemical 8 characteristics, and sensory qualities of kiwifruit juice containing *Lacticaseibacillus paracasei* 9 B31-2. To analyze the data, Gaussian Process Regression (GPR) and Multi-Layer Perceptron 10 (MLP) models were used to predict various factors, including pH, acidity, viable cell counts of 11 *L. paracasei* B31-2, color differences (ΔE), and overall acceptance. Probiotic *L. paracasei* 12 B31-2 was added to the kiwifruit juice at different concentrations (0%, 1% and 2%) and stored 13 at 4 °C. The probiotic juices showed fewer changes in pH, acidity, and color compared to the 14 control juice during storage at room temperature. The sample with a 2% probiotic concentration 15 exhibited the highest viable cell count (7.98 log CFU/mL) and received the most sensory scores 16 among the tested samples. A strong correlation between the predictions made by the GPR 17 model and the actual observed data further validated its effectiveness in similar experimental 18 contexts. This suggests that GPR could offer strategic benefits by lowering laboratory costs 19 and improving analytical efficiency. The GPR model's precision in closely matching real-world 20 data demonstrates its potential as a cost-effective and expedited tool for scientific inquiries. 21 Overall, these findings indicate that kiwifruit juice serves as a promising substrate for carrier 22 of L. paracasei B31-2. 23

Keywords: Kiwifruit Juice; Probiotic Viability; Gaussian Process Regression;
 Lacticaseibacillus paracasei B31-2.

27 **1. Introduction**

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Fruit juices have been widely acknowledged for their nutritional benefits and health-promoting qualities (Xiong et al, 2022a). Packed with essential vitamins, minerals, antioxidants, and phytochemicals, fruit juices have become a popular choice for individuals looking for a

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refreshing and flavorful alternative to traditional drinks (Salehi, 2020). In recent years, plant 31 juices, notably fruit juices, have been gaining attention as a more nutritious option to milk 32 drinks that are high in calories, saturated fat, and cholesterol (Gao et al., 2022). As the focus 33 on plant-based diets increases and lactose intolerance problem, the demand for new alternatives 34 like probiotic fruit juices has also been on the rise (Naseem et al., 2023; Pérez-Rodríguez et al., 35 2023). The rising demand for functional foods has spurred increased interest in probiotic 36 applications for fruit juice processing. Recent studies have focused on fermenting kiwifruit 37 pulp (KP) (Chen et al., 2021; Zhou et al., 2020) and kiwifruit juice (KJ) (Wang et al., 2022) 38 using lactic acid bacteria (LAB). Zhou et al. (2020) and Chen et al. (2021) employed three 39 LAB strains to ferment KP, assessing the transformation of phenolic compounds and in vitro 40 digestibility. Separately, Wang et al. (2022) investigated the fermentation of kiwifruit juices 41 using *Lactiplantibacillus plantarum* and *Lactobacillus acidophilus*, respectively, monitoring 42 changes in bioactive components and volatile aroma profiles. Collectively, these studies 43 44 indicate that LAB fermentation improves the sensory properties of KP and KJ while boosting bioactive compound levels and antioxidant capacity. However, the outcomes varied 45 significantly depending on the LAB strain and kiwifruit cultivar. However, incorporating 46 probiotics into fruit juices presents unique challenges, including low pH, high sugar content, 47 and the presence of antimicrobial compounds, which can reduce bacterial viability. Recent 48 studies, such as the fermentation of kiwifruit juice by probiotic bacteria, highlight its potential 49 to enhance bioactive phenolics, antioxidant activities, and flavor volatiles while maintaining 50 probiotic viability (Wang et al., 2022). 51 Kiwifruit (Actinidia deliciosa), is a nutrient-dense fruit rich in ascorbic acid, dietary fiber, 52 carotenoids, polyphenols, and minerals. Its high phenolic content has been linked to immune 53 system enhancement and prevention of chronic diseases. However, fresh kiwifruit is highly 54 perishable due to its thin peel and climacteric nature, necessitating innovative processing 55 methods to extend shelf life and diversify product applications. Fermentation with probiotics 56 offers a promising solution to address these limitations while amplifying health benefits (Xiong 57 et al, 2022b; Zhang et al., 2021; Sanz et al., 2021; Wang et al., 2021). Additionally, kiwifruit 58 contains actinidin, an enzyme that aids in digestion and may help promote gut health (Zeng et 59 al., 2024; Kaur et al., 2022). Given its unique combination of nutrients and functional 60 properties, kiwifruit has the potential to be a valuable addition to probiotic juices and 61 beverages. 62

Probiotic juices have become increasingly popular due to their potential health benefits,
specifically for digestive health (Kardooni et al., 2023; Mojikon et al., 2022). These beverages

often contain various probiotic strains, such as Lactobacillus and Bifidobacterium, known to 65 support digestive health, enhance the immune system, and improve overall well-being (Gomes 66 & Malcata, 1999). Probiotics are live microorganisms that, when consumed in sufficient 67 quantities, can offer a range of health advantages by improving the balance of beneficial 68 bacteria in the gut (FAO/WHO, 2002). By combining the probiotic properties of these 69 beneficial microorganisms with the nutritional benefits of fruit juices, probiotic juices provide 70 a convenient and delicious way to promote gut health and overall wellness (Pimentel et al., 71 2019). 72 LAB are a group of beneficial bacteria that play a crucial role in the fermentation process of 73 beverages. As Gram-positive, non-spore-forming microbes, LAB are uniquely adapted to 74 thrive in low-pH and anaerobic environments, making them ideal for food fermentation 75 (Alebooye et al., 2023). Their primary metabolic pathway-homolactic or heterolactic 76 fermentation-enables them to convert sugars (e.g., glucose, fructose) into lactic acid, acetic 77 acid, and other bioactive compounds. This acid production not only helps preserve the beverage 78 by inhibiting pathogenic bacteria but also creates a tangy flavor profile characteristic of 79 fermented products. Additionally, LAB exhibit strain-specific probiotic properties, including 80 bile salt hydrolase activity, adhesion to intestinal epithelium, and immunomodulation 81 (Ghazanfari et al., 2024; Wuyts et al., 2018). One specific LAB strain that has shown promise 82 for beverage fortification is *Lacticaseibacillus paracasei*, previously known as *Lactobacillus* 83 *paracasei*. This probiotic bacteria strain has been extensively researched for its ability to 84 survive the acidic stomach environment, colonize the intestines, and provide a variety of health 85

benefits, including immune support, improved digestion, and reduced inflammation (Bengoa et al., 2021). By incorporating *L. paracasei* into fruit juices, beverage manufacturers can
enhance the functional properties of their products and provide consumers with a flavorful and
nutritious way to support their gut health (Mantzourani et al., 2023).

Artificial Neural Networks (ANNs) are revolutionizing the field of food science and 90 technology by providing sophisticated tools for complex problem-solving (Chen et al, 2024). 91 ANNs, inspired by the neural processing of the human brain (Sui et al, 2014), are capable of 92 learning from data, identifying patterns, and making predictions with remarkable accuracy 93 (Chu et al, 2025). In food science, ANNs are being applied to a myriad of tasks, such as 94 predicting the sensory properties of food products, optimizing processing parameters, and 95 ensuring food safety (Neto et al, 2021). For instance, ANNs have been utilized to model 96 microbial growth, thereby aiding in the prediction of food safety and shelf life (Deng et al, 97 2019). They also play a crucial role in interpreting spectroscopic data, which is essential for 98

assessing the quality and authenticity of food products (Feng et al, 2025a). Moreover, ANNs contribute to the understanding of the complex relationships between food components and their functional, chemical, and physical properties during processing and distribution. In the realm of nutrition, ANNs are instrumental in dietary assessment and in analyzing the gut microbiome profile, which is pivotal for personalized nutrition plans (Feng et al, 2025b). They also assist in the toxicity prediction of food ingredients, ensuring consumer safety and compliance with regulatory standards (Gao et al, 2024).

- The integration of ANNs with other technologies, such as electronic noses, electronic tongues, computer vision systems (Sui et al, 2013), and near-infrared spectroscopy (NIR), further enhances their utility in the food industry (He et al, 2025). These combined systems can accurately classify foods, determine quality, and control food processing tools, thereby streamlining operations and improving product consistency (Liu et al, 2023).
- As the demand for food increases with the growing global population, ANNs are set to become an indispensable asset in the food industry (Li et al, 2019). Their ability to handle large datasets and complex calculations makes them ideal for addressing the challenges of food production, safety, and quality assurance (Lu et al, 2024). The future of food science and technology is bright with the continued development and application of ANNs, promising enhanced efficiency, sustainability, and innovation in the field (Shi et al, 2025).

Zhao et al, 2024, applied ANNs to assess the interdependence of information in today's digital 117 landscape. Through model path analysis, it was discovered that both anticipatory confirmation 118 and perceived usefulness significantly contributed to explaining the variance in financial 119 sustainability, at 61% and 74% respectively. The deployment of a deep ANN with a multilayer 120 perceptron architecture enhanced the predictive precision for perceived usefulness, achieving 121 a training accuracy of 87.54% and a testing accuracy of 90.34%. This advancement has 122 enriched the comprehension of the dynamics involved in utilizing green food safety data. 123 Furthermore, a separate investigation conducted by Wang et al, 2023 employed ANNs to 124 determine the key elements influencing the online live sale of fresh produce, guided by a push-125 pull framework. The findings indicated that the neural network model predicted live sale 126 outcomes with an 83.76% accuracy rate. The insights gained from these studies offer a solid 127 theoretical foundation for forecasting consumer behavior in purchasing fresh food via live-128 streaming platforms, considering both consumer and supply chain perspectives. Additionally, 129 these findings suggest strategies for crafting live broadcast content and enhancing the overall 130 131 user experience in e-commerce environments.

132	In this <mark>study</mark> , we aim to produce probiotic kiwifruit juice as an innovative beverage option for
133	individuals looking to enhance their digestive health and overall well-being. Moreover, the
134	effect of probiotic L. paracasei incorporation in kiwifruit juice on its physiochemical and
135	sensory charactrstics was investigated. In the next step, the study employed Gaussian Process
136	Regression (GPR) alongside Multi-Layer Perceptron (MLP) to forecast a range of variables:
137	pH, acidity, viable cells of <i>L. paracasei</i> B31-2, ΔE , and overall acceptance.
138 139	2. Materials and methods
140	2.1. Kiwifruit juice formulation
141	Fresh kiwifruits were obtained from a local market in Mollasani (Khuzestan Province, Iran).
142	The fruits were peeled and juiced using a mechanical juicer. The resulting pulp was then
143	centrifuged (6,000 \times g, 15 min, 4°C) to obtain clarified kiwifruit juice. The juice was
144	standardized to an initial pH of 4.0 using a 50 mg/mL food-grade Na ₂ CO ₃ solution and adjusted
145	to 12.0°Brix with potable water. Prior to fermentation, the juice was pasteurized at 60°C for
146	30 min (Wang et al., 2022). To create the inoculum, the stock culture of <i>L. paracasei</i> B31-2
147	(previously isolated from yogurt samples in our earlier study (Alizadeh Behbahani et al.,
148	2024b) was activated by inoculating 100 μ L of glycerol stock into 100 mL sterile MRS broth
149	(Merck, Germany) in a 250 mL Erlenmeyer flask. The bacterial cells were grown at 37°C for
150	18 h under static conditions until reaching late-log phase (OD ₅₉₀ \approx 0.60, ~9.0 log CFU/mL),
151	as measured using a Jenway 7305 spectrophotometer (England). Prior to inoculation, the
152	cultured cells were as eptically centrifuged $(3,000 \times g, 10 \text{ min}, 4^{\circ}\text{C})$ to pellet the bacteria. The
153	supernatant (MRS broth) was carefully decanted, and the cell pellet was washed twice with
154	sterile phosphate-buffered saline (PBS, pH 7.2) to remove residual medium components that
155	might affect juice fermentation. The final pellet was resuspended in 10 mL of fresh PBS to
156	standardize the cell density. Different concentrations of this standardized inoculum (0%, 1%,
157	and $2\% v/v$, respectively) were aseptically added to pasteurized kiwifruit juices. The probiotic
158	juices were manually stirred to ensure homogeneous distribution of cells and incubated for 21
159	days at 4°C (refrigeration) and 25°C (room temperature) to simulate different storage
160	conditions (Kardooni et al., 2023).

162 **2.2. Changes in pH value**

The pH meter (Metrohm, Switzerland) was used to determine the pH of the probiotic juice samples that had been stored at both 4 °C and 25 °C for 21 days (Vasiee et al., 2025; Han et al., 2015).

2.3. Acidity analysis 166 Titratable acidity was determined by titrating each sample mixture (6 g/50 mL water) with 0.1 167 N NaOH (Merck, Germany) until a pH of 8.2 was reached, and the result was expressed as a 168 percentage of citric acid (% acid) (Falah et al., 2021, Yang et al, 2025). 169 170 2.4. Change in microbial load 171 To determine viable cell counts, samples were serially diluted with sterile peptone water. 172 Following that, 0.1 mL portions of the dilution were plated in triplicate on MRS agar plates. 173 The plates were subsequently incubated at 37°C (for 72 h). After incubation time, plates with 174 20-350 colonies were enumerated, and the outcomes were documented as colony-forming 175 units (CFU) per milliliter of solution, in accordance with the methodology outlined by Pereira 176 et al., (2011). 177 178 2.5. Color analysis 179 The colorimeter (Minolta CR300, Japan) was used to assessment the color of the probiotic 180 juice. The instrument underwent calibration with illuminant D65, and color readings were 181 acquired through an 8-mm port/viewing area. The colorimeter outputted three color metrics: 182 lightness (L*), redness (a*), and yellowness (b*). Subsequently, the numerical values of L*, a*, 183 184 and b* underwent transformation into total color difference (ΔE) through the equation specified by Eq. No 1 (Costa et al, 2013). 185 (1) $\Delta E = \sqrt{\Delta L^{*2} + \Delta b^{*2} + \Delta a^{*2}}$ 186 2.6. Sensory evaluation 187 A group of twenty-five individuals, (15 women and 10 men, 25-35 years old), from the Food 188 Science and Technology department at Agricultural Sciences and Natural Resources University 189 of Khuzestan in Mollasani, Iran, assessed the samples based on their odor, color, texture, and 190 overall acceptability. A nine-point hedonic scale was utilized to assess the sensory 191 characteristics of probiotic juices, including odor, color, and overall acceptability, with 1 192 indicating strong dislike and 9 indicating strong likeness. The samples, were labelled with 193 three-digit numbers, were randomly served in acrylic cups at 10 °C. (Alizadeh Behbahani et 194 al., 2024a). 195

201 **2.7. Statistics**

202 The experiments were conducted thrice, and the data was analyzed using Minitab software

- 203 (version 16) with a completely randomized factorial design. The average values were grouped
- based on the Tukey post-test, with a significance level established at p < 0.05.
- 205

206 2.8. Gaussian process regression (GPR) model

The GPR emerges as a formidable statistical instrument adept at deciphering complex data configurations and offering predictions, even when faced with data that is fragmented or laden with noise (Ni et al, 2025). This technique operates on the principle of a preliminary probabilistic model over a range of plausible functions that may correspond to the observed data, progressively honing this model as additional data is introduced (Sui et al, 2010).

The GPR model's objective is to assimilate training data and adeptly extend the output distribution to new input scenarios (Tian et al, 2013). Here, the model considers noise in the output to account for uncertainties stemming from factors beyond the input variable, like measurement errors. The presumption is that this noise is additive, with a zero mean, stationary, and randomly distributed (Tian et al, 2019). This assumption allows for a more accurate representation of the underlying data structure and enhances the model's predictive capabilities.

<mark>(2)</mark>

<mark>(3)</mark>

The general GPR can be shown by Eqs. No 2 and 3 (Xiang et al, 2025).

$$y = f(x) + \xi$$

$$\xi \square P(0, \mathbf{s}_{\text{noise}}^2)$$

Where s_{noise}^2 is the variance of the noise. The assumption of a Gaussian prior facilitates the interpretation of the function via the mean, denoted as m(x) and the covariance functions (Zhu et al, 2015). Consistent with prevailing academic findings, the configuration of the mean function holds importance primarily in regions without observations and is customarily set to zero. This simplification aids in focusing on the covariance structure, which is pivotal in understanding the relationships within the data (Yang et al, 2011).

226 **2.9. Multilayer Perceptron (MLP) Neural Network**

The MLP, a widely utilized type of neural network, is structured with several layers that include an input layer, one or more hidden layers, and an output layer. Each layer is comprised of neurons, which are the fundamental processing elements (Yao and Chen, 2016; Wang et al, 2025). An example MLP configuration is depicted in **Fig. 1**, showcasing a structure of MLP model with 3 hidden layers. During the network's operation, each neuron applies a specific bias in its calculations.

The input layer receives n distinct input variables denoted as $X = \{X_1, X_2, X_3, ...\}$ while the 233 output layer produces m distinct output variables represented by $Y = \{Y_1, Y_2, Y_3, ...\}$ (Zhiquan et 234 al, 2015). In this research, data normalization precedes the training of the neural network (Yao 235 et al. 2017). The purpose of normalization is to transform the data values to a range between 236 zero and one. This step is crucial for both the training and testing phases of the network, as it 237 ensures the learning algorithm operates effectively (Yao et al, 2014). Without normalization, 238 the network may fail to converge during training, leading to suboptimal outcomes. When 239 employing a sigmoid activation function, the ideal range for data transformation is between 0.9 240 and 1.0 (Zhu et al, 2024). The method of linear normalization was utilized for this data 241 transformation process, which can be mathematically expressed by Eq. No 4 (Taki et al, 2018; 242 Zhao et al, 2012). 243

$$\mathbf{x}_{n} = \frac{\mathbf{x} - \mathbf{x}_{\min}}{\mathbf{x}_{\max} - \mathbf{x}_{\min}} \times (\mathbf{r}_{\max} - \mathbf{r}_{\min}) + \mathbf{r}_{\min}$$
(4)

where, x signifies the unprocessed initial dataset, while x_n denotes the data post-normalization. The highest and lowest values within the original dataset are symbolized by x_{max} and x_{min} , respectively (Zheng et al, 2025). Conversely, x_{max} and x_{min} represent the new data range's maximum and minimum limits. The datasets are subjected to a thorough assessment through these normalization procedures, and the most advantageous technique is selected contingent upon the network's efficacy (Zhao et al, 2011).

- 250 The research utilized the Levenberg-Marquardt, also known as Trainlm, as the backpropagation
- algorithm for the training phase (Zheng and Cao, 2024). The neuron count in the hidden layer
- was varied from 5 to 35, with the optimal number being chosen based on achieving the minimal
- 253 error rate.



Fig. 1. MLP neural network structure with 3 neurons in input, 3 hidden layer and one neuron in output (3-4-4-4-1).

2.10. Performance evaluation criteria 257

To assess the performance of both GPR and MLP models, various statistical metrics were 258

- employed, including the Root Mean Square Error (RMSE), Mean Absolute Percentage Error (MAPE) and the coefficient of determination R^2 as the Eqs. No 5 and 6 (Taki and Rohani,
- 260
- 2022; Zheng et al, 2024). 261

$$MAPE = \frac{1}{n} \sum_{j=1}^{n} \left| \frac{T_{d_j} - T_{p_j}}{T_{d_j}} \right| \times 100$$
(5)

<mark>(6)</mark>

$$R^{2} = \left[\frac{\sum_{j=1}^{n} (T_{d_{j}} - \overline{Td})(T_{p_{j}} - \overline{Tp})}{\sum_{j=1}^{n} (T_{d_{j}} - \overline{Td}) \times \sum_{j=1}^{n} (T_{p_{j}} - \overline{Tp})}\right]^{2}$$

Where n is the experimental data, Tp_j is the predicted data by the models, Td_j is the actual data 262 and \overline{Td} and \overline{Tp} are the average values of actual and predicted data. 263

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3. Results and discussion 265

3.1. Changes in pH value and Acidity analysis 266

It's crucial to carefully control the pH and acidity levels in probiotic juices to maintain the 267 viability of the probiotics, preserve product quality, promote health benefits, and enhance taste 268 and flavour. This close monitoring and control by manufacturers are essential in creating a 269 successful probiotic beverage. The variations in pH and acidity levels of kiwifruit juices during 270 storage are illustrated in Fig. 2. The pH of the control sample of kiwifruit juices stored at 4°C 271 on the first and 21st days was 3.2 and 3, respectively, while for the control sample stored at 272 25°C it was 3.2 and 2.9, respectively. The pH of the with 1% LP sample of fruit juice stored at 273 4°C on the first and 21st days was 3.2 and 2.85, respectively, while for the control sample 274 stored at 25°C it was 3.11and 2.65, respectively. The pH of the with 2% LP sample of fruit 275 juice stored at 4°C on the first and 21st days was 3.17 and 2.75, respectively, while for the 276 control sample stored at 25°C it was 3.1 and 2.55, respectively. With an increase in storage 277 time, temperature and probiotic concentration, the pH value significantly decreased by 12%, 278 4%, and 7%, respectively (p < 0.05) (Fig. 2a). The activity of the probiotic strain and enzymes 279 found in kiwifruit juice may have been enhanced at ambient temperature, resulting in a more 280 noticeable decline in pH. The titratable acidity of kiwifruit juices showed a similar trend to pH, 281 as depicted in Fig. 2b. The observed pH reduction in kiwifruit juice samples correlated with 282 increased acidity, consistent with the known logarithmic inverse relationship between pH and 283 hydrogen ion concentration. According to research by Tieking et al., (2005), L. 284 sanfranciscensis demonstrated the ability to metabolize fructose through microbial processes, 285

leading to a reduction in pH levels and an increase in titratable acidity in the samples. In the 286 juice samples, the rise in acid levels could be attributed to the presence of various by-products 287 resulting from the breakdown of fructose by Lactobacillus, which include lactic acid, acetic 288 acid, and carbon dioxide. This collective influence of by-products positively impacts the 289 overall acidic composition within the juice. A study conducted by Kumar et al., (2015) also 290 noted a similar trend, where mango and sapota juices inoculated with Lactiplantibacillus 291 *plantarum* displayed an increment in acidity throughout the incubation time. Additionally, 292 apart from the production of organic acids, an increase in acidity levels may also stem from the 293 hydrolysis of juice sugars facilitated by enzymes released from lysed probiotic cells. This could 294 be credited to the by-products resulting from the fructose breakdown by *Lactobacillus*, 295 including lactic acid, acetic acid, and carbon dioxide (Peterson & Fred, 1920), all positively 296 influencing the overall acid content in the juice samples. 297



Fig. 2. Fluctuations in pH (a) and acidity (b) of kiwifruit juices loaded with *L. paracasei* B31-209 2 during the storage period. Significant differences (p < 0.05) are represented by different 300 superscript small letters.

3.2. Color analysis 301

During the storage period, a notable increase in the overall color change of the juices was 302 observed (Fig. 3). Additionally, it was noted that the samples containing *L. paracasei* B31-2 303 and stored at 4°C exhibited lower color changes compared to their respective counterparts. 304 These findings align with a study by da Costa et al., (2017), which reported that both control 305 and probiotic pineapple juice underwent darkening became more yellow during storage. Pereira 306 et al. (2011) optimized L. casei NRRL B-442 cultivation in cashew apple juice, determining 307 ideal conditions (pH 6.4, 30°C, 7.48 Log CFU/mL inoculum, 16 h fermentation) and 308 demonstrating its viability (>8 Log CFU/mL) during 42-day refrigerated storage. The study 309 noted increased lightness/yellowness and reduced redness over time, concluding that fermented 310 cashew juice is a viable probiotic functional food, comparable to dairy for L. casei growth. 311 Shah et al. (2010) investigated probiotic survival (Lacticaseibacillus rhamnosus HN001, 312 Bifidobacterium lactis HN001, L. paracasei LPC 37) in a model fruit juice with 313 vitamins/antioxidants. While most additives failed to sustain viability (<10 CFU/mL after 6 314 weeks), vitamin C, grape seed extract, and green tea extract improved survival (4.29–7.41 log 315 CFU/mL). 316

> □0%LP 8 7 6 ab 5 ab ab abc 4 ΔE bcd bcd 3 g g ⊦cd 5 Þ 3 g cq Cd 2 1 0 14-day 14-day 21-day 21-day 7-day 7-day 4°C 25 °C

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■1%LP
        ≥2%LP
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317

321

Fig. 3. Fluctuations in total color difference (ΔE) of kiwifruit juices loaded with *L. paracasei* 318 B31-2 during the storage period. Significant differences (p < 0.05) are represented by different 319 320 superscript small letters.

3.3. Change in microbial load 322

The changes in the number of viable cells of L. paracasei B31-2 in the juice over the storage 323 period are shown in Fig. 4. With increased storage time and temperature, the count of viable 324 cells of *L. paracasei* B31-2 decreased significantly by 17% and 5%, respectively (p < 0.05). 325

- Nevertheless, the samples loaded with 2% LP exhibited the greatest quantity of viable cells 326 (7.98 log CFU/mL) in comparison to those with 1% LP (7.59 log CFU/mL) (p < 0.05). 327 According to Zhu et al. (2020), a decrease in probiotics' survival rate in food products has been 328 linked to increased acidity. The improved preservation of probiotic cells may be attributed to 329 the effective regulation of pH and acidity in kiwifruit juices stored at 4°C throughout the 330 storage period (Fig. 2). In similar, the viability of L. sanfranciscensis in tomato, orange, and 331 apple juices significantly decreased (p < 0.05) after 4 weeks of storage, due to the increasing 332 lactic acid content, which influenced the subsequent viability of the probiotics (Zhu et al., 333 2020). Although probiotic counts decreased, all juice samples maintained viability above the 334 recommended threshold (>10⁶ CFU/mL) after 3 weeks. However, strain-specific acid tolerance 335 must be considered. In our study, L. paracasei B31-2 showed a log reduction of 21 days, 336 aligning with Okina et al. (2018), where L. paracasei ssp. retained >10⁹ CFU/200 mL in grape 337 juice after 21 days. In contrast, Rodrigues et al. (2011) reported variable survival for L. 338 paracasei L26 in low-pH juices, with counts declining below 10⁶ CFU/mL in some 339
- formulations by day 21. These disparities highlight the need for strain- and matrix-specific
- 341 evaluations when designing probiotic juices.



Fig. 4. Fluctuations in microbial load of kiwifruit juices loaded with *L. paracasei* B31-2 during the storage period. Significant differences (p < 0.05) are represented by different superscript small letters.

347 **3.4. Sensory evaluation**

Maintaining the quality of products by adding probiotics is crucial because consumers prefer functional beverages without strange aromas or unpleasant flavors, despite the health benefits (Pimentel et al., 2015). The main effect of storage time on the sensory scores of samples was

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351	significant ($p < 0.05$), and the sensory scores for color, odor, and overall acceptance decreased
352	generally from 8.15 to 4.33, 7.76 to 3.75, and 7.93 to 4.07, respectively, as storage time
353	increased from 1 to 21 days (Fig. 5). The decline in color scores for the control sample (without
354	probiotics) was likely due to non-enzymatic browning (Maillard reaction) and pigment
355	degradation, which are accelerated in the absence of protective compounds produced by
356	probiotics, such as antioxidants or pH-stabilizing metabolites. Additionally, oxidation of
357	phenolic compounds in the juice matrix may have contributed to undesirable color changes.
358	The sensory scores were also impacted by the storage temperature, as cold storage yielded
359	higher scores in comparison to ambient storage. Furthermore, the samples with probiotics
360	exhibited significantly elevated sensory scores relative to the control samples, particularly
361	when stored at 4 °C. This can be ascribed to the probiotics' ability to stabilize acidity and
362	inhibit oxidative reactions, thereby preserving color and flavor. Additionally, it was stated by
363	Alizadeh Behbahani et al. (2024a) that peach juices containing Levilactobacillus brevis HL6
364	and stored at 4 °C demonstrated the highest levels of sensory scores.

365

366 3.5. GPR and MLP models

The results of Fig. 6, show that GPR model outperformed than MLP in predicting pH, acidity, 367 viable cells of *L. paracasei* B31-2, ΔE , and overall acceptance. This superior performance can 368 be attributed to several factors inherent to GPR. Unlike MLP, GPR is a non-parametric 369 approach, which means it does not make strong assumptions about the underlying function 370 form, allowing for greater flexibility and adaptability to the data's intrinsic patterns. GPR's 371 ability to incorporate prior knowledge through kernels enables it to reflect assumptions about 372 the function's behavior, enhancing its predictive power. Furthermore, GPR provides 373 uncertainty quantification for its predictions, a feature not typically available in MLP models, 374 offering valuable insights for risk assessment and decision-making processes. The optimization 375 of GPR's hyper parameters is generally more straightforward, avoiding the complex 376 architecture tuning required by MLPs. Lastly, GPR's probabilistic foundation renders it more 377 robust against overfitting, a common pitfall for MLP, particularly when dealing with limited 378 or noisy datasets. The MAPE values, ranging from 1.62% for ∆E to 2.67% for Log, underscore 379 GPR's precision in output prediction, which is crucial for the reliability and accuracy required 380 in food science and technology applications. These factors collectively contribute to the GPR 381 model's higher accuracy in this context. 382

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Fig. 5. Fluctuations in color (a), color (b), and overall acceptance (c) of kiwifruit juices loaded with *L. paracasei* B31-2 during the storage period. Significant differences (p < 0.05) are represented by different superscript small letters.



Fig. 6. The results of modeling (A and C: using GPR model based on the MAPE and R² factors;
B and D: using MLP model based on the MAPE and R² factors.

400 4. Conclusions

This particular LAB were tested for its ability to create probiotic kiwifruit juice. When stored 401 at 4 °C, the probiotic juice retained its pH and acidity without any detrimental alterations. 402 Moreover, it exhibited minimal color deterioration and sustained the highest viable cells at the 403 conclusion of the storage duration. Additionally, the probiotic kiwifruit juice garnered the most 404 favorable sensory scores. Based on the findings of this study, it can be concluded that L. 405 *paracasei* B31-2 could be utilized as a probiotic culture for producing a healthy beverage from 406 kiwifruit, which would be suitable for vegetarians or individuals allergic to lactose found in 407 probiotic dairy products. In the conducted research, the efficacy of GPR was compared with 408 MLP in forecasting a set of laboratory parameters such as pH, acidity, viable cells of L. 409 *paracasei* B31-2, ΔE , and overall acceptance. The findings revealed that GPR stands out as an 410 exceptionally precise method for output prediction. This is particularly notable in situations 411 where the dataset is limited in size. The robust analytical capabilities of GPR ensure that even 412 with a smaller pool of data, the model can deliver reliable and accurate predictions, making it 413 a valuable tool for laboratory and research applications where data scarcity is a common 414 challenge. 415 416 Acknowledgements 417 The authors would like to express their sincere gratitude to the Vice-chancellor for Research 418

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422 **References**

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