Conductometry based models for the prediction of probiotic yogurt quality indices

Lale Amini Kanisiran [1], Mohammad Alizadeh Khaled abad [2], Mahmoud Rezazad Bari[3] , Nasim Kheshti [4] Najme Aberoon[5]

[1.] Department Faculty of Agriculture, Urmia University, Urmia, Iran, amini123lale@yahoo.com
[2.] Department Faculty of Agriculture, Urmia University, Urmia, Iran, m.alizadeh@mail.urmia.ac.ir
[3.] Department Faculty of Agriculture, Urmia University, Urmia, Iran, mahmoud114@hotmail.com
[4.] Department Faculty of Agriculture, Urmia University, Urmia, Iran, N-kheshti@gmail.com
[5]. Department Faculty of Agriculture, Urmia University, Urmia, Iran, aberoon52@yahoo.com

Abstract: Models for prediction of probiotic yogurt quality indices as a function of incubation temperature, storage time, and concentration of Lactobacillus acidophilus inoculation were investigated in this study. The models were developed using response surface methodology and their relation with electrical conductivity for the yogurt classification was assessed as well. The results showed that the electrical conductivity and pH of the yogurt has no significant relation. At a fixed level of the probiotic concentration, L. acidophilus counts were reduced. On the other hand, the increase in the storage time and the inoculation temperature caused a rise in syneresis. No significant relation between the probiotic concentration and the pH was found. The electrical conductivity has a significant relation with both the syneresis and acidity. Using linear discriminant model, it was possible to classify the yogurt samples regarding the syneresis and the probiotic counts. Another linear model was also developed for classifying the yogurt samples with respect to Streptococcus termophillus counts.
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1. Introduction

Probiotic yogurt is an enriched food with high health benefits [1]. This product can provide beneficial effects on the host and cure illnesses [1-4] by improving their intestinal microflora balance [5]. To take advantages of the yogurt to the most, its quality is of high importance to the food agencies for hygiene issues as well as to wide consumer's acceptance. Various quality indices of the yogurt as pH, viscosity, amount of probiotic and the other bacterial type, syneresis, and acidity was defined by the food agencies [6] and forced to be checked by producers before sale. These indices are being analyzed by different analytical methods developed by the researchers. A method with rapid and sensitive detection of each quality indices is of high significance in this regard.

Conductometry is a rapid, inexpensive, and sensitive method for conductivity measurement of dairy products especially the yogurt. The conductivity of dairy products is because of such inorganic ions as Na, K, Cl and organic molecules as proteins, fats, and amino acids of the milk [7]. The method had been employed for the assay of other qualities by the researchers as well. St-Gelais et al. [8] used this method as an indication of acidification in the process of fermentation. Lanzanova et al. [9] used the conductometry as an accurate and sensitive method for the measurement of growth and activity of 119 acid lactic bacterial types in milk. Mabrrok and Petty [10] made use of the method for the water content measurement of milk. Romero et al. [11] used the automatic electrical conductivity measurements during milking for mastitis detection in goats.

The quality indices as pH, bacterial counts, and acidity have an influence on the conductivity. The replacement of the different methods for the assay of sanitary quality indices of the yogurt with the conductometry can lead to rapid, sensitive, and inexpensive measurement of sanitary quality of the yogurt and its classification. The changes of such variables as incubation temperature, storage time, and the concentration of probiotic microorganism Lactobacillus acidophilus inoculation on the sanitary quality indices of the yogurt was investigated and the correlation models for the prediction of the qualities was presented in this study using response surface methodology.

2. Materials and methods

2-1. Materials

Milk composed of ($3.7 \pm 0.21\%$) of lipids, ($3.18 \pm 0.11\%$) of proteins, (8.67 ± 0.29) of dry weight without lipids, and water was bought from Faculty of Agriculture (Urmia University). Commercial starter including Streptococcus termophillus and Lactobacillus delbrueckii was

purchased in DVS package from Chr.Hansen (Hørsholm, Denmark). Probiotic starter La-5 including probiotic microorganism of L. acidophilus was purchased in DVS package from Chr.Hansen (Hørsholm, Denmark). Peptone water, culture media, and all the other reagents were bought from Merck (Darmstadt, Germany) and used as received without any further purification.

2-2.Instruments

Apparent **viscosity** of all yogurt samples was measured by Brookfield viscometer (Brookfield DVII, USA) at 8oC according to [12]. Slip velocity and spindle in all measurements were 30 rpm and 64, respectively. Syneresis was determined from the weight difference of supernatant and the initial yogurt sample after centrifugation at 222g for 10min with 15g of the sample [13]. pH of all the samples were measured at 8°C by pH meter (Eutecht, Singapore) calibrated with commercial phosphate buffers at pH 4 and pH 7. Titratable acidity expressed as concentration of lactic acid in g/100ml was measured by diluting 10gr of the yogurt with 10ml of distilled water and titrating with 0.1N NaOH in the presence of phenolphthalein as an indicator to an end point of faint pink color [14]. The conductivity of each well mixed yogurt sample was measured by the Conductometer (Metrohm, Swiss) calibrated with 0.1N KOH solution at 20oC. All the measurements were done in triplicates.

2-3. Experiment design

Design-Expert 7.0 was used and factorial design with three factors was performed: probiotic concentrations with levels of 0.01, 0.02, and 0.03, incubation temperature with levels of 38, 41, 44oC, and storage time with levels of 1st, 8th, and 15th day yielding 15 different formulas (Table 1). The response of the system was explained by the following second-degree polynomial model.

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad (1)$$

Where, Y, β_0, β_i, β_{ij} (I and j =1-n), Xi (i=1-n) are the response, constant coefficient, linear interaction coefficients, quadratic interaction coefficients, and non-coded variables, respectively. The data for Equ 1 was processed using the Design Expert program, including ANOVA to assess the interaction between the process variables and the response (a quality index). The various quality indices of the samples were analyzed in order to find the system response with the variables under study. The electrical conductivity of the samples was also evaluated to find the relation between conductivity and the other quality indices. The quality of the fitted linear model to the actual data was expressed by the correlation coefficient. The statistical significance of the model at α 0.05 was checked by the F-test in the program.

Table 1: Factorial design for the probiotic yogurt formulations

Run	First factor, probiotic	Second factor, incubation temperature	Third factor, storage time
1	0.02	41	8
2	0.03	41	15
3	0.02	44	1
4	0.02	41	8
5	0.03	44	8
6	0.02	41	8
7	0.03	38	8
8	0.02	38	1
9	0.02	44	15
10	0.01	41	15
11	0.01	44	8
12	0.02	41	8
13	0.01	41	1
14	0.02	38	15
15	0.03	41	1

2-4. Probiotic yogurt preparation

The milk was pasteurized by continuous heating and mixing for 15 minute to the temperature of 85oC in water bath. The milk was then cooled to the temperature of 43°C. The commercial starter was then added according to the guidelines of the producer company, and the starter of probiotic yogurt was added at three inoculations of 0.01, 0.02, and 0.03. The samples were then poured into 250ml vials and incubated at three temperatures of 38, 41, and 44°C so as to reach the pH of 4.6.

2-5. Microbial counts

The probiotic bacteria L. acidophilus was incubated in MRS-agar culture media with 0.15 Biliary bile in aerobic conditions at temperature of 37°C for 72hr. S. termophillus was incubated in the same conditions in the M17-agar. A 1ml aliquot of the sample was then moved to 0.1% sterile peptone water, and diluted sequentially to 6, 7, and 8 stage. A 1ml aliquot of each diluent was poured and incubated onto plates. The plates with colonies more than 20-200 were then counted.

3. Results and discussion

The relation between the probiotic yogurt quality indices and the variables (the storage time, the

incubation temperature, and the probiotic concentration) were assessed and presented in the following.

3-1. The relation of viscosity with the variables

No significant relation was found in the linear model adopted in this study. The correlation coefficient of the model is 0.1298 indicating the low contribution of the model to the population.

3-2. The relation of electrical conductivity with the variables

The electrical conductivity has a significant relation with the probiotic concentration and storage time. According to Fig. 1, the distribution of actual data around the linear predicting model is an indication of the high variance of the model. The correlation coefficient of the model is 0.8449 indicating that the predicting model and the actual experimental data are in agreement. Where, the electrical conductivity can be predicted by the following linear model.

$$Conductivity = 6.15 - 0.013A - 0.15C - 0.082A^2 - 0.51C^2 \quad (2)$$

Where, A and C show the storage time and the probiotic concentration, respectively. Fig. 2a shows the relation of electrical conductivity with the storage time. The electrical conductivity has direct relation till day 8th and has reverse relation after that. Fig. 2b shows the direct relation of conductivity up to probiotic concentration of 0.02, and the reverse one after that.

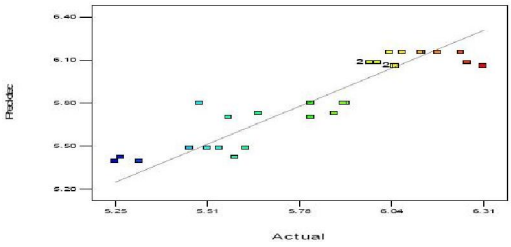
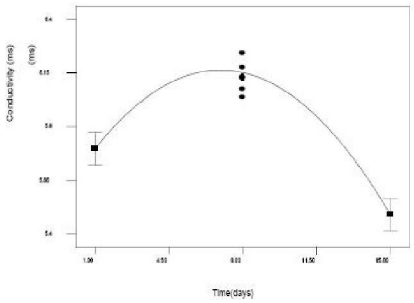
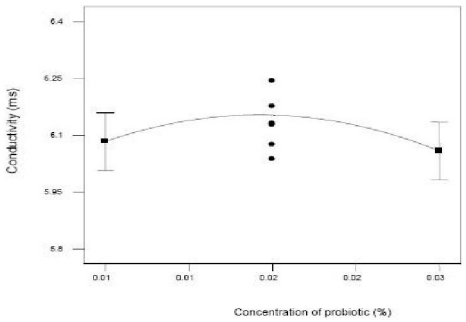


Fig. 1. Actual vs. predicted values of the electrical conductivity



(a)



(b)

Fig. 2. Electrical conductivity relation with the storage time (a) and the probiotic concentration (b)

3-3. The relation of pH with the variables

The significant relation between the pH and the variables under study was found. Fig. 3 shows the distribution of experimental data around the predicting linear model. The correlation coefficient of the model is 0.95 that is an indication of high contribution of the model to the variance of population. The pH increases with temperature up to 41oC and then decreases (Fig. 4a). The pH decreases up to day 11 and becomes constant thereafter (Fig. 4b). The pH shows slight decrease with probiotic concentration up to 0.02 and slight increase at higher concentrations (Fig. 4c). All of these variables can be included in the following equation for the pH prediction.

$$pH = 4.2 - 0.018A - 0.027B - 0.16C + 0.041A^2 - 0.11B^2 + 0.15C^2 \quad (3)$$

Where, A, B, and C represent the probiotic concentration, incubation temperature, and retention time, respectively.

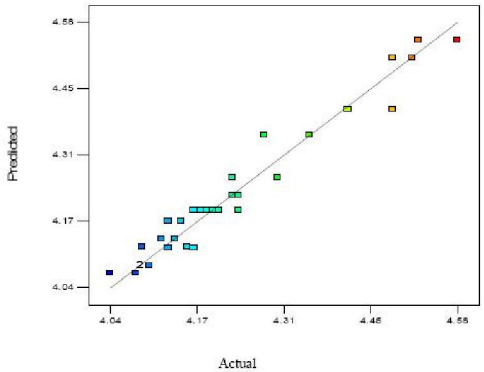
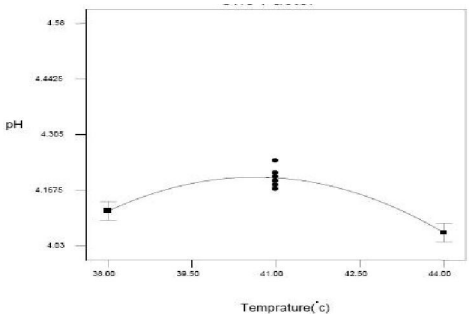
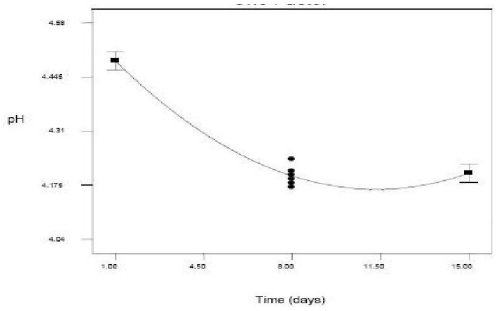


Fig. 3. Actual vs. predicted values of the pH



(a)



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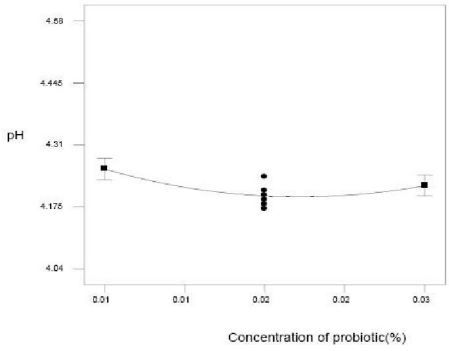


(c)

Fig. 4. pH relation with the incubation temperature (a), storage time (b) and probiotic concentration (c)

3-4. The relation of syneresis with the variables

The syneresis has significant relation with the probiotic concentration and simultaneously with both the incubation temperature and the storage time. Fig. 5 shows the distribution of data around the predicting regression model. The correlation coefficient of the model is 0.85 that is an indication of high contribution of the model to the variance of population. The syneresis decreases with the probiotic concentration till 0.02 and then increases (Fig 6a). The combined effect of the temperature and the storage time on the syneresis shows the lowest syneresis at 1st day and 38oC and highest one at 15th day and 44°C (Fig 6b).

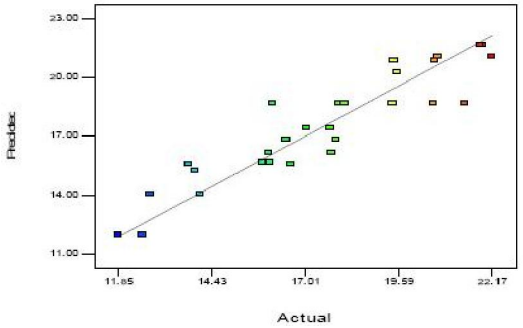
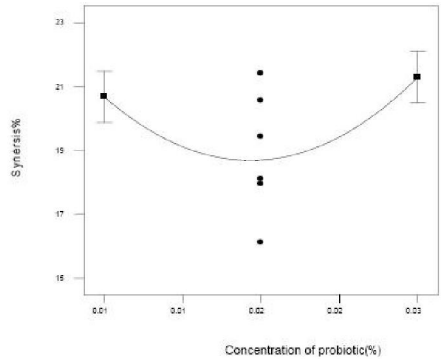
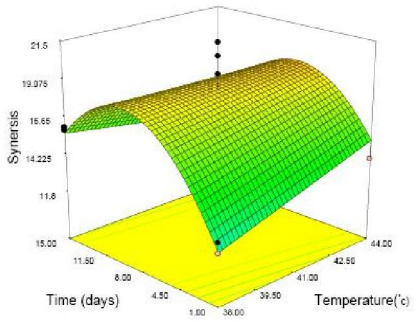


Fig. 5. Actual vs. predicted values of the syneresis



(a)



(b)

Fig. 6. Syneresis relation with the probiotic concentration (a) and both temperature and storage time (b)

The predicting model of the syneresis was determined by discriminant analysis. Incorporation of the sample electrical conductivity in the model and the one with higher order was used with correct determinant coefficient of 0.714 for syneresis prediction. Two type of chemical behavior in relation to syneresis was observed which made the modeling with serious difficulty. Thus, the syneresis higher and lower than 16.94 was classified high and low respectively with the following results.

High syneresis:

$$Y = -216.55 + 72.94X \quad (4)$$

Low syneresis:

$$Y = -197.26 + 69.61X \quad (5)$$

Where, X and Y show the syneresis and electrical conductivity, respectively.

3-5. The relation of probiotic bacterial counts with the variables

The bacterial counts of L. acidophilus have significant relation with the variables under study so that the correlation coefficient of the model is 0.84. Fig. 7 shows the distribution of data around the regression axis. The combined effect of the incubation temperature and the storage time on the bacterial counts was shown in Fig. 8. According to the Fig. 8, the number of probiotic bacteria decreases with a decline in the incubation temperature at a constant storage time. The number of probiotic bacteria decreases with an increase in the storage time at a constant incubation temperature. The minimum bacterial counts were found in the 15th day of the test and 38oC and the maximum was found in the 1st day and 44°C. The number of bacteria can be correlated by the following equation as a function of the variables.

$$N_{L.acidophilus} = 6.55\times10^{7} + 1.44\times10^{7} A + 6.72\times10^{7} B - 1.53\times10^{7} C - 2\times10^{7} A.C + 1.2\times10^{7} B.C - 1.58\times10^{7} A^{2} - 1.83\times10^{7} C^{2} \quad (6)$$

Where, A, B, and C represent the probiotic concentration, incubation temperature, and the retention time, respectively.

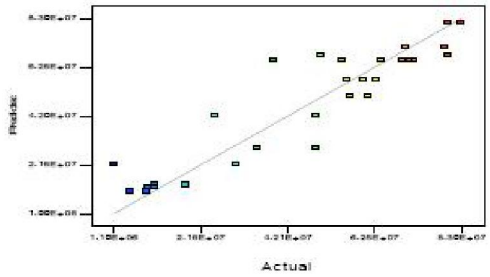


Fig. 7. Actual vs. predicted values of the probiotic bacterial counts

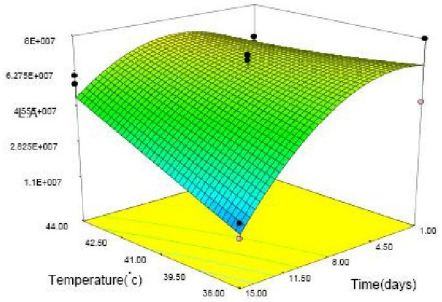


Fig. 8. Probiotic bacterial counts relation with both the storage time and the incubation temperature

3-6. The relation of S. termophillus counts with the variables

Fig. 9 shows the interaction effect of the incubation temperature and the storage time on S. termophillus counts so that a significant relation was noticed. The B+ line shows the effect at the temperature of 44oC and the B- line shows that of 38°C. The effect of temperature on the number of probiotic microorganisms depends on the time. Where no significant difference was observed at the first day, significant relation was arisen in the 15th day. The number of the microorganisms was increased with time at the incubation temperature of 44oC. But at the temperature of 38°C, the bacterial counts have an inverse relation with the time. The predicting model of the bacterial counts was determined by discriminant analysis and the following two variable models were obtained. Incorporation of the storage time and acidity of the samples in the models, the one with higher order can be used with correct determinant coefficient of 0.83 for bacterial counts prediction.

Two variable model was designed in this study so that the microorganisms was classified into two groups low and high depending to the number of bacteria lower or higher than 865×10^{6} as follows.

Low:

$$Y = -39.12 + 0.53X_1 + 102.16X_2 \quad (7)$$

High:

$$Y = -47.3 + 0.81X_1 + 109.35X_2 \quad (8)$$

Where, X1, X2, and Y represents the incubation temperature, acidity, and the bacterial counts, respectively.

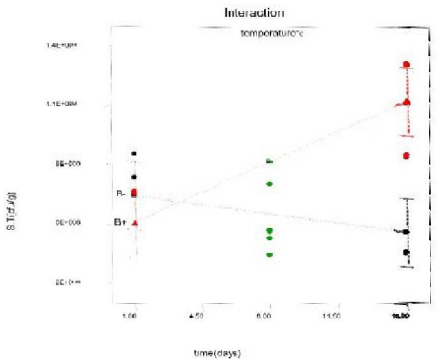


Fig. 9. The interaction effect of storage time and incubation temperature on S. termophillus counts

3-7. Acidity relation with the variables

The significant relation was observed between acidity with the temperature, the time, and the probiotic concentration so that the correlation coefficient is 0.85. Fig. 10 shows the distribution of actual data around the linear predicting model that is an indication of the high contribution of the model to the variance of population. There is a slight dependence between acidity and the storage time till the 8th day (Fig. 11a). But, a direct relation can be observed between the acidity and the storage time at later days. The acidity and probiotic concentration have a direct relation till 8th day and a reverse one thereafter. The acidity shows a decreasing trend with a rise in incubation temperature from 38 to 41oC and then an increasing trend from 41 to 44oC (Fig. 11b). The following equation can be used to show the dependence of acidity to the variables.

$$Acidity = 0.73 + 9.57\times10^{-3}A + 4.57\times10^{-3}B + 0.016C - 0.045A^2 + 0.055B^2 + 0.023C^2 \quad (9)$$

Where, A, B, and C represent the probiotic concentration, the incubation temperature, and the storage time.

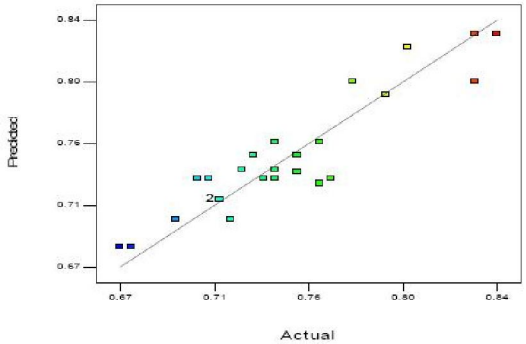
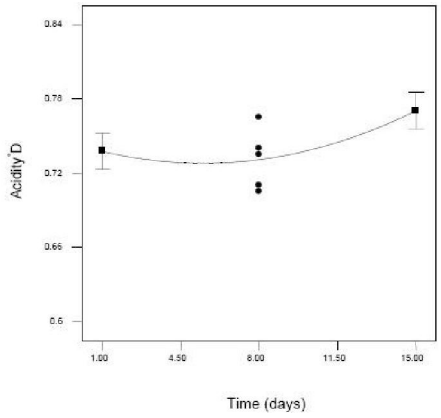
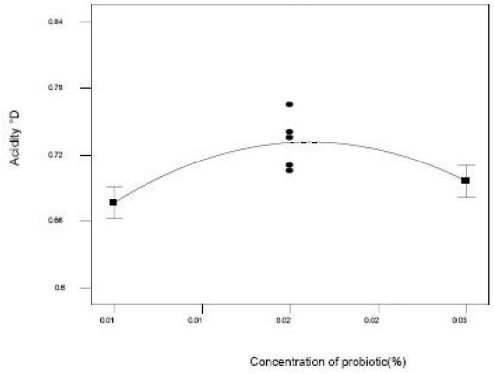


Fig. 10. Actual vs. predicted values of the acidity



(a)



(b)

Fig. 11. Acidity relation with the storage time (a) and the incubation temperature (b)

4. Conclusion

The relation between the probiotic yogurt quality indices and such important variables as the storage time, the incubation temperature, and the probiotic concentration were investigated. No significant relation was found between the yogurt viscosity and the variables. The electrical conductivity has significant relation with both the probiotic concentration and the storage time. The pH and syneresis of the yogurt have been studied and significant relations with the variables were found as well. The electrical conductivity has a significant relation with both the syneresis and acidity. The yogurt samples were classified with some linear models regarding syneresis, probiotic counts, and S. termophillus counts.

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Corresponding Author:
Lale Amini Kanisiran
Department Faculty of Agriculture, Urmia University, Urmia, Iran,
Email: amini123lale@yahoo.com

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