Artificial intelligence-based model for evaluating the inhibition of *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* in kefir matrix

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Abstract

The present study aimed to inhibit the activity of some foodborne pathogens by probiotic lactic acid bacteria (LAB) in kefir. The antimicrobial effect of probiotic LAB was evaluated by using Artificial Intelligence (AI)-based models, Artificial Neural Network (ANN), and Adaptive Network-based Fuzzy Inference System (ANFIS). The experiment was performed on fermentation day 0, 1, and 2, and storage day 1, 3, 7, and 10 of kefir. The average inhibition results obtained for *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* at training stage was 2.4 $\log_{10}$ CFU/g, 2.0 $\log_{10}$ CFU/g, and 2.4 $\log_{10}$ CFU/g in Artificial Neural Network, respectively, and 2.33 $\log_{10}$ CFU/g, 2.04 $\log_{10}$ CFU/g, and 2.03 $\log_{10}$ CFU/g in Adaptive Network-based Fuzzy Inference System, respectively. The average result obtained in the case of tested LAB was 4.9 $\log_{10}$ CFU/g, 4.8 $\log_{10}$ CFU/g, and 4.9 $\log_{10}$ CFU/g, respectively, in Artificial Neural Network in each organism; while similar result was observed in Adaptive Network-based Fuzzy Inference System. The results indicate that the activity of all targeted foodborne pathogens was reduced during fermentation and storage days by the potential probiotic LAB present in kefir. Based on the experiment, it was concluded that the activity of foodborne pathogens can be inhibited by probiotic LAB in kefir. In addition, it was suggested that probiotic bacteria in kefir are promising bio-controlling agents that can be used in the food industry.

Keywords: *Listeria monocytogenes*; *Staphylococcus aureus*; *Escherichia coli*; kefir; probiotic; lactic acid bacteria

Introduction

Foodborne pathogens are known to be the most common food contaminating microorganisms that cause foodborne illnesses. The occurrence of foodborne illnesses is mostly arising from contamination of fresh agricultural produce that intensify disease outbreaks globally (Yang *et al*., 2024a). The contamination of food by pathogens is mostly associated with inappropriate food handling during production, processing, storage, and transportation. Moreover, international trade fairs and diverse food supply chains also widely enhance the spread of foodborne diseases across borders, resulting in health and related risks to the consumers, especially people of vulnerable groups, such as pregnant women, elders, and infants. As a result, the issue of foodborne illnesses has reached the alarming stage of food safety because of global public health issue (Choi *et al*., 2020). The issue is not only of global health concern but also of its considerable economic burden, particularly in

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developing countries, because of the risk associated with the safety and quality of food products (Keba *et al*., 2020).

Among the common foodborne pathogens of public health and economic issue are *Listeria monocytogenes, Staphylococus auerus*, and coliform bacteria that cause serious foodborne infections and damage food products. *L. monocytogenes* is the most serious and deadly pathogen that causes foodborne infections (Rivas-Macho *et al*., 2024). The pathogen is ubiquitous in nature, and can live and persist in different conditions, including farm environments, food production environments, food products, food contact surfaces and utensils, as it is a biofilm-forming organism in monospecies or in conjunction with other microbes (Fagerlund *et al.,* 2020; Gu *et al*., 2024; Kallipolitis *et al.,* 2020; Kannan *et al*., 2020). It causes listeriosis in humans, particularly in venerable groups, such as pregnant women, elders, and people with debilitated or compromised immune system. It spreads following consumption of either contaminated or undercooked foods, pasteurized milk, soft cheese, semi-soft cheese, and cooked meat (Chen *et al*., 2020; Cufaoglu *et al*., 2021; Olaimat *et al*., 2021).

It has been suggested by some research findings that foods with low moisture content (foods with water activity < 0.85) are found to be a potential source of this pathogen (Ly *et al*., 2019; Taylor and Zhu, 2021); foods that are kept under cold conditions for more than the recommended period can enhance the chance of survival for the pathogen in the food products. Another finding has indicated that prevalence of the pathogen in dairy products is high and negatively affects the dairy industry (El Hag *et al*., 2021). For the most part, since the pathogen is capable to contaminate food products along the food chain from production stages to consumption, a large number of recalls are observed in the food processing industry (Duze *et al*., 2021). Besides the infections associated with consumption of food products, the pathogen is responsible for other illnesses, such as meningitis (meningoencephalitis), central nervous system infection, silent diabetes mellitus, and other complications in immune-compromised groups (Steinbrecher *et al*., 2023).

Another foodborne pathogen is *Staphylococcus aureus.* It is well characterized by its colonization on the skin and the upper respiratory tract in humans under normal conditions (Flora *et al*., 2019). The pathogen causes staphylococcal food poisoning by the consumption of staphylococcal enterotoxins produced in foods and subsequent exposure of consumers to various health problems, resulting in huge economic losses to the food industry (Farha *et al*., 2020). The level of poisoning depends on the secretion of multiple toxic proteins, especially if they are produced $>10^5$ CFU/g, representing pathogenic toxins (Zhao *et al*., 2020). The staphylococcal enterotoxins are often reported in dairy milk products, and are observed as the major cause of infections associated with food poisoning in humans and as mastitis in animals (Zhao *et al*., 2021). The pathogen is opportunistic as it can survive in the food processing environments because of its tolerance to desiccation and other stress conditions (Wang *et al*., 2023). Similar to *L. monocytogenes*, *S. aureus* is a ubiquitous foodborne pathogen and biofilm-forming bacteria that persistently attach to food contact surfaces during food production, thereby contaminating food products (Rubab *et al*., 2018; Titouche *et al*., 2019). In dairy farms, the pathogen is most commonly known to cause mastitis in dairy cattle, leading to potential risk to public health as well as product loss and economic crises to the dairy industry.

Coliforms are also among the foodborne bacteria that are abundantly present in the environment as well as in the fecal matter of humans and warm-blooded animals. However, the presence of total coliform bacteria in food products interfere the quality of products, leading to the loss of physicochemical characteristics, particularly that of dairy products (Selover *et al*., 2021). Among the group of coliforms, the presence of toxin-producing *E. coli* in dairy products (raw and processed milk) has become a public health concern due to its ability to produce infective shigatoxin (Rosario *et al*., 2021). Thus, food products, including dairy products, are among the various food matrices where pathogens are detected and lead to the majority of food borne illnesses in humans (Fallah *et al.,* 2021; Tian *et al*., 2022).

Several mechanisms and approaches are established to mitigate the activity and spread of foodborne pathogens. Biological controlling approach is considered as an effective way of combating the potential risk of pathogens to ensure food safety and quality. Probiotic bacteria are one of the biological agents that a number of scientific researchers have addressed for their antimicrobial effects on foodborne pathogens (Abdelhamid and El-Dougdoug, 2020; Jara *et al*., 2020; Kaya and Simsek, 2019; Rajabi *et al*., 2020). These probiotic bacteria produce different metabolites, such as bacteriocins, organic acids, and other components that inhibit the growth of pathogenic microorganisms. Meanwhile, some research findings have reported that some probiotics have traits (genes) that might be transferred to their hosts. In light of this, some research reports identified that probiotic bacteria, such as Bacillus species, have virulence genes/factors, such as *nheABC*, *entFM enterotoxin* genes, *hbIA*, *hbIC*, *hbID*, and *cytK* (Anokyewaa *et al*., 2021) as well as *nheABC*, *hblCDA*, and *cytotoxin cytK2* (Deng *et al*., 2021) that enable them constrain the activity of microbes and resist antibiotics. In another investigation, it was identified that isolates of some probiotic *Enterococcus faecium* from Tulum cheese traditionally produced in Turkey

exhibited their antibacterial activity against *L. monocytogenes* ATCC 7644 and *S. aureus* ATCC 25923 because of the presence of virulence gene determinants, such as *asal*, *gel*E, *Cyl*A, *cyl*$L_{L,s}$, *cyl*M, *Hyl*$_{emf}$, and *gel*E, detected in the probiotic bacteria (Özkan *et al*., 2021). The majority of these probiotics, particularly LAB, are naturally present in fermented dairy and other food products, and these are considered as desirable and beneficial microorganisms (Klimko *et al*., 2020). Kefir is a valuable probiotic dairy product; many researchers studied bacteriostatic and bactericidal effects against bacterial pathogens as well as preventive effect on spoilage fungi (González-Orozco *et al*., 2022; Gut *et al.*, 2022; John and Deeseenthum, 2015; Rosa *et al.*, 2017; Ulusoy *et al*., 2007).

Regarding technological requirements, these bacteria are suitable for addition to food products because of their viability and efficacy as well as their resistance to several processing conditions employed in the food industry (Soares *et al*., 2023). Thus, probiotics are considered as alternative antimicrobial agents when incorporated into food products to antagonize the activity of foodborne pathogens (Ağagündüz *et al*., 2022; Khaneghah *et al*., 2020; Vahdat *et al.,* 2024). In the present study, kefir was used as a product that contains probiotic microorganisms. It is a traditionally fermented dairy product produced by the action of bacteria and yeast existing in symbiotic association with kefir grains, and characterized by probiotic properties and antimicrobial activity (Gökmen *et al*., 2022; González-Orozco *et al*., 2022; Yilmaz *et al*., 2022). Limited research findings exist regarding the inhibition of targeted foodborne pathogens by using probiotic LAB in kefir milk. Therefore, the aim of this research was to inhibit the activity and growth ability of *L. monocytogenes*, *S. aureus*, and coliform bacteria, more specifically *E. coli* bacteria, by using probiotic LAB, naturally present in kefir milk. Feasible benefits of inhibiting these foodborne pathogens from the perspective of food safety are also identified. Rapid development of Artificial Intelligence (AI) provides significant benefits for research studies, especially for finding the way and obtaining the hypothesis of the research by predicting. In this research supporting help of AI was used for our hypothesis.

Materials and Methods

Activation of pathogenic bacterial suspensions

Pure cultures of *Listeria monocytogenes* (ATCC 19111) and *Staphylococcus aureus* (ATCC 19212) were obtained from Ankara University, Turkey. Activation of pathogenic bacteria was performed on nutrient agar media through the streaking technique, or alternatively, about 1 mL of bacterial suspension from each pathogen was suspended in 9 mL of Maximum Recovery Dilution (MRD) (Lab M Limited, Lancashire, United Kingdom). The plates containing bacterial inoculum were incubated at 37°C for 24–48 h. A fecal sample was collected for the cultivation of coliform bacteria, which was swabbed on Violet Red Bile (VRB) agar (Merck kGaA, Darmstadt, Germany) and incubated at 37°C for 24–48 h. The suspension of both *Listeria monocytogenes* and *Staphylococcus aureus* pathogens was separately adjusted to 0.5 McFarland turbidity standards to approximate bacterial concentration to 10^6 CFU/mL as described by Prezzi *et al.* (2020).

Kefir grains and preparing kefir milk

Kefir grains were donated by the Department of Food Hygiene and Technology of Near East University, Nicosia, Cyprus. The grains were maintained and activated in full-fat ultra high temperature (UHT) milk at 25°C for 18-h fermentation period. The grains were strained through a sterile sieve and washed with sterile normal saline solution to remove curdle part of the milk as described by Angelidis *et al*. (2020) and fermented kefir milk was obtained by following the process of straining kefir grains.

Experimental contamination of kefir

Four sterile glass jars (beakers) were used to prepare experimental groups. For each group, 5 g of kefir grains was weighed aseptically and mixed with 50 mL of UHT milk. About 0.1 mL of bacterial suspension of *L. monocytogenes*, *S. aureus*, and coliforms was added to each jar. Inoculation was not done in one of the jars, which was considered as the control group. All the jars containing homogenized solutions were kept in an incubator at 30°C for 2 days of fermentation process as described by Dimitreli and Antoniou (2011) and Gökmen *et al*. (2022). After complete removal of grains, the kefir milk in each jar was refrigerated at 4°C for microbiological analysis.

Microbiological analysis

The results of microbiological analysis of LAB in *L. monocytogenes, S. aureus, coliforms*/*E. coli* are described in Table 1.

Lactic acid bacteria

Serial dilutions were prepared with minimum required dilution (MRD) in the ratio of 1:9 in aseptic conditions for microbially contaminated solutions. About 0.1 mL of solution representing the targeted microbe was spread on selective agar plates. The microbiological analysis of LAB was carried out aseptically by pouring onto the pre-prepared De Man–Rogosa–Sharpe (MRS) agar medium

Table 1. Medium, incubation conditions, and analytical references of methods used for microbiological analysis of LAB.

Micro-organisms	Analytical reference method	Media name	Incubation conditions		
			Incubation temperature	Incubation period	O_2 requirement
Staphylococci *Staphylococcus aureus*	ISO 6888-1:1999 + A1:2003	Baird Parker Medium Agar (LAB 085) + Egg Yolk Tellurite Emulsion (X 085)	35–37ºC	24 ± 2 h	Aerobic
		Brain Heart Infusion Broth (LAB 049)	Confirmation for *Staphylococcus aureus*		
		Rabbit Plasma (X086)			
Coliform bacteria	ISO 4832:2006	Violet Red Bile Glucose Agar (LAB 031)	30–37ºC	24 ± 2 h	Microaerophilic
		Brilliant Green Bile Broth (LAB051)	Confirmation		
Listeria monocytogenes	ISO 11290-1: 1996 + A1:2004	Half Fraser Broth Base (LAB 164)	30ºC	24 ± 2 h	Aerobic
		Fraser Broth Base (LAB 164)	37ºC	24 h	
		Palcam Agar (LAB 148)	37ºC	24 ± 3 h	
		Tryptone Soya Yeast Extract Broth (LAB004)	Confirmation for *Listeria* spp.		
		Sheep Blood Agar (LAB028)	Confirmation for *L. monocytogenes*		
Lactic acid bacteria	ISO 15214:1998	MRS Agar (LAB223)	30ºC	2–3 days	Anaerobic

(Merck kGaA) under anaerobic conditions. The plates were incubated at 37°C for 48 h. Microbial enumeration was conducted by using colony counting machine after completion of incubation. The enumerated LAB was analyzed and compared with the control group.

Pathogens

From the serial dilutions prepared for microbial contaminated solutions, about 0.1 mL of solution of each pathogen was added into the designated growth media and spread on the surfaces of agar plates. *L. monocytogenes* was grown on polymyxin acriflavin lithium-chloride ceftazidime esculin mannitol (PALCAM) agar (Lab M Limited, Lancashire, UK), while *S. aureus* and coliform/*E. coli* bacteria were allowed to grow on Baird–Parker agar supplemented with egg yolk tellurite emulsion (20%) (Merck kGaA) and VRB agar (Merck kGaA, 64271 Darmstadt, EMD Millipore Corporation, Germany), respectively. The procedure was carried out during fermentation and storage (post-fermentation) of dairy kefir. The plates were kept in an incubator at 37°C for 24–48 h as described by Angelidis *et al*. (2020) with some modifications. Following the removal of colony counting plates from the incubator, bacterial colonies were enumerated on the surface of plates. The enumeration of coliforms was done by pour plating method by cultivating bacteria onto VRB agar in anaerobic conditions. The identification of *E. coli* was done by incubating VRB agar plate containing coliform bacteria at a temperature of 44°C for 24–48 h.

Data analysis by AI

The experimental analysis of the present study was conducted in triplicate. AI data-based approaches (artificial neural network [ANN] and adaptive network-based fuzzy inference system [ANFIS] models) were applied to analyze inhibition of *L. monocytogenes*, *S. aureus*, and *E. coli* foodborne pathogenic bacteria by using probiotic LAB present in dairy kefir milk.

Results

Listeria monocytogenes

The ANN model analyzed the inhibition of *L. monocytogenes* by using probiotic dairy kefir, and the inhibition was evaluated at a specified time. The average of obtained results for LAB control, tested LAB, and *L. monocytogenes* were 5.23, 4.95, and 2.41 $\log_{10}$ CFU/g, respectively, at training stage whereas at testing stage, the recorded values were 5.80, 5.38, and 2.04 $\log_{10}$ CFU/g, respectively. As described in Table 2, reduction in the number of pathogens was observed on SD1, SD3, SD7, and SD10 whereas the number of tested LAB increased contrarily on stated days. In addition, the obtained result for the reduction of number of *L. monocytogenes* was also supported by regression analysis at training, validation, and testing stages, with regression (R) = 0.9783, 0.9991, 0.9815, respectively (Figure 1). Furthermore, the ANN model revealed the inhibitory activity of LAB against the pathogen with the best validation performance of 0.2298 at epoch 4 (Figure 2) and error of the model as 0.02395 as shown in Figure 3. Similarly, the ANFIS model also simulated the overall modelling of inhibition of *L. monocytogenes* using probiotic dairy kefir. In this model, the average number of the bacteria was 5.23, 4.93, and 2.33 $\log_{10}$ CFU/g for LAB control, tested LAB, and *L. monocytogenes*, respectively, for the training stage, and 5.65,

Table 2. ANN model inhibition of *L. monocytogenes* by probiotic dairy kefir (in $\log_{10}$ CFU/g).

Days	LAB control	Tested LAB	*L. monocytogenes*
Training stage			
FD0	3.22	3.1554	2.92988
FD1	4.1	3.81108	3.16429
FD2	4.96	4.70434	3.9069
SD1	5.1	4.95914	2.62325
SD3	5.2	5.03216	2.25576
SD7	5.29	5.04857	2.17457
SD10	5.4	5.0539	2.15349
FD0	4.2	4.09884	3.72184
FD1	5.1	4.95914	2.62325
FD2	5.86	5.25682	2.02536
SD1	5.98	5.41074	1.91552
SD3	6.11	5.52634	1.77089
SD7	6.34	6.01235	1.29927
SD10	6.43	6.25392	1.18377
Average	5.2350	4.9488	2.4106
Testing stage			
FD0	4.18	4.03903	3.6057
FD1	4.97	4.72179	3.81903
FD2	5.78	5.16275	2.08419
SD1	5.88	5.28384	2.00786
SD3	6.1	5.51774	1.78513
SD7	6.23	5.69512	1.53622
SD10	6.41	6.20991	1.20264
Average	5.8081	5.3876	2.0497

5.29, and 2.45 $\log_{10}$ CFU/g, respectively, at the testing stage (Table 3). The number of pathogens reduced starting from fermentation day 2 (FD2) to storage day 10 (SD10) whereas the number of LAB increased from FD0 to SD10, unlike the number of pathogens.

Staphylococcus aureus

Similar to *L. monocytogenes*, the ANN and ANFIS models also analyzed the inhibition of *S. aureus* by using probiotic dairy kefir during fermentation and storage days. The models evaluated at the specified time interval as displayed in Tables 4 and 5, respectively. On average, *S. aureus* was simulated by ANN model in relation to LAB control (5.23 $\log_{10}$ CFU/g) and tested LAB (4.89 $\log_{10}$ CFU/g), arriving at an average reduction of 2.04 $\log_{10}$ CFU/g. The inhibition of *S. aureus* by probiotic LAB in dairy kefir was also supported by regression analysis at training, validation, and testing stages, with R = 0.9842, 0.9905, 0.8873, respectively, as shown in Figure 5, and with the best validation performance of 0.071812 at epoch 21 (Figure 6). The inhibition of *S. aureus* by probiotic LAB was also analyzed by ANFIS model. As shown in Table 5, reduction in number of *S. aureus* was observed from FD2 to SD10 at the training stage, while the number of LAB increased during the above-stated days.

Escherichia coli

The inhibition of *E. coli* by probiotic LAB present in dairy kefir was analyzed using ANN model. The results showed reduction in the number of *E. coli* from FD2 to SD10 whereas an increment in the number of LAB was observed from FD0 to SD10 (Table 6) at the training stage. Likewise, reduction in the number of *E. coli* was observed on days FD0–SD10 at the testing stage. The average result obtained for LAB control, tested LAB, and *E. coli* was 5.23, 4.96, and 2.46 $\log_{10}$ CFU/g, respectively, at the training stage whereas it was 5.81, 5.46, and 1.93 $\log_{10}$ CFU/g, respectively, at the testing stage as displayed in Table 6. In addition, the inhibition of the targeted pathogen was also braced by regression analysis, with R = 0.9702, 0.9514 and 0.9537 at training, validation, and testing stages, respectively (Figure 9). The best validation performance for the inhibition of the pathogen was obtained at 0.18637 as shown in Figure 10.

Similarly, the inhibition of *E. coli* by biological means, namely probiotic LAB naturally present in dairy kefir milk and used in the present study, was analyzed using ANFIS model. The model simulated with a complete inhibition of *E. coli* at the training stage with an average number of LAB in control (5.23 $\log_{10}$ CFU/g), tested LAB (4.93 $\log_{10}$ CFU/g), and *E. coli* (2.03 $\log_{10}$ CFU/g) as shown in Table 7. Likewise, the average value of LAB control, tested LAB, and *E. coli* obtained at the testing stage were 5.65, 5.25, and 1.34 $\log_{10}$ CFU/g, respectively. For this model, the inhibition of the activity of *E. coli* was observed during fermentation and storage days of kefir milk, which was confirmed by the reduced number of the pathogen from FD2 to SD10.

Discussion

Biological approach for controlling the activity of foodborne pathogens has attracted the attention researchers. The present study was conducted to investigatr the activity of targeted foodborne pathogens, namely *L. monocytogens*, *S. aureus*, and coliform/*E. coli* by using biological controlling approach, which include probiotic LAB present naturally in dairy kefir milk. Evidence showed that various probiotic products were used as alternative controlling means to overcome the risks associated with the effect of pathogens present the foods (Abdelhamid and El-Dougdoug, 2020; Hossain *et al*., 2020;

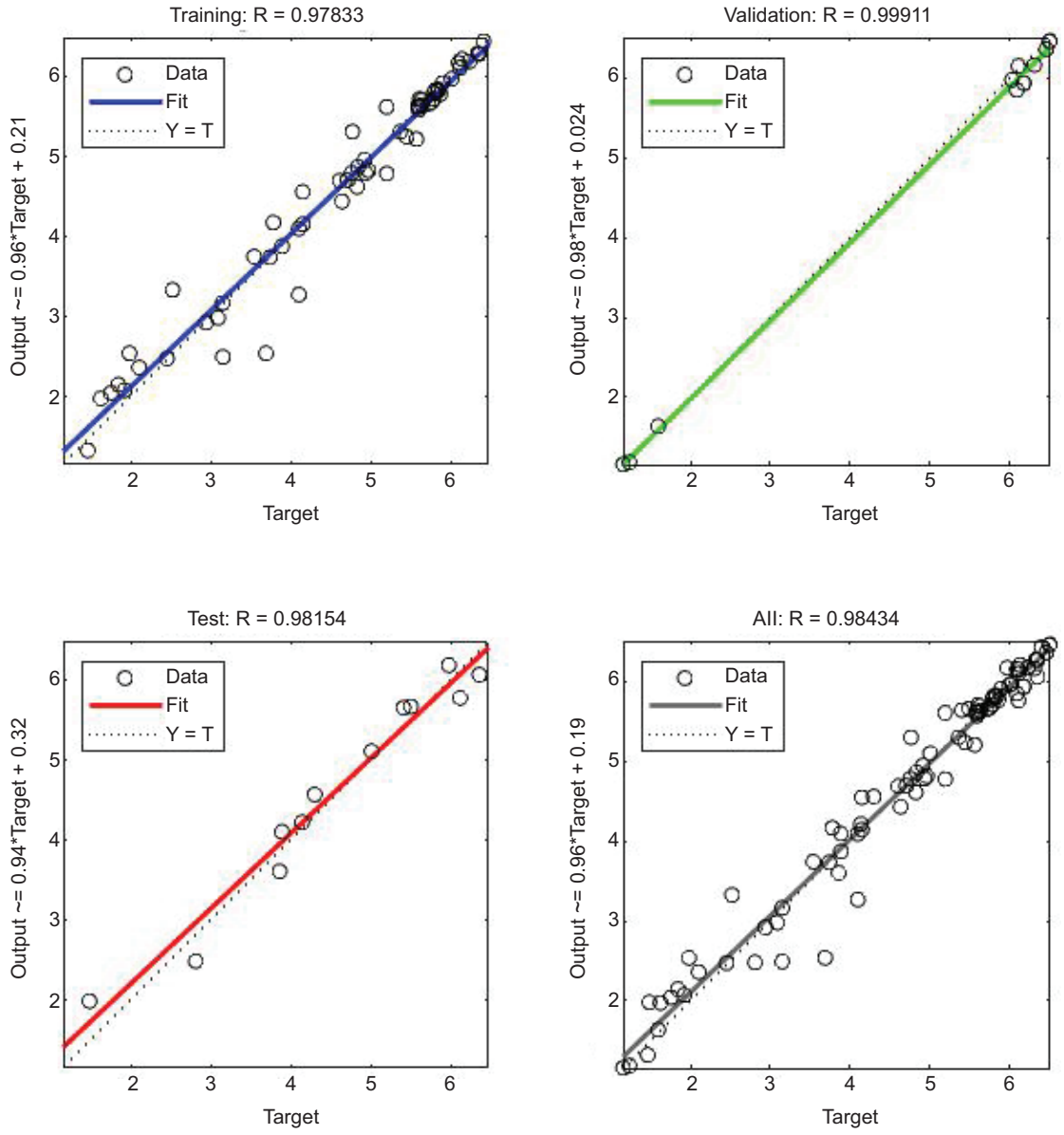

Figure 1. Regression graph of ANN model prediction for the inhibition of *L. monocytogenes* (in log_{10} CFU/g) using probiotic dairy product kefir.

Kouhi *et al*., 2022; Martín *et al*., 2022; Muñoz *et al*., 2019). More specifically, the report of Prezzi *et al*. (2020), who evaluated the effect of *Lactobacillus rhamnosus* GG on the growth of two foodborne pathogens (*S. aureus* and *L. monocytogenes*) by inoculating the surface of Minas Frescal cheese, indicated that the addition of *Lactobacillus rhamnosus* in the cheese was found to be responsible for the inhibition of *L. monocytogenes*.

Probiotic LAB is naturally present in different types of fermented dairy products and other food matrices, providing health benefits and microbial balance in the gastrointestinal tract of consumers (Colombo *et al*., 2018; Kefyalew *et al*., 2021). Because of the presence of different metabolites, such as organic acids, carbon dioxide, hydrogen peroxide, and bacteriocin (nisin), these beneficial microorganisms are able to inhibit the activity of foodborne pathogens. Therefore, the agricultural sector and food processing plants use beneficial microorganisms as alternative bio-control agents to mitigate the potential risk of foodborne pathogenic bacteria and their biofilms (Abdelhamid and El-Dougdoug, 2020; Kaya and Simsek, 2019).

In the present study, the inhibition of these targeted pathogens was evaluated by using ANN and ANFIS models during fermentation and storage days of dairy kefir. The activity of all selected foodborne pathogens

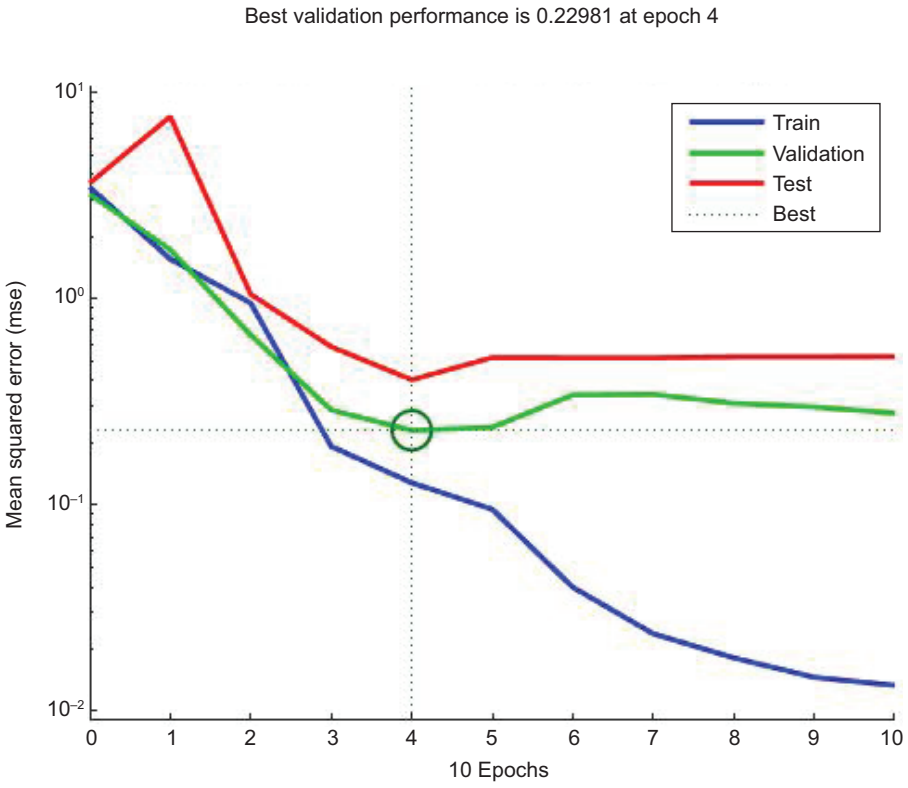

Figure 2. Plot performance graph of ANN model prediction for the inhibition of *L. monocytogenes* (in $\log_{10}$ CFU/g) using probiotic dairy product kefir.

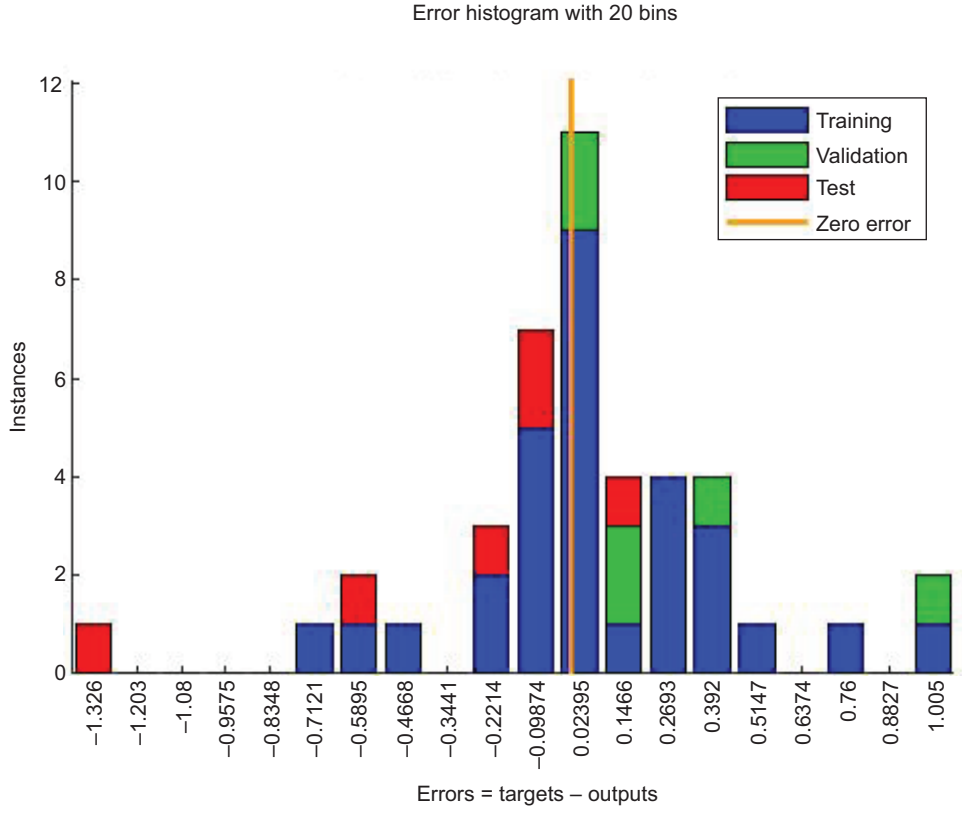

Figure 3. Histogram of ANN model prediction for the inhibition of *L. monocytogenes* (in $\log_{10}$ CFU/g) using probiotic dairy product kefir.

Table 3. ANFIS model inhibition of *L. monocytogenes* by probiotic dairy kefir (in log_{10} CFU/g).

Days	LAB control	Tested LAB	*L. monocytogenes*
Training stage			
FD0	3.22	3.15	3.223
FD1	4.1	3.9478	3.2745
FD2	4.96	4.6715	2.7313
SD1	5.1	4.816	2.608
SD3	5.2	4.8571	2.6016
SD7	5.29	4.9144	1.8703
SD10	5.4	5.0067	1.3871
FD0	4.2	4.0791	3.3261
FD1	5.1	4.8143	2.7302
FD2	5.86	5.5105	2.8393
SD1	5.98	5.6223	1.6358
SD3	6.11	5.7867	1.6372
SD7	6.34	5.8936	1.2656
SD10	6.43	5.9725	1.5433
Average	5.2350	4.9316	2.3338
Testing stage			
FD0	4.18	4.1123	3.1567
FD1	4.97	4.611	2.8691
FD2	5.78	5.4321	1.6415
SD1	5.88	5.5083	1.8067
SD3	6.1	5.6885	1.9563
SD7	6.23	5.804	2.7218
SD10	6.41	5.8989	3.0079
Average	5.6500	5.2936	2.4514

Table 4. ANN model inhibition of *S. aureus* using probiotic dairy product kefir (in log_{10} CFU/g).

Days	LAB control	Tested LAB	*S. aureus*
Training stage			
FD0	3.22	3.099997	2.729985
FD1	4.1	3.860021	2.890063
FD2	4.96	4.574421	2.371017
SD1	5.1	4.601372	2.29078
SD3	5.2	4.870955	1.455232
SD7	5.29	5.011625	1.042168
SD10	5.4	5.051125	1.099865
FD0	4.2	4.051899	3.792911
FD1	5.1	4.601372	2.29078
FD2	5.86	5.495737	2.373361
SD1	5.98	5.599042	2.122545
SD3	6.11	5.728212	1.802943
SD7	6.34	5.947762	1.258809
SD10	6.43	6.015005	1.092137
Average	5.2350	4.8935	2.0438
Testing stage			
FD0	4.18	4.010105	3.69915
FD1	4.97	4.574777	2.370089
FD2	5.78	5.437892	2.496509
SD1	5.88	5.511534	2.336036
SD3	6.1	5.717981	1.828292
SD7	6.23	5.84888	1.503898
SD10	6.41	6.001234	1.12627
Average	5.8081	5.4447	2.0041

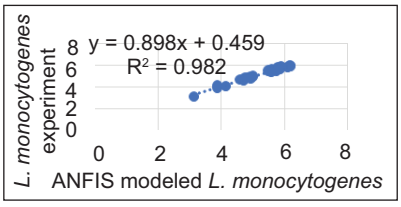

Figure 4. Scatter plots of ANFIS model prediction for the inhibition of *L. monocytogenes* using probiotic dairy product kefir.

was found to be inhibited by probiotic LAB present in dairy kefir, particularly during storage periods of kefir milk. Different research findings suggested that potential probiotic LAB isolated from various dairy products possess antimicrobial effects on foodborne pathogens, including those discussed in the present study (Mkadem *et al.*, 2023; Wu *et al., 2022*; Yang *et al.*, 2024a). For instance, the inhibitory activity of certain strains of LAB, such as *Lactobacillus rhamnosus* (Kamal *et al.*, 2018), *Lactobacillus paracasei* (Shahverdi *et al.*, 2023), *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Enterococcus faecium*, *Enterococcus faecalis*, and *Limosilactobacillus fermentum* (Roldán-Pérez *et al.*, 2023), on different foodborne pathogens, such as *E. coli* O157:H7, *S. aureus*, *Yersinia enterocolitica*, *Salmonella enterica serovar Typhimurium*, and other strains identified as *Lactobacillus plantarum* strains 4–10 (Yang *et al.*, 2024b), is taken as supportive findings for the present study.

Similarly, the findings of Mulaw *et al.* (2019) on the inhibitory activity of probiotic LAB against the growth/impact of *S. aureus* ATCC 25923, *L. monocytogenes*, and *E. coli* ATCC 25922 are evidences regarding the antimicrobial activity of LAB. More such types of probiotic bacteria are required to incorporate into foods as well as for developing functional foods. *In vitro* investigations revealed that LAB that survived in different bile concentrations (0.3% and 0.8%) (Paongphan *et al.*, 2023) and was tolerant to acid (Klimko *et al.*, 2020) exhibited antagonizing effect on the growth of foodborne pathogens. In addition to their inhibitory activity, probiotics are supplemented

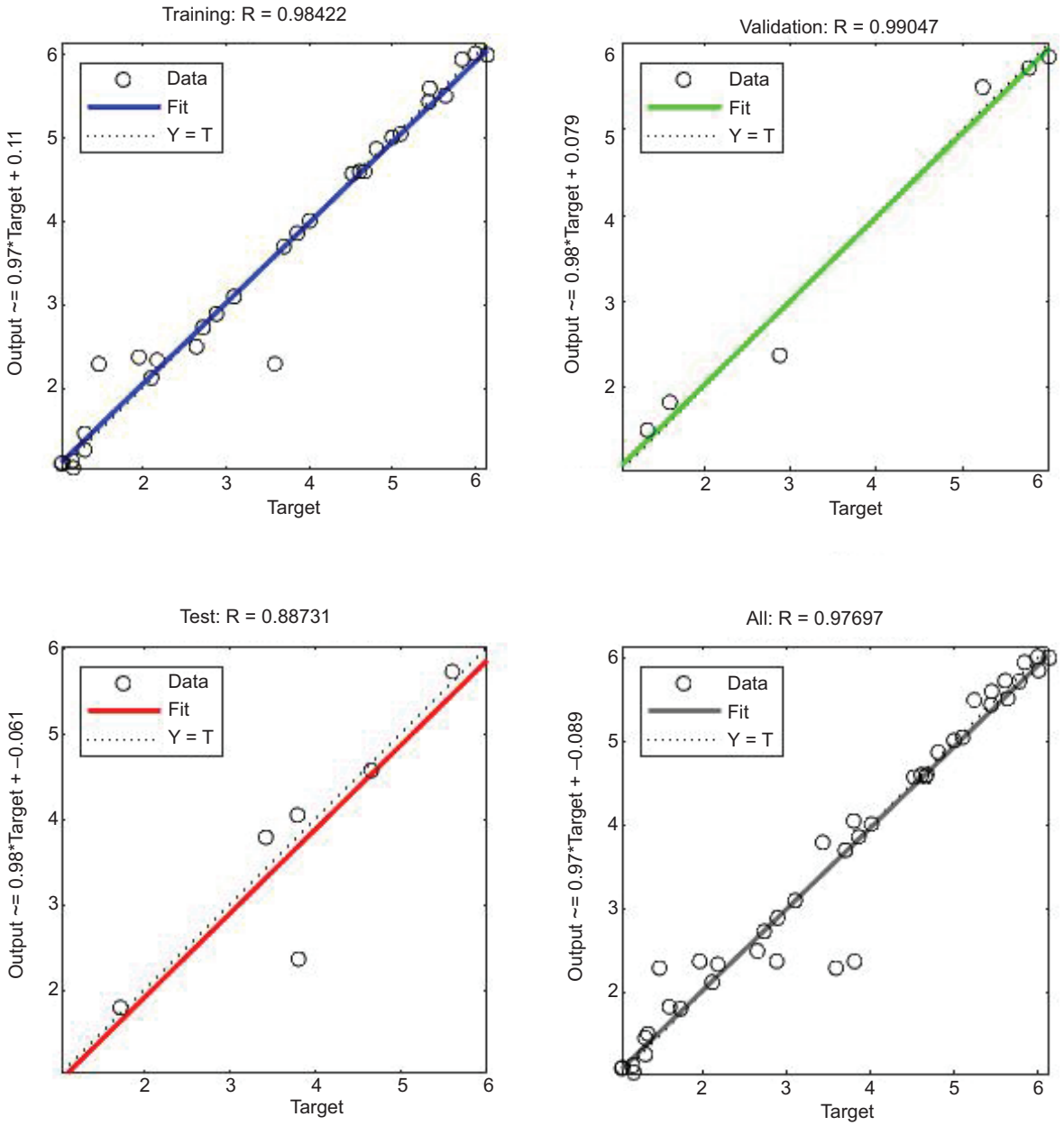

Figure 5. The regression graph of ANN model prediction for the inhibition of *S. aureus* (in $\log_{10}$ CFU/g) using probiotic dairy product kefir.

into foods to contribute to their organoleptic characteristics as well as to extend their shelf life during fermentation processes. Probiotics implement this function by maintaining their viability and efficacy. In the present study, the growth and activity of *L. monocytogenes* were tested by LAB present in dairy kefir from FD0 to FD2 and SD1 to SD10 by analyzing using both ANN and ANFIS models. The count of pathogens reduced from SD1 to SD10 at the training stage and from FD2 to SD10 at the testing stage of ANN model, while count of the same pathogens reduced for FD2–SD10 at the training stage and for FD1–SD10 at the testing stage of ANFIS model. Contrarily, the number of LAB in experimentally contaminated kefir with pathogens increased from day zero (0) of fermentation to day 10 of storage in refrigerator (Tables 2 and 3). The reduced number of pathogens indicated that the selected probiotic LAB in kefir milk antagonized the activity and growth of pathogens. Hence, the findings of the present study were in close agreement to the findings of Lim *et al*. (2020), who identified two strains of probiotic bacteria, *Leuconostoc mesenteroides* and *Lactobacillus curvatus*, isolated from *kimchi*, and were responsible to antagonize the growth of *L. monocytogenes*.

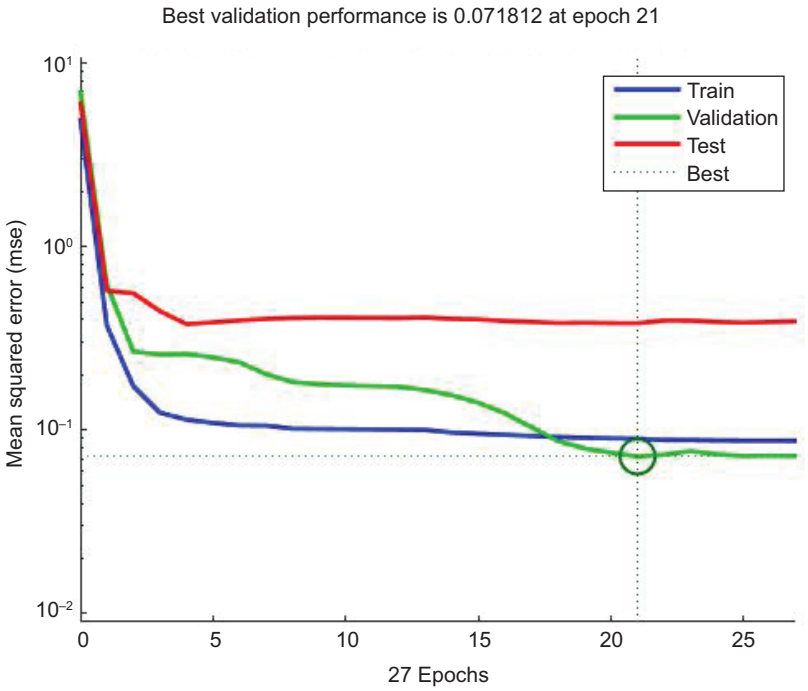

Figure 6. Plot performance graph of ANN model prediction for the inhibition of *S. aureus* (in $\log_{10}$ CFU/g) using probiotic dairy product kefir.

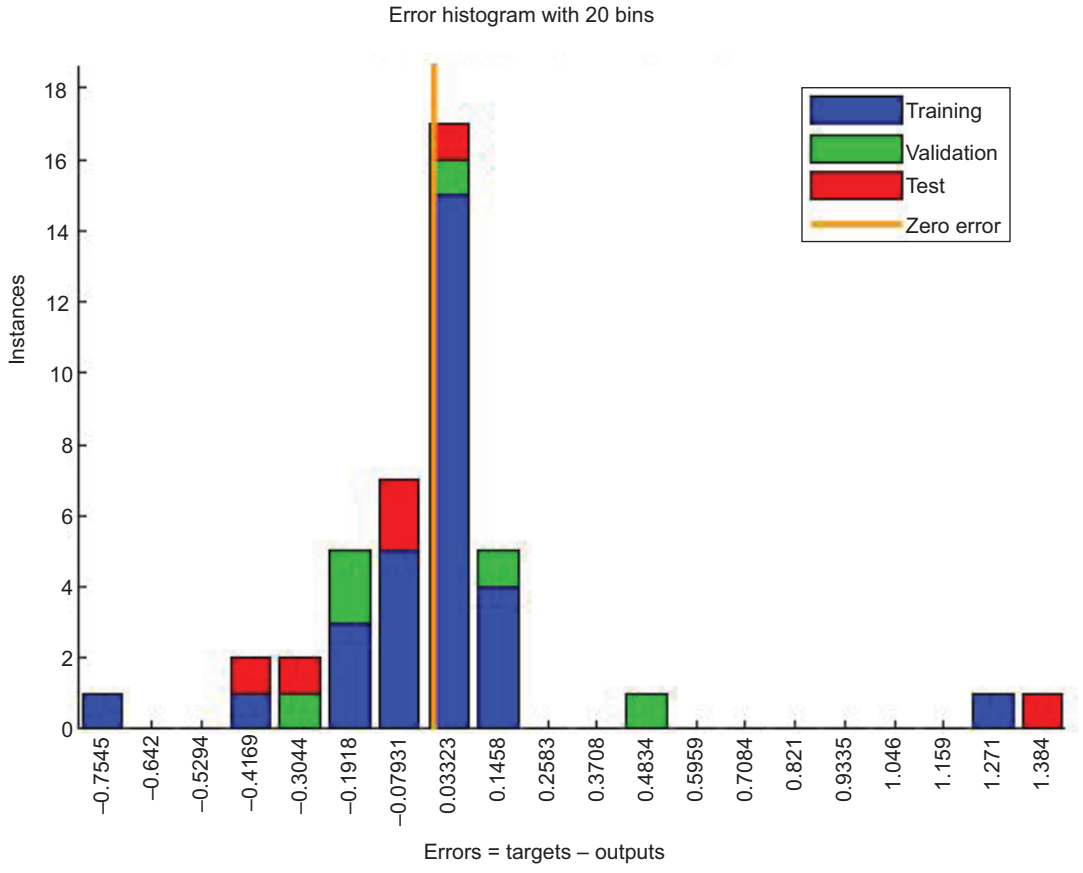

Figure 7. Histogram of ANN model prediction for the inhibition of *S. aureus* (in $\log_{10}$ CFU/g) using probiotic dairy product kefir.

Table 5. ANFIS model inhibition of *S. aureus* by probiotic dairy kefir (in log_{10} CFU/g).

Days	LAB control	Test LAB	*S. aureus*
Training stage			
FD0	3.22	3.1	2.73
FD1	4.1	3.7966	3.1944
FD2	4.96	4.5281	2.3997
SD1	5.1	4.6312	2.2107
SD3	5.2	4.8051	1.1803
SD7	5.29	4.951	1.1873
SD10	5.4	5.1009	1.2163
FD0	4.2	3.873	3.081
FD1	5.1	4.6364	2.6065
FD2	5.86	5.2431	2.9766
SD1	5.98	5.5245	1.3546
SD3	6.11	5.6451	1.6865
SD7	6.34	5.854	1.3672
SD10	6.43	5.9209	1.469
Average	5.2350	4.8293	2.0472
Testing stage			
FD0	4.18	3.8731	3.7969
FD1	4.97	4.6098	3.9406
FD2	5.78	5.3633	2.1166
SD1	5.88	5.4137	1.6937
SD3	6.1	5.5426	1.1974
SD7	6.23	5.5781	1.1467
SD10	6.41	5.7215	1.0504
Average	5.6500	5.1574	2.134

Table 6. ANN model inhibition of *E. coli* using probiotic dairy product kefir (in log_{10} CFU/g).

Days	LAB control	Tested LAB	*E. coli*
Training stage			
FD0	3.22	3.22781	2.61819
FD1	4.1	3.79129	3.17393
FD2	4.96	4.43822	3.23498
SD1	5.1	4.92613	2.95347
SD3	5.2	5.07217	2.88304
SD7	5.29	5.12174	2.85996
SD10	5.4	5.14246	2.84803
FD0	4.2	3.64604	3.42336
FD1	5.1	4.92613	2.95347
FD2	5.86	5.44944	2.14581
SD1	5.98	5.67452	1.62552
SD3	6.11	5.79773	1.36348
SD7	6.34	6.01353	1.25022
SD10	6.43	6.23813	1.20511
Average	5.2350	4.9618	2.4670
Testing stage			
FD0	4.18	3.68151	3.36319
FD1	4.97	4.48445	3.20556
FD2	5.78	5.30451	2.48313
SD1	5.88	5.49074	2.04982
SD3	6.1	5.79159	1.37418
SD7	6.23	5.87139	1.29047
SD10	6.41	6.18071	1.21626
Average	5.8081	5.4569	1.9261

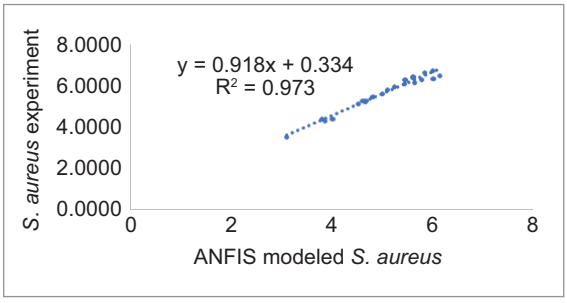

Figure 8. Scatter plots of ANFIS model prediction for the inhibition of *S. aureus* (in log_{10} CFU/g) using probiotic dairy product kefir.

In the present study, the average count obtained in LAB control, tested LAB, and *L. monocytogenes* was 5.23, 4.94, and 2.41 log_{10} CFU/g, respectively, at the training stage, while at the testing stage, the respective values were 5.80, 5.38, and 2.04 log_{10} CFU/g (Table 2). However, in a study conducted by Gökmen *et al*. (2022), the obtained counts of strains of LAB, namely lactobacilli and lactic streptococci, were 9.64–7.91 log CFU/mL and 9.64–8.69 log CFU/mL, respectively. This result indicates that there is observable variation in the number of probiotic LAB, compared to the findings of the present study. However, results of the present study closely agreed with the findings of Jara *et al*. (2020), who identified the potential of probiotic *Lactobacillus fermentum* MP26 and *Lactobacillus salivarius* MP14, both inhibiting the activity and growth of *Listeria monocytogenes.* In addition, in the investigation conducted by Morandi *et al*. (2020), the inhibitory activity of LAB against *L. monocytogenes* in Gorgonzola cheese indicated counts of the pathogen as <2.0 log_{10} CFU/g, and this result was close to the result obtained during storage period at both training and testing stages of the present study.

In a previous study, the inhibitory effect of *Lactobacillus rhamnosus* in probiotic Minas Frescal cheese against *L. monocytogenes* was reported as 1.1–1.6 log CFU/g (Prezzi *et al*., 2020); this result was similar to the result obtained

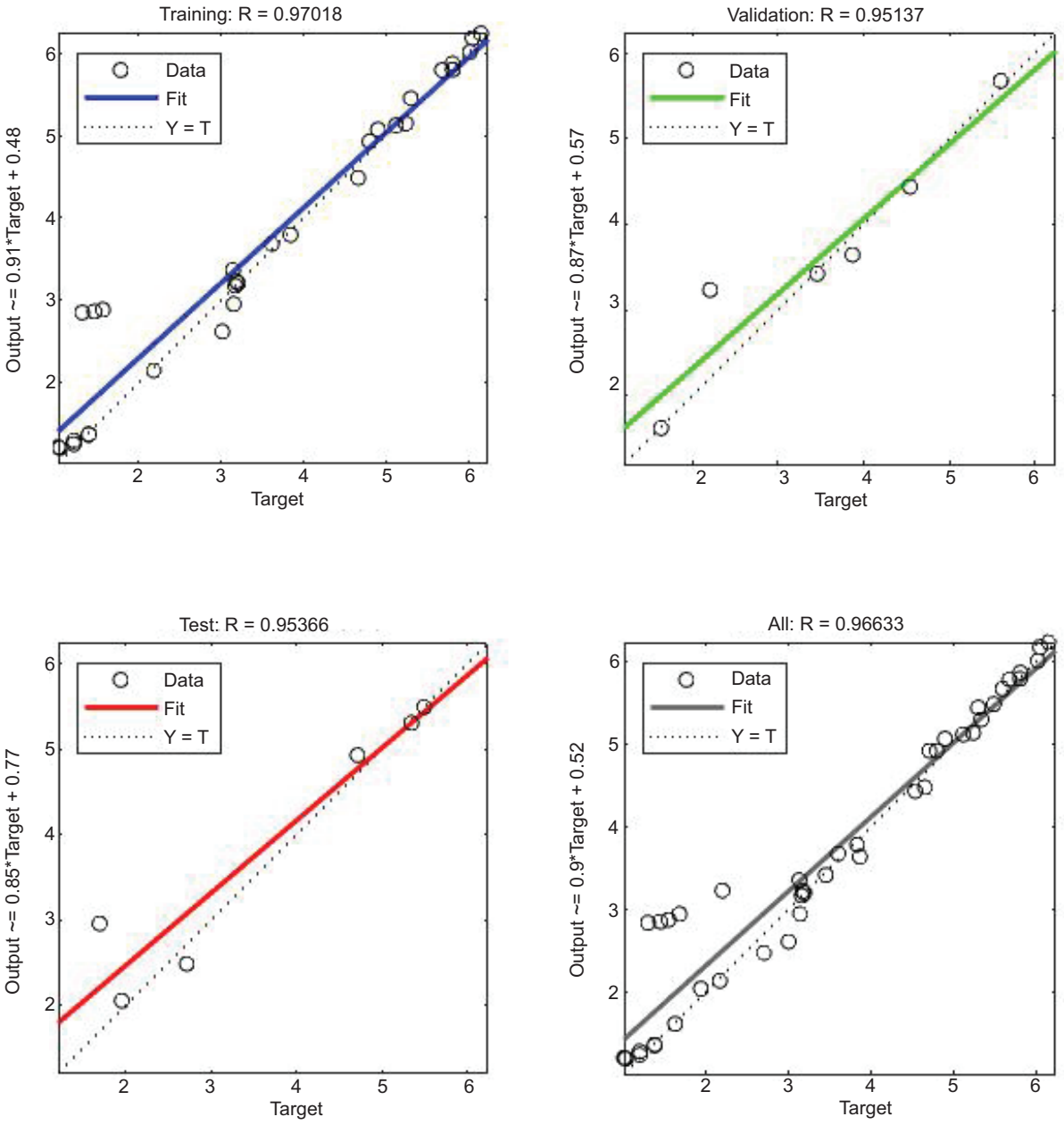

Figure 9. The regression graph of ANN model prediction for the inhibition of *E. coli* (in $\log_{10}$ CFU/g) using probiotic dairy product kefir.

in the present study at SD1 (1.6 $\log_{10}$ CFU/g) and SD3 (1.6 $\log_{10}$ CFU/g) with ANFIS model, and at SD10 (1.18 $\log_{10}$ CFU/g) with ANN model. The inhibitory potential of probiotic LAB was further confirmed on biofilm-forming pathogenic *L. monocytogenes* present in vegetables and in the food industry without any risk to consumers (Hossain *et al.*, 2020). Moreover, Martín *et al.* (2022) suggested that the strain of *Lactiplantibacillus plantarum* B2 alone or combined with *Lactiplantibacillus* spp. B4 are good candidates against growth of *L. monocytogenes* in traditional soft cheese obtained from dairy milk during their refrigerated storage. The survival of some foodborne pathogens, including both Gram-positive and Gram-negative bacteria, in kefir produced by microbial level and pullulan was determined by Gökmen *et al.* (2022), of which *L. monocytogenes* was the most susceptible bacterium to the metabolites of LAB during storage, with maximum reduction of pathogen after 24-h fermentation at 30°C.

The other targeted foodborne pathogen involved in the present study was *S. aureus.* The microbiological profile of this pathogen was evaluated and analyzed by both ANN and ANFIS models at specified time intervals as

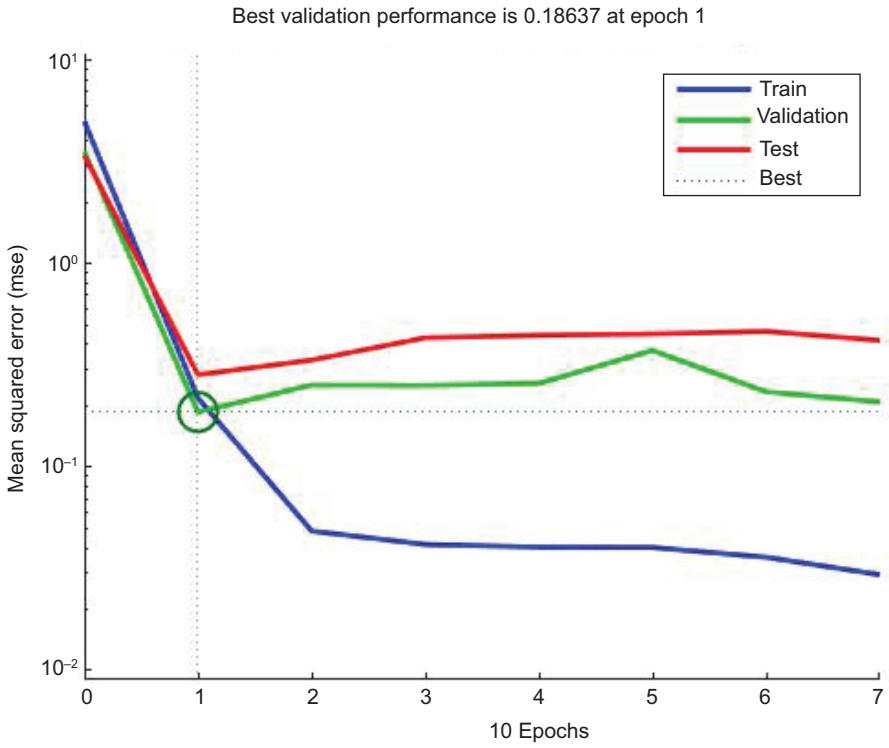

Figure 10. Plot performance graph of ANN model prediction for the inhibition of *E. coli* (in $\log_{10}$ CFU/g) using probiotic dairy product kefir.

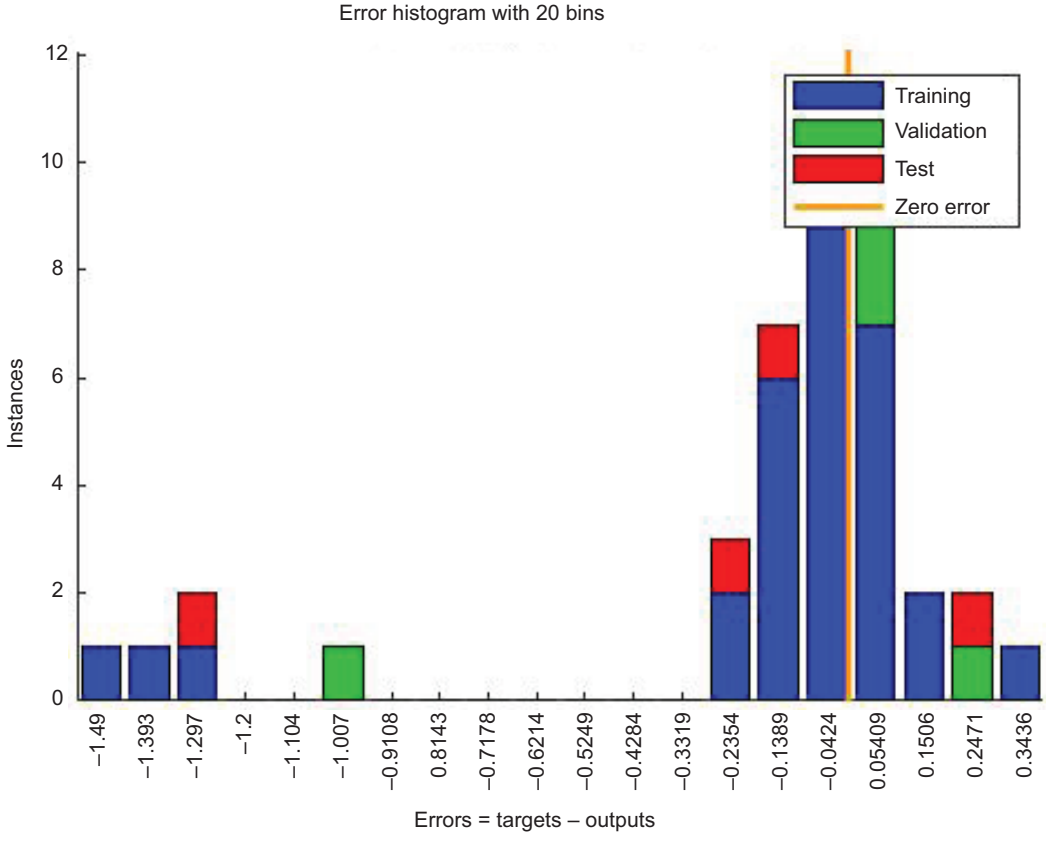

Figure 11. Histogram of ANN model prediction for the inhibition of *E. coli* (in $\log_{10}$ CFU/g) using probiotic dairy product kefir.

Table 7. ANFIS model inhibition of *E. coli* using probiotic dairy kefir (in log_{10} CFU/g).

Days	LAB control	Tested LAB	*E. coli*
Training stage			
FD0	3.22	3.1800	3.0100
FD1	4.1	3.8091	3.3488
FD2	4.96	4.6054	2.5160
SD1	5.1	4.7106	1.9043
SD3	5.2	4.9028	1.4241
SD7	5.29	5.0724	1.4142
SD10	5.4	5.2434	1.4378
FD0	4.2	3.9059	3.2552
FD1	5.1	4.7187	2.7691
FD2	5.86	5.3117	2.2918
SD1	5.98	5.6904	1.2978
SD3	6.11	5.8206	1.3768
SD7	6.34	6.0181	1.2115
SD10	6.43	6.0909	1.2226
Average	5.2350	4.9343	2.0343
Testing stage			
FD0	4.18	3.9910	2.1385
FD1	4.97	4.6777	1.4653
FD2	5.78	5.5236	1.3392
SD1	5.88	5.5680	1.2989
SD3	6.1	5.6617	1.1773
SD7	6.23	5.6334	1.0062
SD10	6.41	5.7466	1.0029
Average	5.6500	5.2574	1.3469

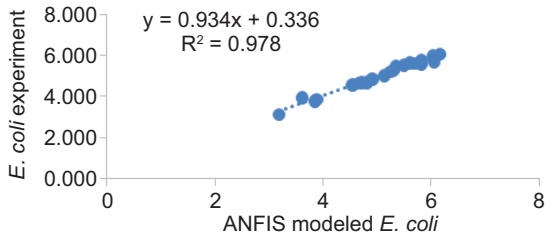

Figure 12. Scatter plots of ANFIS model prediction for the inhibition of *E. coli* (in log_{10} CFU/g) using probiotic dairy product kefir.

displayed in Tables 5 and 6. The analysis was also carried out for the evaluation of LAB control and test LAB for both fermentation and storage periods. Based on the analysis with ANN model, the average number of *S. aureus* was found as 2.04 log_{10} CFU/g, while the number of LAB control and test LAB was 5.23 log_{10} CFU/g and 4.89 log_{10} CFU/g, respectively, at the testing stage whereas with ANFIS model, the average number of *S. aureus* was recorded as 2.04 log_{10} CFU/g, while the number of LAB control and test LAB was 5.23 log_{10} CFU/g and 4.82 log_{10} CFU/g, respectively.

Recently published articles have indicated that the potential of LAB to inhibit the growth of *S. aureus* has been confirmed in various food products, such as cheese, yoghurt, kefir, and milk. Jiang *et al*. (2022) reported that strains of LAB isolated from traditional fermented yoghurt were found to have antibacterial and antibiofilm activity against *S. aureus*, indicating that the obtained result was similar to the finding of the present study. Likewise, the application of probiotic LAB to decrease the growth of *S. aureus* in the co-culture of sheep's milk reported by Rodríguez-Sánchez *et al*. (2022) was nearly similar to the findings of the present study. In contrary to the present study, Prezzi *et al*. (2020) indicated that the efficacy of probiotic *Lactobacillus rhamnosus* isolated from Minas Frescal cheese during the storage period of 21 days at 7°C was not observed against pathogenic *S. aureus*

In the present study, the growth and activity of *S. aureus* was evaluated by testing in LAB of dairy kefir during fermentation days (FD0–FD2) and storage days (SD1–SD10) with both ANN and ANFIS models. As a result, count of the pathogen was found to decrease from FD2 to SD10 at the training stage and from FD1 to SD10 at the testing stage with ANN model (Table 5). On the other hand, the number of *S. aureus* reduced from FD2 to SD10 at both training and testing stages with ANFIS model (Table 6). Contrarily, the number of test LAB and LAB control increased from FD0 to SD10 during the refrigerated storage of experimental kefir. The inhibition of *S. aureus* could involve different metabolites of LAB, which are the most important compounds that inhibit the growth of undesirable microorganisms, particularly *S. aureus*, in food and pharmaceutical industries (Nataraj *et al*., 2021). Thus, the inhibition of *S. aureus* enhances food safety and hygiene to ensure the health of consumers.

Different studies have revealed that strains of probiotic LAB isolated from dairy food products possess antagonizing activity against *S. aureus*, particularly in food production environments (Folliero *et al*., 2022; Jiang *et al*., 2021; Nataraj *et al*., 2021; Tarique *et al*., 2022). For example, the efficacy of probiotic *L. brevis* gp104, which was isolated from Iranian traditional cheese, had a promising potential against the growth of *S. aureus*, and has potential health benefits for its application as a novel biotherapeutic and biopreserving agent (Hojjati *et al*., 2020). Similarly, the antagonizing activity of some probiotic LAB isolated from traditional high acid and low moisture yogurt-like products, including *Streptococcus thermophilus*, *Lactobacillus delbrueckii*, *Enterococcus faecium*, and

Lacticaseibacillus rhamnosus, was reported against *S. aureus* in a study conducted by Tarique *et al*. (2022).

Moreover, in the present study, the inhibition of *S. auerus* was also confirmed by regression analysis at training, validation, and testing stages, with respective R = 0.9842, 0.9905, 0.8873, as indicated in Figure 6. In addition, the model reflected the evaluation of *S. auerus* with the best validation performance of 0.071812 at epoch 21 (Figure 7). Evaluation of the inhibitory activity of potential probiotic LAB of dairy kefir against *S. aureus* was also analyzed with ANFIS model as described in Table 6. The findings showed that reduction in the count of the targeted pathogen was observed from FD2 to SD10 at both training and testing stages whereas the number of LAB increased during the above-stated days. In this analysis, reduction in the count of *S. auerus* was high during storage days than during fermentation days of the experimental kefir. A study conducted by (Yan *et al*. (2019) confirmed that several strains of LAB exhibited antagonizing potential against foodborne pathogens; among of these strains were *Pediococcus acidilactic* and *Lactococcus plantarum*, both being promising probiotics against *S. aureus* CMCC 26003. Thus, the findings of the cited investigation were in close agreement to the results of the present study.

The inhibition of *E. coli* by probiotic LAB present in dairy kefir was analyzed using ANN model, with reduced number of pathogen from FD2 to SD10 whereas an increment in the number of LAB was observed from FD0 to SD10 (Table 7) at the training stage. Likewise, decrease in the number of *E. coli* was observed from FD0 to SD10 at the testing stage. However, the average result obtained for LAB control, tested LAB, and *E. coli* was 5.23, 4.96, and 2.46 $\log_{10}$ CFU/g, respectively, at the training stage whereas for the testing stage, the respective values were 5.80, 5.45, and 1.92 $\log_{10}$ CFU/g, as displayed in Table 7. Findings of the study conducted by Esfandiari *et al*. (2024) regarding the inhibitory activity of probiotic LAB against *E. coli* were in close agreement with the findings of the present study. Similarly, investigation done by de Amorim Trindade *et al*. (2022) and Darvishi *et al*. (2021) indicated that the strains of LAB exhibited probiotic potential against the growth of *E. coli* and other foodborne pathogens. Thus, it is obvious that the effects of various probiotic bacterial strains against the growth and activity of foodborne pathogenic bacteria, usually presenting in food products and the environment, are documented in different food processing plants. The strains of *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, and *Enterococcus* are also responsible for inhibiting the growth of biofilm-forming *E. coli* and are applied as bio-control agents against spoilage and pathogenic bacteria in meat and meat products (Abouloifa *et al*., 2023; Darvishi *et al*., 2021).

In addition, inhibition of *E. coli* was also braced by regression analysis, with R = 0.9702, 0.9514 and 0.9537 at training, validation and testing stages, respectively. The best validation performance for the inhibition of *E. coli* was obtained at 0.18637. Similarly, the inhibition of pathogenic *E. coli* by biological means, namely probiotic LAB, which are naturally present in dairy kefir milk and used in the present study, was analyzed with ANFIS model. The model simulated with inhibition of *E. coli* at training stages with the average number of LAB in control (5.23 $\log_{10}$ CFU/g), tested LAB (4.93 $\log_{10}$ CFU/g), and *E. coli* (2.03 $\log_{10}$ CFU/g). Likewise, the average value of LAB control, tested LAB, and *E. coli* obtained at the testing stage were 5.65, 5.25, and 1.34 $\log_{10}$ CFU/g, respectively. In this model, inhibition of the activity of *E. coli* was observed along with the fermentation and storage days of kefir milk, which was confirmed by the reduced number of the pathogen from FD2 to SD10.

E. coli is among the well-known and most serious foodborne bacteria, causing severe health problems to consumers through adherence to the mucosal membrane of host's intestines. To combat the activity of this pathogen, the application of probiotic food products, such as dairy products, is more reliable, as demonstrated in various studies. As evidenced, the strains of some probiotic LAB, reported in previous studies, including *Lactobacillus sakei*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Lactobacillus fermentum*, and *Lactobacillus casei*, have an antagonizing activity toward pathogenic *E. coli* (Darvishi *et al*., 2021; de Amorim Trindade *et al*., 2022; Hansen *et al*., 2021). These strains of LAB possess desirable potential for passing through the low pH of the stomach and entering the intestines to inhibit the adherence activity of infectious *E. coli* (Behbahani *et al*., 2019). In the present study, the inhibition of *E. coli* was more observable during the storage period of dairy kefir stored at 4°C. This finding was in close agreement with the investigations of Choi *et al*. (2021), who confirmed the antibacterial influence of probiotic *Leuconostoc mesenteroides* (KCTC 13374) and *Lactobacillus plantarum* (KCTC 33133) isolated from commercially manufactured *kimichi* during fermentation at respective temperatures of 10°C and 25°C.

Conclusions and Recommendations

The present study was conducted on the inhibition of pathogenic foodborne pathogens, viz. *L. monocytogenes*, *S. aureus*, and *E. coli*, by probiotic LAB present naturally in dairy kefir using artificial intelligence models (ANN and ANFIS). The activity and growth of these foodborne pathogens was repressed by LAB present in dairy kefir. Thus, probiotic dairy kefir products are biological controlling means that can be applied to both food industry

and agricultural sector. Based on the present study, the antibacterial activity of probiotic LAB in kefir was more observable during storage period than fermentation period. Therefore, based on the results, the following steps are recommended: first, more research work must be emphasized on the investigation of kefir as a potential probiotic antagonizing the activity of serious foodborne pathogens. Second, Artificial Intelligence-based approaches for the inhibition of targeted pathogens could be a baseline for more attention to the research.

Author Contributions

BC Kefyalew and BH Ulusoy designed the study. BC Kefyalew and F Kaya Yıldırım conducted the data analysis. BC Kefyalew drafted the manuscript and F Kaya Yıldırım revised the same. All authors read and approved the final manuscript.

Data Availability

The datasets used in this study are available from corresponding authors upon reasonable request.

Conflicts of Interest

The authors reported no conflict of interest.

Funding Statement

Not applicable

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