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Artificial neural network and response surface methodology for modeling and optimization of activation of lactoperoxidase system

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ABSTRACT

In the present study, the multi-component lactoperoxidase system (LPS) is used for improving milk safety and requires thiocyanate (SCN^-) as a substrate for the generation of antimicrobial hypothiocyanite ($OSCN^-$). The influence of four independent variables for activation of lactoperoxidase system on the improving the quality of raw goat milk were investigated and optimized using an artificial neural network and response surface methodology on the growth of total coliform count and bacterial count. The two models' predictive capabilities were compared in terms of root mean square error, mean absolute error, standard error of prediction, absolute average deviation, and coefficient of determination based on the validation data set. The results showed that properly trained artificial neural network model is more accurate in prediction than the RSM model. The optimum conditions were a temperature of 25 °C, storage time of 10 hr, NaSCN of 30 ppm and hydrogen peroxide of 18 ppm. For these conditions, an experimental total coliform count of 4.51×10^2cfu/mL and total bacteria count of 5.44×10^4cfu/mLwas obtained, which was in reasonable agreement with the predicted content.The results indicate that the model is in substantial agreement with current research, and activating the LP System can extend the storage period of goat milkfor up to10hr when stored at 25 °C.The results revealed no significant differences in milk composition (protein content, fat content, lactose content, total solids, moisture content and ash content) were observed among activated and control goat milk samples.

1. Introduction

Milk is one of the foremost valuable foods regularly consumed among people. Thanks to high nutrient composition, milk production has been popular and played a crucial role in global food security (Naing et al., al.,2019). Milk and milk products have made very significant contributions to human nutrition ever since the earliest civilizations. However, there are wide variations within the traditional role of milk within individuals' diet in several subcontinents (Seifu et al., al.,2005). Thanks to its high nutritional value, raw milk provides as a good medium for microbial growth that degrades the milk quality and shelf life. This situation forces producers to look for alternative methods for raw milk preservation that is simple to use, relatively inexpensive and not pose any risk for thebuyer. And also protect milk from spoilage for periods long enough to the processing plants (Najim, 2015).

The growth of contaminants in milk poses a threat to consumer health, whereas the rapid spoilage reduces the milk's market price, resulting in income losses to both producers and vendors (Njage et al., 2010). The collection and transportation of milk in developing countries presenta variety of technical and organizational problems. Chemical preservation of milk would be the simplest solution to the present problem (Jandal, 1996). The combination of poor hygienic standards, high ambient temperatures and lack of refrigeration facilities render raw goat milk considerably vulnerable to spoilage due to common lactic acid bacteria (Murigu et al., 2008).In seeking to address the present problem, an alternate way to enhance milk storage at high ambient temperatures has been developed (Nigussie and Seifu, 2007).

The method that would help farmers preserve their milk during storage and transportation of raw goat milk to the dairy processing plant would help minimize milk spoilage andenhances its utilization. The lactoperoxidase system (LPS) (LP thiocyanate- H_2O_2) is a natural antimicrobial system present in milk (Abbes et al., 2018), which is activated by increasing the concentrations of two components or activators (thiocyanate and hydrogen peroxide) to promote the reacting with one

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another. This reaction is catalyzed by the enzyme lactoperoxidase, which is present in milk and results in the formation of antibacterial compounds (Seifu et al., 2005). The LPS has been recognized as crucial temporary preservation of raw milk by inhibiting spoilage and provides resistance to pathogenic microorganisms(Zapico et al., 1995).

The LPS consists of three compounds; the enzyme lactoperoxidase, hydrogen peroxide (H_2O_2), andthiocyanate ($SCN-$), and is active only in the presence of all three components (Chiraz et al., 2013; Seifu et al., 2005). Antimicrobial activity occurs in a LPS when three components are present, thus acting as a natural inhibitor in milk and affecting many gram-positive and gram-negative microorganisms (Althaus et al., al., 2001). Its use has been recommended as an antimicrobial agent for preserving food for safe human consumption (Jasour et al., 2015).

Research reports indicate that the LPSis amongst the most cost-effective approaches to extend the stability of pasteurized and raw milk quality by adding thiocyanate and hydrogen peroxide (Musa et al., 2013; Seifu et al., 2004, 2005). This LPS antimicrobial effect might be of great interest in milk preservation, especially in areas where refrigeration is not readily available and for economic reasons (such as in developing countries). In addition to this, improved the bacteriological quality of refrigerated milk (Althaus et al., 2001).

The RSM is a statistical, mathematical toolwidely employed to examine multiple regression analysis usingquantitative data obtained from appropriate experiments to determine and simultaneously solve multi variant equations. In recent years, artificial neural network (ANN) has arisen as an efficient and attractive approach for nonlinear multi-factor modeling due to its generic structure and ability to learn from historical data (Yang et al., 2015).ANN is a powerful mathematical method suitable for modeling and simulation of various processes in real engineering applications. Optimization technique offers several advantages over conventional modeling techniques because they can model based on no assumptions concerning the nature of the phenomenological mechanisms and understanding the mathematical background of the problem (Maran et al., 2013). ANN has already been applied in food science, biochemical, and food engineering to simulate processes such as papaya (Maran et al., 2013), fermentation of vitamin C (Yang et al., 2015), pomegranate juice and coconut oil ethyl ester (Samuel et al., al., 2018).

In the present study, RSM and ANN linked genetic algorithm-based models have been developed to predict the relationship between the input variables (temperature, storage time, sodium thiocyanate and hydrogen peroxide) and the output variables. Subsequently, the results predicted by the ANN and RSM techniques were compared statistically to the coefficient of determination (R^2), root means square error (RMSE),mean absolute error (MAE), standard error of prediction (SEP %), and absolute average deviation (AAD%) based on the validation data set for their predictive and generalization capabilities. Many researchers have evaluated LPS on maintaining the quality of milk, however, there were no reporting on the detailed analysis of variables for activation of the LPS on the milk by coupling RSM and ANN methodology. A useful RSM model and a feedforward neural network on back-propagation were developed utilizing the experimental data, and the efficiency of both models were compared. Ultimately the main objective was to find the best operational condition for the activation of the LPS that yields minimum growth of total coliform count (TCC) and total bacteria count (TBC).

2. Materials and methods

The raw goat milk used for this study was collected from the Ilica area of Bishoftutown, Ethiopia. Milk samples were collected in the early morning (7:00 –7: 30 am) to ensure freshness just after the milking was done. Samples were kept in sterilized bottles and transported to laboratories in iceboxes at 4 °C before being prepared for analysis. Prior to experimentation, the sample was tested for freshness by using the alcohol test, clot-on-boiling test, titratable acidity test and pH value. Freezing point test and bacterial load was determined according to the standard methods (Table 1). Upon arrival, the samples were divided into two:non-LPS treated and LPS activated. The activation of LPS was carried out at different temperatures, storage time, sodium thiocyanate, and hydrogen peroxide.

2.1. Chemical analysis of the milk samples

The milk samples' freshness, physicochemical composition (fat, total solid, solids not fat, moisture, protein, ash and lactose) of the milk were determined according to the standard methods described by AOAC Official methods of analysis (Table 1).

2.2. Method for activation of the lactoperoxidase system

The activation of LPS of milk samples was carried out by the methods proposed by Musa et al.(2013). After the analyses for milk freshness, lactoperoxidase was activated by adding (15 - 30) ppm sodium thiocyanate (NaSCN) as a source of *SCN*. After 1 min of thorough mixing approximately (10 - 20) ppm of hydrogen peroxide was added to the100 mlmilk sample, respectively.Then chemical and microbiological analyses were carried out during a storage period of (2 – 10) hr at 19 – 43 °C.

2.3. Microbiological analysis

The microbiological analysis was carried out at the dairy laboratory of the Ethiopian Meat and Dairy Industry Development Institute and Jije laboratory, Ethiopia. Samples of raw goat milk were analyzed for TBC and TCC was performed according to the standard procedures described byMarshall, RT (1992).

2.4. Response surface methodology modeling

In the present work, activation of the LPSwas studied to determine the optimized conditions for the minimum growth of TCC and TBC. The influence of temperature, storage time, sodium thiocyanate, and hydrogen peroxide were determined through RSM i.e. central composite design (CCD) was selected requiring a total of 30 experimental runsto determine the best combination of parameters for the activation of LPS. The responses and the process variables were modeled and optimized using analysis of variance (ANOVA) to predict the statistical parameters. The independent process variables ranges were selected basedon the work by Haddadin et al. (1996).Generally, CCD involves sixteen factorial points, eight axial points and six points at the center were carried out with the alpha factor of 1.414. All factors have to be adjusted at five coded levels (-α, −1, 0, +1, + α)(Faraji et al., 2017). The variables were coded according to the Equation:

Table 1
Standards methods used for physicochemical, microbiological and freshness analysis of raw and LP activated goat milk.

S. No.	Parameters	Methods
1	Alcohol test	(Connor, 1995)
2	Clot-on-boiling test	(Connor, 1995)
3	Titratable acidity test	(AOAC 947.05, 2006)
4	pH value	(AOAC 947.05, 2000)
5	Freezing point test	CTS 570,538 (1998)
6	Fat content	(AOAC 920.39, 2000).
7	Total solid content	(AOAC, 2000)
8	Moisture content	(AOAC, 2000)
9	Solids, not fat (SNF)content	(AOAC, 2000)
10	Protein content	(AOAC, 2000)
11	Ash content	(AOAC, 2000)
12	Lactose content	(AOAC 920.39, 2000).
13	Total bacterial count	APHA, Marshall, RT(1992)
14	Total coliform count	APHA, Marshall, RT(1992)

$$N = 2^n + 2n + nc = 16 + 2 \times 4 + 6 = 30 \quad (1)$$

where N is the total number of experiments required, n is the number of variables, and n_c is the number of replicates. The relationship of the variables and response was calculated by second-order polynomial multiple the quadratic regression equation.

$$Y = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} x_i x_j \quad (2)$$

where Y is the predicted response (i.e., TCC and TBC), n is the number of independent variables, b_0 is the constant coefficient, b_i is the linear coefficients, b_{ij} is the second-order interaction coefficients, b_{ii} is the quadratic coefficients and x_i, and $x_j$are the coded values of the independent variables.

The results were summarized and statistically analyzed using Design-Expertversion 11software (Stat-Ease Inc., Minneapolis, USA) and Minitab 17. The ANOVA test was employed to estimate the statistical significance of the regression model.In the quadratic polynomial, non-significant terms ($p > 0.05$) were deleted, and a new polynomial was recalculated to obtain a predictive model for each dependent variable (Shu et al., 2016). The coefficient of determination R^2, adjusted R^2, and predicted coefficient R^2, lack of fit from ANOVA, were used to determine the quality of the developed model. The numerical optimization technique was applied for the optimization of multiple responses. All the independent variables were kept within range, while the responses were set to the minimized. The desirability function method was applied for generating optimum conditions with specific desirability values. The experiments were carried out in triplicate.

2.5. Artificial neural network modeling

In the present study, the ANN was developed to describe the activation of LPS to enhance the shelf life of milk. The data generated from the experimental design planned through CCD (Table 3) were used to constitute the optimal architecture of ANN.ANN has been applied for simulation on the same experimental data used for RSM. ANNs consists of highly interconnected and elementary processors called neurons which are a computational structureinspired by biological neural systems (Chamoli, 2015).

In the current study, the tan-sigmoidal transfer function was applied between the input layer and the hidden layers and purely was applied between the hidden andouter layers.The neural network architectures were trained by the Levenberg–Marquardt back-propagation algorithm. The network architecture consisted of an input layer of four neurons (temperature, NaSCN, storage time, and H_2O_2 concentration), an output layer of two neurons (TCC and TBC), and a hidden layer. Eighty percent of data points selected for training to develop the neural network, 10% of the data set used for validation and 10% for testing. It is clear that more data sets in training reduce processing time in ANN learning and improve models' generalization capability (Sildir et al., 2020). This makes possible the assessment of the generality of the ANN model.The number of neurons in the hidden layer can be calculated from the following expression.

$$2(n + m)^{0.5} \text{to } 2n + 1 \quad (3)$$

where n is the number of neurons in the input layer and m is the number of neurons in the output layer (Valente et al., 2014). Each networkis trained separately, and therefore,the best network is selected based on the accuracy of the predictions within the testing phase. The network inputs and target have been normalized before training. The individual ANN nodes' input and target data were normalized within a range of 0 (new x_{min}) to 1(new x_{max}) to achieve fast convergence to obtain the minimal RMSE values. The following equation ensures uniform attention during the training process.

$$x_{norm} = \frac{x_i - x_{min}}{x_{max} - x_{min}} (new\, x_{max} - new\, x_{min}) + new\, x_{min} \quad (4)$$

where x_{norm} is the normalized data, x_i is the input/output data (data of independent and dependent variables), x_{max} and x_{min} are the maximum and minimum values of the particular variable, respectively.

The normalization of inputs and target was performed to avoid overflows that may appear due to very large or minimal weights. The training process was run until a minimum of the MSE was reached in the validation process. The trained network's performance was estimated based on the accuracy of the network with the test data.Feed forward with backward propagation neural network wasused in the current study. All calculations were done using the Neural Network Toolbox of MAT LAB version 8.1(R2013a) (Razmi-rad et al., 2007).

2.6. Comparative analysis of RSM and ANN models

ANN and RSM models' performance was compared by using the RMSE, MAE, R^2, SEP, and AAD were calculated between experimental and predicted data. The formula used for error analysis was calculated by equation (5) to (9)(Sarve et al., 2015).

$$SEP = \frac{RMSE}{Y_e} \times 100 \quad (5)$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n} (Y_{i,p} - Y_{i,e})^2}{\sum_{i=1}^{n} (Y_{i,p} - Y_{i,e})^2} \quad (6)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (Y_{i,e} - Y_{i,p})^2}{n}} \quad (7)$$

$$MAE = \sum_{i=1}^{n} \left(\frac{Y_{i,e} - Y_{i,p}}{n} \right) \quad (8)$$

$$AAD = \frac{100}{n} \sum_{i=1}^{n} \frac{|Y_{i,p} - Y_{i,e}|}{|Y_{i,e}|} \quad (9)$$

where, Yi,e is the experimental data, Yi,p is he predicted data obtained from either RSM or ANN, Ye is the mean value of experimental data, and n is the experimental data. The final network was selected based on the lowest training error and depending upon the test data.

3. Result and discussion

3.1. Freshness analysis of raw goat milk

The freshness of the raw goat and preserved (LP activated) goat milk had a negative response they weren't solidified, clotting or precipitation using alcohol test and clot-on-boiling test (COB) up to 10 hr (Table 2). It showed that negative stability at 68% (v/v) alcohol concentration during testing.Alcohol test and clot-on-boiling testis an indicator that its freshness meets the quality standards for further processing.This supported the previous finding by Dajanta et al. (2008), who reported that alcohol stability can be used as a good indicator of milk freshness due to

Table 2
Comparison of freshness analysis of raw and LP activated goat milk.

Parameters	Raw goat milk	LP activated milk up to 10 hr
Alcohol test	-ve	-ve
Clot on boiling test	-ve COB	-ve COB
pH	6.6	6.52
Titratable acidity test	0.168	0.179
Freezing point	−0.52	−0.53

its reliable and consistent results. This result shows that the acid development by the conversion of lactose is low due to microbial inhibition by the LPS. LPS can keep goats 'milk fresh for up to 10 hr. but the control milk didn't coagulate or precipitate for to 4 h when stored at 25 °C.

According to FAO (2005), the inhibitory effect of the treatment is dependent on the temperature of the stored milk (30, 25, 20, and 15 °C) and keeps the quality of milk up to 7–8, 11–12, 16–17 and 24–26 hr. respectively. The experiment was carried out in different countries with raw milk of an initial good hygienic standard.

The current finding was in agreement with an earlier finding of (Prajapati et al., 2017), who reported that the alcohol test of raw goat, cow, and buffalo milk was negative at 68% v/v alcohol concentration. The alcohol test showed that the utilization of LPS treatment has significantly improved ($p < 0.05$) the shelf life of milk compared to untreated milk. The LPS treated milk stored at temperatures of 25 °C had additional 6hr shelf life compared to the untreated raw goat milk stored in similar conditions. This result indicated that the storage period had a significant ($P < 0.05$) effect on an alcohol test of the various treatments' milk samples.

The freezing point of raw goat milk was found to be −0.52 °C. The present study's value was significantly higher than the previous findings of Janštová et al. (2007). However, the current finding was in agreement with an earlier finding by Prajapati et al. (2017), who reported the freezing points of raw goat milk (−0.550 to −0.468 °C), cow milk (−0.564 to −0.516 °C) and buffalo milk (−0.584 and −0.532 °C). The value obtained in the LP-activated milk is comparable with the value reported by FAO (2005). The difference of these results may be due to many factors, such as the difference in initial microbial load of the goat milk, storage temperature, health status of the goat, and NaSCN and H_2O_2concentration.

According to the result obtained within this study, the pH of milk samples from milk producer households (6.6) was in the range of fresh cow milk (Table 2). These results agreed with the findings by Bendary et al. (2017) and Mahmood and Usman (2010), who reported the pH value of milk is 6.63–6.90. Above 6.8 shows mastitis of the milk, and pH values below 6.6 indicate increased milk acidity due to bacterial multiplication (Connor, 1995). In this study, a significant ($P > 0.05$) change in pH was recorded in the control samples, and LP-treated goat's milk was kept at 25 °C.

3.2. Effect of process variables on the microbial growth

The effects of process variables such as temperature, NaSCN, storage time, and H_2O_2 were examined as factors to investigate the correlation between the LPS variables and the growth of TCC and TBC by using CCD. The complete experiment variables design matrix together with the values of experimental responses was presented in Table 3. The ANOVA was carried out to investigate the model terms, select a suitable model, and detect the model equation's significances.

3.3. Statistical analysis

Table 4 shows the models Eqs. (10) and (11) and their significant coefficients of the responses, where all the models are significant at a level of less than 0.0001, thus confirming the adequacy of the fitted models. With these models, it is possible to predict the responses under any given experimental conditions within the limits of the variables studied.The statistical analyses show that quadratic models fit very well into the data for the response. The predicted quadratic model for the two responses was highly significant ($p < 0.0001$).The Model F value of 138.43 and 145.81 implies the model is significant for TCC and TBC growth, respectively. For total coliform count A, B, C, D, BC, CD, B^2, and D^2 were found to significantly affect the TCC, while A^2, C^2, AB, AC, AD, and BD were not significant. In the case of total bacteria count A, B, C, D, AC, BC,A^2, B^2 and D^2 were found to have a significant effect on the TBC, while C^2, AB, CD, AD, and BD were not significant (Table 4). The analysis of variance for the lack of fit test did not show the inadequacy of the model concerning the growth of TCC, and TBC ($P > 0.05$), indicating that the model could adequately fit the experimental data (Table 4). The

Table 3
Central Composite design matrix and experimental results.

Run	Coded variable					Decoded Variable			Dependent Variable	
	A	B	C	D	Temp(°C)	Time(hr)	NaSCN(ppm)	H_2O_2(ppm)	TCCcfu/mL	TBC cfu/mL
1	−1	−1	1	−1	25	8	30	10	763.6	78,986.0
2	1	1	1	1	37	8	15	20	1228.6	115,235.0
3	1	−1	−1	−1	31	6	22.5	15	720.6	80,238.0
4	1	1	1	−1	25	8	15	20	895.4	89,125.0
5	α	0	0	0	37	8	30	20	920.2	95,825.0
6	−1	−1	1	−1	31	6	22.5	15	740.9	78,285.0
7	1	1	−1	1	31	6	22.5	25	836.4	85,819.0
8	−1	1	−1	1	37	8	15	10	1210.0	119,789.0
9	0	0	0	0	37	8	30	10	1022.6	102,389.0
10	1	1	−1	−1	31	6	22.5	15	754.5	78,364.0
11	0	0	-α	0	25	8	15	10	924.9	93,123.0
12	−1	1	−1	−1	25	4	30	10	513.6	61,498.0
13	0	0	α	0	37	4	15	10	823.9	89,789.0
14	0	0	0	0	37	4	30	10	754.5	78,856.0
15	0	0	0	0	25	4	15	20	489.8	55,897.0
16	1	−1	−1	1	31	6	22.5	15	736.4	79,178.0
17	0	0	0	0	25	10	30	20	454.5	55,469.0
18	−1	−1	−1	1	31	6	22.5	15	746.8	79,569.0
19	0	0	0	0	19	6	22.5	15	468.9	53,125.0
20	−1	1	1	−1	31	6	22.5	5	988.7	95,129.0
21	-α	0	0	0	25	4	30	20	610.8	69,998.0
22	−1	−1	1	1	43	6	22.5	15	965.9	96,238.0
23	0	A	0	0	31	10	22.5	15	1121.0	111,689.0
24	−1	1	1	1	37	4	30	20	715.2	72,781.0
25	0	-α	0	0	37	4	15	20	824.9	85,636.0
26	−1	−1	−1	−1	31	6	22.5	15	711.9	76,892.0
27	1	−1	1	1	31	6	37.5	15	540.2	69,654.0
28	0	0	0	α	31	2	22.5	15	518.2	63,345.0
29	0	0	0	-α	31	6	7.5	15	898.6	95,151.0
30	0	0	0	0	25	4	15	10	487.9	59,102.0

Table 4
Analysis of variance for response surface quadratic model of TCC and TBC.

Source	TCC (cfu/mL) F-value	p-value	TBC (cfu/mL) F-value	p-value
Model	138.43	< 0.0001	145.81	< 0.0001
A-Temperature	687.54	< 0.0001	780.44	< 0.0001
B- Time	844.90	< 0.0001	887.46	< 0.0001
C - NaSCN	208.62	< 0.0001	198.50	< 0.0001
D – H_2O_2	27.14	0.0001	37.60	< 0.0001
AB	0.0180	0.8951	0.6963	0.4171
AC	4.41	0.0529	12.52	0.0030
AD	1.26	0.2786	0.0111	0.9173
BC	52.00	< 0.0001	33.96	< 0.0001
BD	2.67	0.1231	0.3142	0.5834
CD	10.95	0.0048	2.01	0.1765
A^2	0.9330	0.3494	9.11	0.0086
B^2	17.30	0.0008	26.00	0.0001
C^2	0.7492	0.4004	3.48	0.0819
D^2	77.82	< 0.0001	48.57	< 0.0001
Residual				
Lack of Fit	3.44	0.0925	4.17	0.1643

Table 5
Regression coefficients of the predicted second-order model for the response variables.

S.No	Response parameter	Total coliform count	Total bacteria count
1	Std. Dev.	26.11	20.72
2	Mean	779.65	8220.80
3	C.V%	3.35	2.52
4	R^2	0.9923	0.9927
5	Adjusted R^2	0.9852	0.9859
6	Predicted R^2	0.9600	0.9614
7	Adeq Precision	43.3464	48.10
8	Model suggested	Quadratic	Quadratic

results show that the models are suitable to predict the growth of TCC and TBC within the range of factors studied.

Table 5 shows that the variation (CV%) coefficient was reasonably low and acceptable, indicating a better precision and reliability of the experiment. The goodness of fit of the models was further inspected using the R^2 value. It had been suggested that the R^2 value should be at least 0.80 for a good fit of a model. The regression model found to be highly significant, with the R^2 value of TCC and TBC was 0.9912, and 0.9922, respectively, indicating a close agreement between the observed and the theoretical values predicted by the model equation (Fig. 1 and 2).Moreover, the value of the adjusted R^2 for TCC and TBC was 0.9830, and 0.9849 respectively, and this confirms that the model was highly significant, which indicated a good agreement between the experimental and predicted values.

3.4. Development of regression model equation

The experimental results obtained from the activation of LPS of goat milk-based on CCD are presented in Table 3. To build a simple model with a minimum of equation terms and prevent over-fitting, the insignificant coefficients (CE), which have values nearest to zero, are eliminated from the models. The developed model equation that correlates TCC and TBC's growth to the activated LPS of the goat milk process parameters in terms of coded factors after excluding the insignificant terms were given in Eq. (10) and (11),respectively. It should be noted that Eqs. (10) and (11) are only valid within the range of tested conditions: 25 °C < temperature < 37 °C, 4hr < storage time < 8hr, 15PPM <NaSCN< 30PPM and 10PPM < H_2O_2< 20PPM.Following the quadratic Eqs. (10) and (11) has been predicted based on the software suggestion from the RSM analysis.

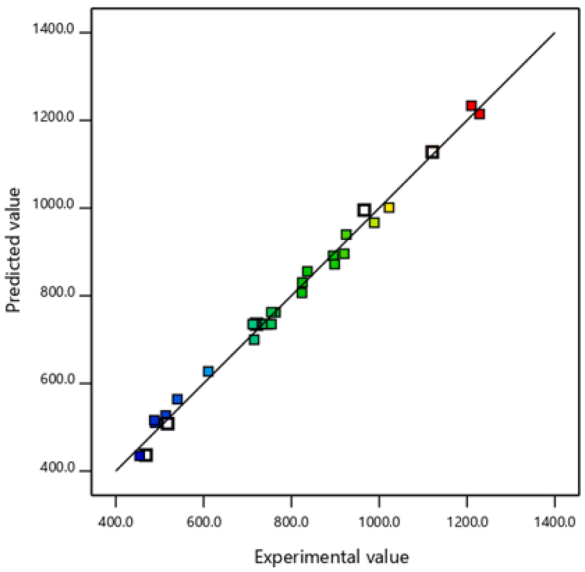

Fig. 1. Correlation between the experimental and predicted value of total coliform count.

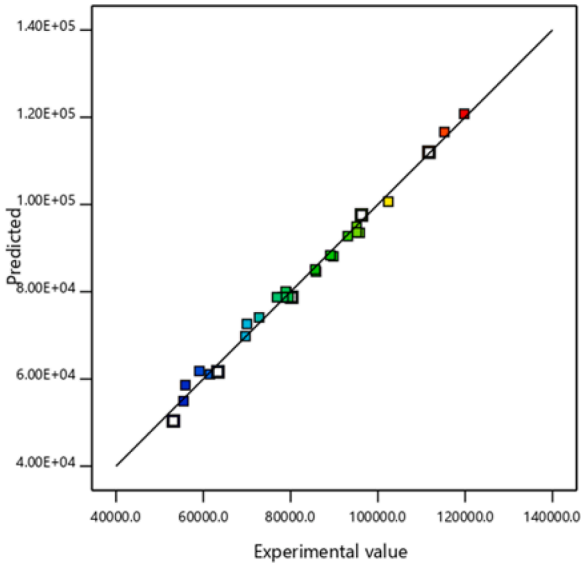

Fig. 2. Correlation between the experimental and predicted value of total bacteria count.

Total Coliform Count (Y_C, CFU/mL)

$$Yc(CFU/mL) = +735.18 + 139.73A + 154.89B - 76.97C - 27.76D - 46.07BC - 21.60CD + 20.74B^2 + 43.97D^2 \quad (10)$$

Total Bacteria Count (Y_b, CFU/mL)

$$Y_b(CFU/mL) = +78754.33 + 11805.33A + 12588.75B - 5953.67C - 2591.08D - 1831.38AC - 3015.88BC - 1193.23A^2 + 2015.65B^2 + 2754.90D^2 \quad (11)$$

where A is temperature (°C), B is storage time (hr), C is sodium thiocyanate (ppm), and D is hydrogen peroxide (ppm).

The positive signs in the models signify the factor's synergetic effects, while the negative sign indicates the antagonistic effect. The positive coefficient of temperature and storage time indicated increasing the growth of TCC and TBC, while NaSCNand H_2O_2have a negative effect. The linear, interaction and quadratic values of F represent the significant impact of variables on response parameters.

Figs. 1 and 2demonstrated that the predicted values are much closer to the experimental values. All the data points areconcentrated near the diagonal line,and no scattered points were observed. The points of all predicted and actual responses fell in 45° lines, indicating that the developed model is appropriate to predict TCC and TBC growth.

3.5. Analysis of response surfaces

Based on the model equations developed Eqs. (10) and (11) for TCC and TBC, the effects of the independent variables and their interactions were represented in 3D response surface plots. The regression models developed in this study have four independent variables; two variablesareheld constant atthe optimum level, whereas the other two factors vary within their experimental range. The significant interaction effects on responses are demonstrated in Figs. 3 and 4 through 3D response surface plots.

3.6. Effect of process parameters on the growth of total coliform count

The present work attempted to investigate and compare the microbiological quality of inactivated control and LPS activated raw goat milk at different storage time intervals, NaSCNconcentration, and H_2O_2 in different storage temperatures. The result shows that the growth of TCC of LPS activated raw goat milk was in the range of 4.55×10^2–1.23×10^3cfu/mL (Table 3). These results are significantly lower than the reported values of raw goat milk (4.5×10^3cfu/mL)(Bendary et al., 2017), raw cow milk (1.81×10^2–3.08×10^6cfu/mL) (Tekilegiorgis, 2018), raw cattle milk(1.59×10^5cfu/mL)(Naing et al., 2019),raw goat milk (1.3×10^2 - 5.7×10^6cfu/mL)(Abdel-Hameed, 2011), (1.48×10^4–8.71×10^4cfu/mL)(Mohamed et al., 2016)and (6.9×10^6 –3.7×10^7cfu/mL) (Nigussie and Seifu, 2007).The growth of TCC was comparable to the LP-activated raw goat milk (0.06×10^4 to 15×10^6 cfu/mL) (Naceur et al., 2018).

The current finding is in agreement with an earlier finding (Srisaikham et al., 2017a).The lower TCC may be due to a sound cleaning system, good packaging, and fair handling from farms to the plant, as was reported by (Chye et al., 2004). However,high numbers of TCC are commonly used as an indicator of poor hygiene and improper handling. It is also remarkable to emphasize that food poisoning cases may happen when the bacteria counts are increases (Elhosseny et al., 2018).

The TCC was inversely proportional to NaSCN and H_2O_2,and indicated that increasing any of those parameters would decrease the growth of TCC until the optimum value was achieved(Eq. (10)). The growth of TCC always decreases if NaSCNand H_2O_2 are increased with the other remaining constant because both of these factors inhibited bacterial multiplication (Eq. (10)).The temperature, storage time, NaSCN, and H_2O_2shows a significant ($p < 0.0001$) effect on the growths of TCC. Storage time, compared to the other process variable, had the most significant effect on the growths of TCC as it showed the F-value (844.90) is larger, and the effect is within the order of $B > A > C > D^2 > D >$ BC> B^2>CD.

The effect of NaSCNon the activation of theLPSvaried in ranges from 15 ppm to 30 ppm was studied by maintaining other parameters as constant. As the NaSCNwas increased from 15 ppm to 30 ppm, the growth of TCC was found to be reduce from 8.58×10^2cfu/mL to 7.2×10^2cfu/mL.The results suggest that the external addition of NaSCNand H_2O_2increased the LPS in the raw goat milk, which in turnreduced bacterial growth, lactic acid production,and led to a quality of milk.This is because excessive intake of NaSCNmay have atoxic effect and cause disturbances in the thyroid function indirectly through interference by iodine metabolism. Similar effects were noted in the activation of LPS which significantly reduced the total count of mesophilic aerobic bacteria in raw milk Amenu et al. (2017), Srisaikham (2015) and ZapicP

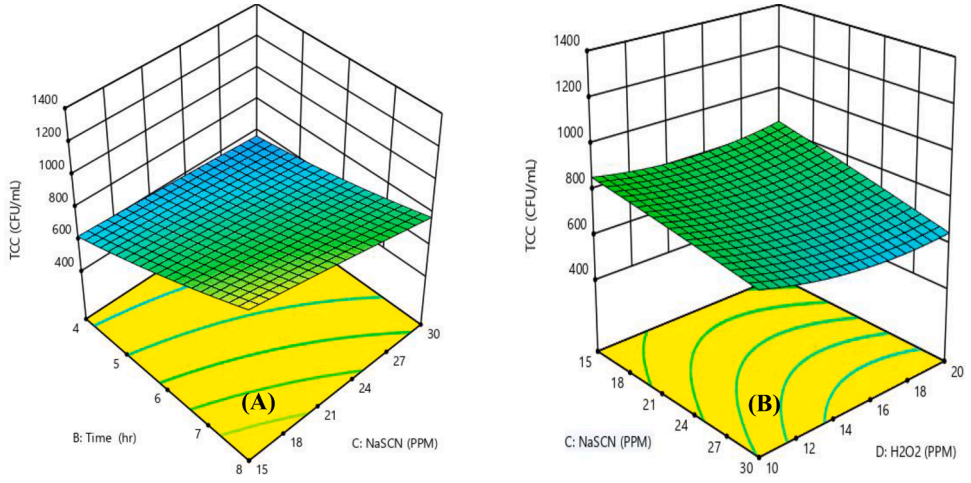

Fig. 3. 3D response surface plots of growth of TCC (A) effect of storage time and NaSCN at 31 °C and 15PPM $H_2O_2$and (B) effect of H_2O_2 and NaSCN at 31 °C and 6 h storage time.

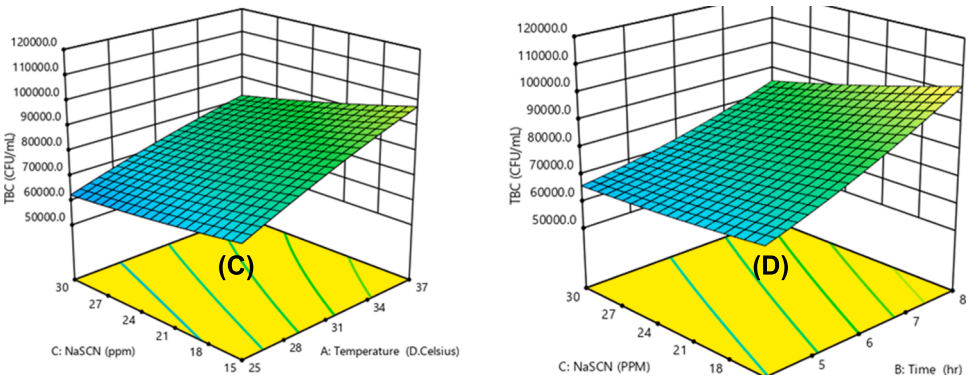

Fig. 4. 3D response surface plots of growth of TBC (C) effect of NaSCN and temperature at 6hr storage time and 15 PPM H_2O_2 and (D) effect NaSCNand storage time at 31 °C temperature and 15 PPM H_2O_2.

et al. (1991).

The TCC was directly proportional to storage temperature and time, i.e., when the storage temperature and time increase, the growth of TCC would be increased if all other factors remain constant (Eq. (10)). This is due to the growth of coliform bacteria which is accelerated when suitable temperature conditions; i.e. they recover their damaged cytoplasmic membrane. The effect of storage temperature and period in this study is similar to previous work Hartley et al. (1968) and Naceur et al. (2018), which worked on the effect of storage period and temperature on the growth of TCC of raw Milk.

The analysis reveals that the interaction of storage time with NaSCN has a significant ($p < 0.05$) effect on the growth of TCC, followed by H_2O_2 and NaSCN (Table 3).This is because of the high F value (33.96) for the interaction of storage time with NaSCN. Fig. 3(A) shows that storage time and NaSCN concentration has a significant combined effect on the growth of TCC, with storage time exhibiting the highest effect. However, the growth of TCC shows good effect too.As the storage time and NaSCN concentration increases, the growth of TCC significantly decreases until a specific optimum condition.This is because H_2O_2 and NaSCN inhibited bacterial metabolism, thereby preventing bacteria multiplication present in the milk.

Fig. 3(B) shows that the two factors have a significant effect on the growth of TCC. It was observed that, at higher NaSCN, increasing H_2O_2 greatly decreases the growth of TCC than at lower NaSCN. LPS can catalyze the oxidation of SCN by H_2O_2 with the production of antibacterial hypothiocyanate (OSCN-)(Schoos et al., 1999).The TCC decreased with the external addition of NaSCN and H_2O_2 until the end of the optimum conditions. They increase the concentrations of two components reacting with each other, which reduced the microbial count and increased milk quality during storage and transportation to processing plants. These products have more effectiveness in reducing bacterial growth activity (Naidu, 2000) by damaging the cell membranes and inhibiting metabolic enzyme activity. This suggests that the activation of LP by adding NaSCN and H_2O_2might be used as aneffective treatment to increase milk quality during storage by reducing the microbial population caused by the spoilage present in milk. A similar effect was detected by ZapicP et al. (1991) and AY and Bostan (2017).

3.7. Effect of process parameters on the total bacteria count

The present study was undertaken to elucidate the effect of selected independent variables on the counts of microorganisms in raw goat's milk. The result shows that the growth of TBC of the LP treated goat milk was in the range of 5.31×10^4 - 1.19×10^5cfu/mL(Table 3), which is significantly lower than the reported values of 3.8×10^7cfu/mL) for cows' milk (Tassew and Seifu, 2011). This study's TBC is comparable to the raw milk of 5×10^3–3.8×10^8cfu/mL and 13×10^4 to 14×10^6cfu/mL as suggested by Naceur et al. (2018) and Tekilegiorgis (2018), respectively. TBC may be high due tocontaminated udders, maintaining an unclean milking and housing environment. The study revealed that the milking system's poor cleaning significantly affects the TBC of raw goat's milk. The present study was in agreement with an earlier finding by Chye et al. (2004).

The TBC is significantly affected by the temperature, storage time, NaSCN and H_2O_2, and the quadratic effect of temperature, time and H_2O_2. The storage time was the most significant operational variable to the TBC. It showed whose F-value (887.46) is higher than the others and, followed by storage temperature, NaSCN and H_2O_2(Table 4). TBC is inversely proportional to NaSCN and $H_2O_2$and indicated that increasing any of these process parameters would decrease TBC if all other factors remained constant (Eq. (11)). Similarly, TBC's growth always increased if storage temperature and time increased (with the other remaining constant), which produce acid and gas and disrupt milk proteins by fermenting lactose to cause rapid milk deterioration. The TBC level increases from a temperature between 25 and 31 °C to an average of 1.02×10^5cfu/mL, and at a higher temperature (37 °C), the TBC becomes 1.19×10^5 cfu/mL. A similar observation was reported in the literature (Connell et al., 2016) who reported when milk was stored at 4 °C, 6 °C, and 8 °C, the TBC increased from a starting value of 3.2×10^3cfu/mLto 5.2×10^5 at four °C, 3.3×10^6 at 6 °C, and 1.0×10^7 at8 °C, after 105 hr of storage.The study revealed that all the parameters, i.e., temperature, storage time, NaSCN and H_2O_2individually affect the TBC significantly compared to the combined effect.

Storage time is known to play a significant role in the growth of TBC in lactoperoxidase activated raw goat milk. The reason is that the antibacterial products hypothiocyanate ion and hypothiocyanous acid are unstable and short-lived products. As time increases, their availability decreases and results in a decrease in the effectiveness of antimicrobial. Therefore, the LPs' efficiency and effectiveness depend upon the initial microbiological quality of milk and perseveres for a limited period Althaus et al. (2001) and M.I.M (A 2005). Besides, the length of this time depends on intermediate products formed as a result of oxidation of thiocyanate by H_2O_2 concentration. This finding was similar to those reported by Stefano et al. (1995), who reported the bacteriostatic effect generatedfrom the activation of LP-s in milk had extended the shelf-life by decreasing acidity caused by microbial flora present in milk.

Fig. 4(C) shows that temperature and NaSCN have a relatively small effect on TBC.It was observed that the effect of temperature is relatively higher than an external addition of NaSCN and an increase in any of the two factors decreases TBC growth. These results were in agreement with the study carried by Naceur et al. (2018). The present study results revealed that the difference in elevated temperature was influential in the quality of activated raw milk tested. The interaction between storage time and NaSCNhas a significant ($p<0.0001$) effect on the TBC, followed

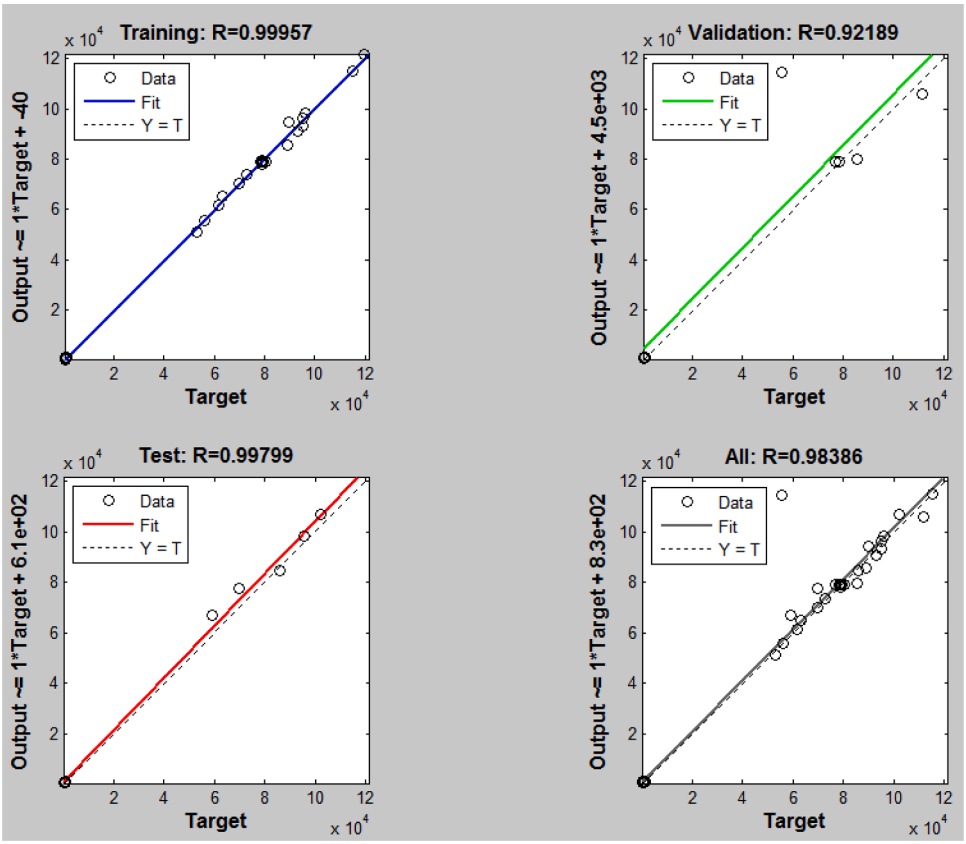

Fig. 5. Neural Network model with training, validation, test and all prediction set.

Table 6
Validation data set for experimentally determined ANN and RSM predicted values of TCC and TBC.

Exp. No	Total coliform count (cfu/mL)			Total bacteria count (cfu/mL)		
	Experimental	Predicted value		Experimental	Predicted value	
		RSM	ANN		RSM	ANN
1	763.6	762.10	763.90	78,986.0	79,952.00	78,996.00
2	1228.6	1214.51	1226.80	115,235.0	1.166×10^5	115,245.52
3	720.6	735.18	722.27	80,238.0	78,754.33	80,238.57
4	895.4	891.21	894.34	89,125.0	88,356.67	89,115.96
5	920.2	895.83	910.52	95,825.0	93,533.00	95,825.45
6	740.9	735.18	739.91	78,285.0	78,754.33	78,285.00
7	836.4	855.56	836.42	85,819.0	84,591.75	85,879.98
8	1210.0	1233.48	1213.32	119,789.0	1.208×10^5	119,789.00
9	1022.6	1001.20	1022.64	102,389.0	1.007×10^5	102,321.23
10	754.5	735.18	748.51	78,364.0	78,754.33	78,364.00
11	924.9	939.53	925.52	93,123.0	92,760.08	93,028.69
12	513.6	526.86	513.65	61,498.0	61,089.75	61,498.00
13	823.9	806.50	821.87	89,789.0	88,134.75	89,778.98
14	754.5	762.46	756.78	78,856.0	80,064.67	78,766.58
15	489.8	510.38	487.98	55,897.0	58,591.42	55,867.12
16	736.4	735.18	736.20	79,178.0	78,754.33	79,187.75
17	454.5	434.80	454.61	55,469.0	54,910.33	55,459.00
18	746.8	735.18	745.75	79,569.0	78,754.33	79,569.00
19	468.9	436.48	466.42	53,125.0	50,370.75	53,125.00
20	988.7	966.59	978.32	95,129.0	94,956.08	95,119.00
21	610.8	627.38	612.63	69,998.0	72,612.08	70,021.56
22	965.9	995.38	970.81	96,238.0	97,592.08	96,298.38
23	1121.0	1127.91	1120.32	111,689.0	1.120×10^5	111,679.78
24	715.2	699.75	714.78	72,781.0	74,103.75	72,788.85
25	824.9	830.18	823.95	85,636.0	85,110.33	85,636.00
26	711.9	735.18	713.75	76,892.0	78,754.33	76,798.36
27	540.2	563.99	541.53	69,654.0	69,795.08	69,654.00
28	518.2	508.34	516.52	63,345.0	61,639.42	63,443.85
29	898.6	871.86	897.72	95,151.0	93,609.75	95,189.53
30	487.9	516.05	488.32	59,102.0	61,834.33	59,102.56

by storage temperature and NaSCN(Table 4).

Fig. 4(D), the interaction effect of both NaSCNand storage time was observed. Storage time significantly controls the combined effect of the two factors, with NaSCNhavinga significant effecton TBC andincreases in any factor that reasonably decreasesTBC growth.Regardless of the temperature effect, TBC's response to addition of NaSCN and H_2O_2 depended on incubation time as TBC increased with increasing incubation time.The microbial growth inhibition by activation of the LP-system in raw goat milk was comparable with results obtained by M.I. M (2005), which reported that at 30 °C, the stabilized milk samples in LP-activated cow milk remained unchanged for at least 10hr. The current result was in agreement with the finding Nigussie and Seifu (2007), who suggested that activation of LPS in raw goat milk can significantly decreaseTBC level compared to non LPS activated raw goat milk. According to Amenu et al.(2017), activation of LPS in camel milk extends the shelf life for 12 h and cow's milk for 6 hrs. Generally, the result encouraged the use of activating LPS to preserve milk as it has been found useful in extending the shelf life of raw goat milk.

3.8. Artificial neural network

Fig. 5 shows the experimental versus the computed ANN data's spread plot in both training, testing and validation networks. The correlation coefficients (R) values for training (0.99957), validation (0.92189), testing (0.99799) and all prediction sets (0.98386) indicates that the ANN model shows better regression and fitting compared to the RSM model. Nearly each data point has been scattered around the 45° line indicating excellent compatibility between the experimental and predicted output data values by ANN. Therefore, the ANN prediction for training, validation, and testing is highly substantial and respected in terms of correlation and implies that the predicted model was more precise in predicting the responses.

Table 6 shows the best combination of the ANN parameters to predict the output parameter most accurately. The value of MSE obtained from the ANNs for both batch and continuous modes was 0.00078, close to the acceptance limit for the MSE, which was set to 0. The closeness of the training and testing errors validates the accuracy of the model.

3.9. Comparative evaluation of ANN and RSM models

The ANN and RSM models' predictive competence was determined and compared based on prediction accuracy and various parameters such as RMSE, R^2, SEP, MAE and AAD. Table 7 shows the predictive indices for RSM and ANN models compared to TCC and TBC growth. Both the models performed reasonably well, but ANN models have the superior modeling capability compared to the RSM models for both TCC and TBC. Fig. 6(A) and (B) depicted the experimental values and predicted values of RSM and ANN.RSM modeling is more comfortable compared to ANN, as ANN needs a higher number of inputs than RSM for better predictions. However, as indicate values, the ANN predicted value is much closer to that of the experimentally measured data, suggesting that the ANN model has superior prediction ability than the RSM model. The present study was in agreement with an earlier finding of (Samuel et al., 2018).

Table 7
Comparison of predictive abilities of RSM and ANN models.

	Total coliform count (TCC)		Total bacteria count (TBC)	
Parameters	RSM	ANN	RSM	ANN
RMSE	18.4590	3.2396	1462.299	41.2936
R^2	0.9922	0.9997	0.9996	1
AAD (%)	2.3461	0.2540	1.6374	0.0308
MAE	16.4743	2.0280	1227.4916	24.8433
SEP	0.0789	0.4155	1.7788	0.0502

3.10. Optimization of activation of the lactoperoxidase system by response surface modeling

This study's main objectives were to determine the optimal operational conditions for the minimum TCC and TBC from raw goat milk. CCD performed the numerical optimization of the lactoperoxidase system's activation by setting the desired goal for each process variableand response. The optimum combinations for theprocess parameters were selected in order to obtain the minimum amount of TCC and TBC. By selecting the desired values, the appropriate process conditions could be determined by the statistical software (Design Expert ®). Table 8 shows model validation for the growth of TCC and TBC. The minimum TCC and TBC were obtained at sodium thiocyanate, and hydrogen peroxide concentration of 30 ppm and 17.80 ppm, respectively, at 25 °C and a storage time of 8 hr. At these optimum conditions, TCC and TBC's growth were found to be 4.51×10^2 cfu/mL and 5.44×10^4 cfu/mL, respectively. The value of desirability (0.991) shows its approach to unity and with low error. Theresult indicated that there was good agreement between the predicted and experimental results verified the validity of the model and confirmed the optimal point's existence.

3.11. Comparison of physicochemical characterization of raw and LP treated goat milk

The comparison between control and lactoperoxidase treated raw goat's milk were presented in Table 9. The physicochemical characterization of LP treated goat milk was done at optimized operating conditions. The optimum activation of lactoperoxidase conditions predicted for lower TCC, and TBC growth was 30 ppm NaSCNand 17.80 ppm H_2O_2, at a storage temperature of 25 °C and time of 10 hr. The results (Table 9)showed that the values of moisture, protein, fat, ash, TS, lactose and SNF in raw and preserved goat milk samples had no significant ($P > 0.05$) effect.The current finding was in agreement with an earlier finding of (Srisaikham et al., al.,2017b), who reported that the milk composition obtained from the tank of raw milk and the quality of LP-treated milk were similar to those of regular raw milk. Thus, the current result suggested that the lactoperoxidase system can increase the shelf life of the raw goat milk, but there is no effect on the milk's chemical composition. Other studies have also reported similar results.

The protein, fat, ash, SNF and TS of the raw goat milk samples found in the current study significantly higher than the previous findings of (Lai et al., al.,2016) and (Seifu et al., 2004).The composition of goat milk in the present study was compared favorably with the average composition of goat milk in the USA, which contained TS of 13.20%, fat of 4.36%, SNF of 8.83%,lactose of 4.70%, a proteinof 3.53%,ashof 0.80% (Clark and Mora García, 2017). The water content in the raw and preserved goat milk samples was comparable to the reference range of 80% to 90% (Lai et al., 2016).

Significant ($P < 0.05$) difference in titratable acidity was observed in LPS activated milk than non LPS activated samples, this might be due to the action of LPS. The control milk sample had the highest titratable acidity compared to the activated milk sample. This could be due to the sufficient concentrations of the lactoperoxidase agents. The present study was in agreement with an earlier finding by Amenu et al. (2017) who reported camel and cow milk have a significant ($P < 0.05$) difference in titratable acidity.

The treated goat milk significantly ($P < 0.05$) retarded lactic acid production compared to the control milk samples at 10 hr of storage. The results indicated that the storage period had a significant ($P < 0.05$) effect on the milk samples' titratable acidity.

In the present study, LP-activated raw goat milk was in agreement with an earlier finding of(Nigussie and Seifu, 2007), where there was a significant decrease in lactic acid production in LP-activated raw goat milk during different storage time at 22–23 °C. The value obtained in the current study was comparable with the acidity of cow and buffalo milk (Prajapati et al., 2017).

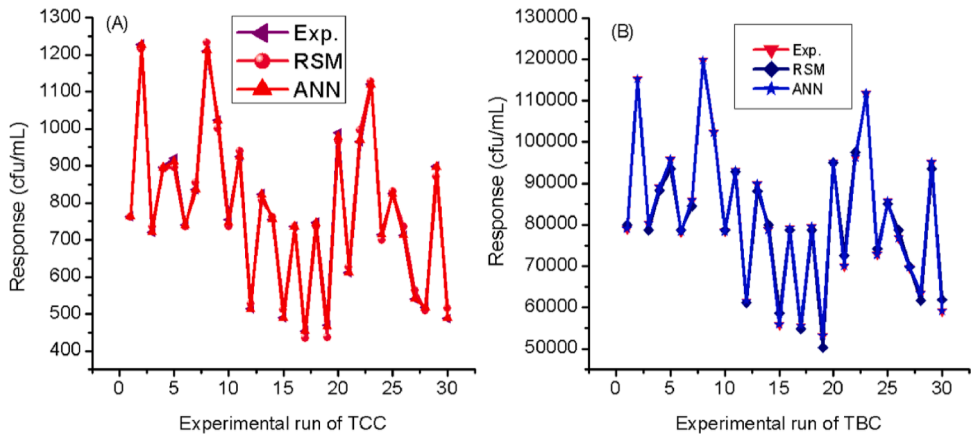

Fig. 6. Comparison of experimental with a predicted value obtained by (A) the RSM and ANN model for predicting TCC and (B) the RSM and ANN model for the prediction of TBC for each experimental run.

Table 8
Model validation for optimization of Total coliform count and total bacteria count.

Model Desirability	TCC Experimental (%)	Predicted (%)	Error (%)	TBC Experimental (%)	Predicted (%)	Error (%)
0.991	451.36	454.50	0.69	54,379.02	53,125.00	2.30

Table 9
Chemical composition of raw and LP activated goat milk.

Storage time (hr)	Initial	10hr	
Composition	Raw goat milk	Preserved milk	P-value
Moisture (%)	84.9 ± 0.06	84.9 ± 0.02	0.539
Protein (%)	3.88 ± 0.02	3.84 ± 0.04	0.128
Fat (%)	5.65 ± 0.03	5.61 ± 0.02	0.132
Ash (%)	0.88 ± 0.02	0.87 ± 0.02	0.326
TS (%)	15.13 ± 0.06	15.11 ± 0.02	0.539
Lactose%	4.79 ± 0.1	4.72 ± 0.03	0.282
SNF%	9.48 ± 0.08	9.45 ± 0.03	0.807
Titratable acidity	0.173 ± 0.001	0.204 ± 0.006	0.005

4. Conclusion

In this study, the modeling, predictive and generalization capabilities of RSM and ANN models were compared for activation of lactoperoxidase system to enhance the shelf life of raw goat milk.The performance of both the models was compared based on the prediction accuracy of TCC and TBC growth.The study revealed that all thevariables(temperature, storage time, sodium thiocyanate and hydrogen peroxide)individually affect the growth ofTCC and TBC significantly compared to the combined and squared effect. The most significant parameters that affect TCC and TBC's growth were storage time, temperature, sodium thiocyanate and hydrogen peroxide, respectively. Based on the values of R^2(0.9997), RMSE (3.2396), SEP(0.4155), MAE (2.0280), AAD (0.2540)for validation data sets, the ANN model was demonstrated to be more efficient than the RSM model both in data fitting and prediction capabilities. The optimum conditions of TCC and TBC growth were achieved at a storage time, temperature, sodium thiocyanate and hydrogen peroxide concentration of 10hr, 25 °C, 30 ppm and 17.80 ppm, respectively, with the desirability of 0.991. Under these conditions, the minimum growth of total coliform count and total bacteria count of 4.51×10^2 cfu/mL and 5.44×10^4cfu/mL, respectively. The result indicates that no significant differences in milk composition (protein content, fat content, lactose content, total solids, moisture content and ash content) were observed among activated and control goat milk samples. The present study demonstrated that activation of raw goat milk with lactoperoxidase system significantly ($P <$ 0.05) decreased the microbial load and prolonging the shelf life of the milk ranging from 4 to 10 h depending on the storage temperatures, and provides opportunities for rural farmers who usually do not have milk cooling facilities. Therefore, under good hygienic milking and handling conditions, it is possible to use the LP system as an alternative means to preserve raw goat milk quality during transportation or in the area with a lack of cooling facility.

Credit author statement

Ermias Girma is responsible for ensuring that the descriptions are accurate and agreed by all authors.

Declaration of Competing Interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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