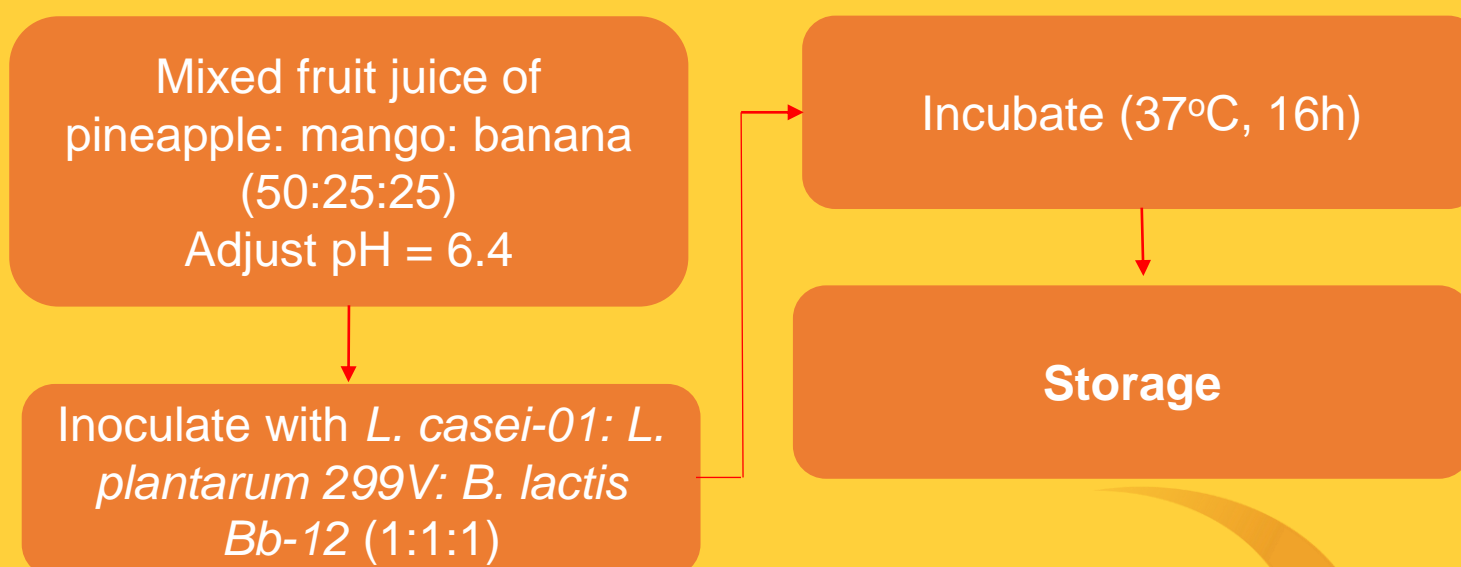


Introduction

Fruit contains numerous nutrients and sugars which may serve as a suitable medium to cultivate probiotic bacteria to enhance the health benefits of the food product [1]. Furthermore, the juice is a rich source of phenolic compounds which are catechins, quercetin, anthocyanins, gallic and ellagic acids [2]. Recently, probiotic fruit juices have been known as a functional food and nutraceutical with health beneficial effect. Since fermented fruit juices are known as a novel probiotic product, shelf-life evaluation is important to ensure a quality product during the storage period.

Materials and methods



Storage temperature	Time sample taken (day)								
5°C	0	6	12	18	24	30	36	42	45
15°C	0	3	6	9	12	15	18		
25°C	0	1	2	3	4	5	6	7	
35°C	0	0.5	1	1.5	2	2.5	3	3.5	

RESULTS

Change of pH and microbial population

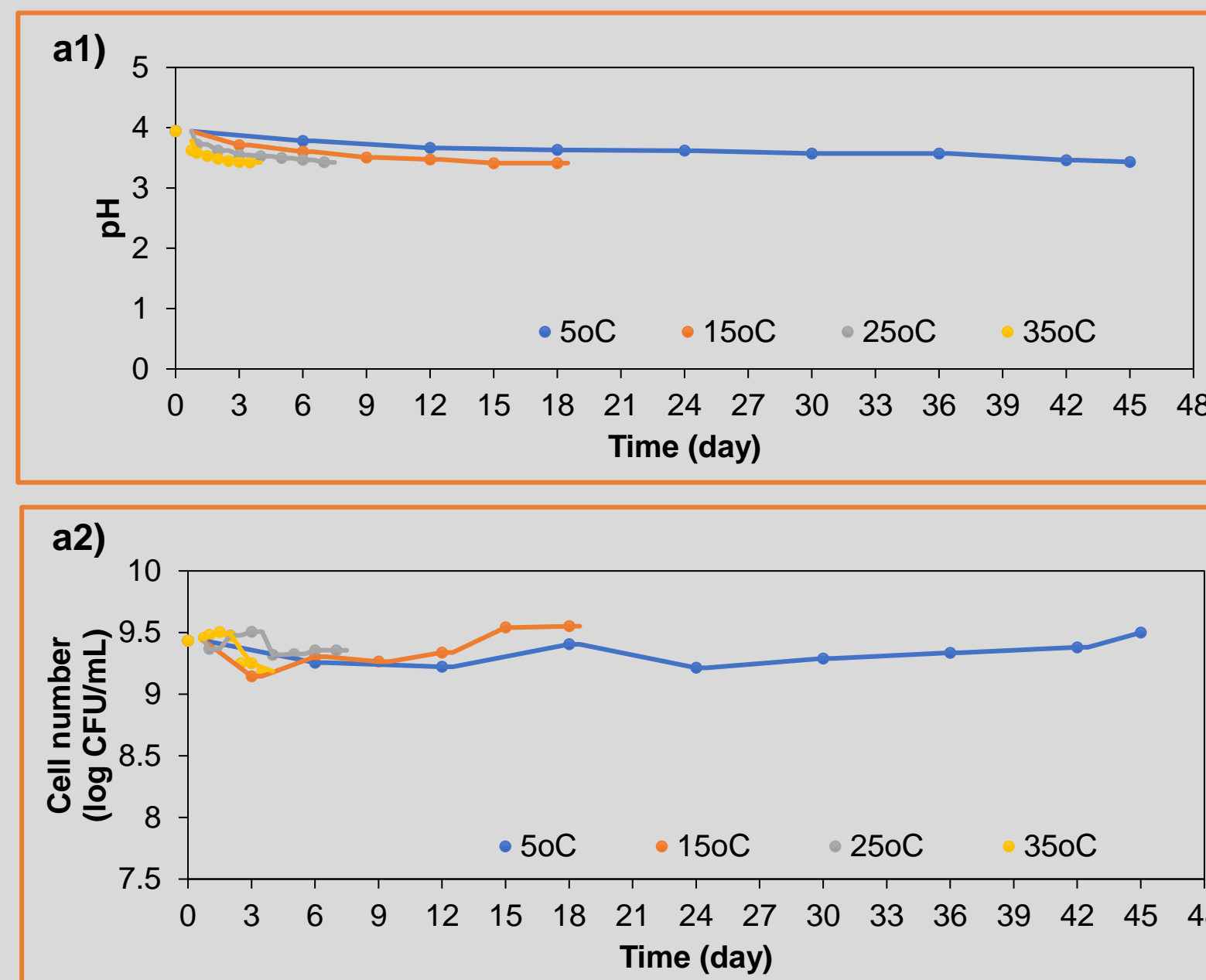


Figure 1: pH value a1) and microbial population (log CFU/mL) a2) of mixed fruit juice of pineapple: mango: banana ratio 50P:25M:25B fermented by mixed culture of *L. casei-01*: *L. plantarum* 299V: *B. lactis* Bb-12 (1:1:1) during storage at different temperatures (5°C, 15°C, 25°C and 35°C)

During the storage time at different temperatures (5°C, 15°C, 25°C and 35°C), the fermented mixed fruit juice with mixed culture showed a reduce sharp in pH value (Fig. 1 a1)). The pH dropped from 3.94 at the initial storage time to around 3.4 at the end. At this pH value, the product may not be accepted in sensory evaluation.

Change in viable cell counts of fermented mixed fruit juice during storage are presented in Fig. 1 a2). The population of bacteria in fermented mixed fruit juice showed different trends depending on the storage temperature. The initial cell count of bacteria was 9.43 log CFU/mL

After storage, the population of all samples were over 9 log CFU/mL. It could consider that the microbial population in the products after stored at different temperature still could fulfil the standard of probiotic products in term of viable cells.

Shelf-life estimation

Table 1: Nonlinear and linear equations of zeroth-order, first-order, second-order, and third-order model

Models	Nonlinear equation	Linear equation
0 order	$\frac{dA_t}{dt} = -k_o$	$A = -k_o t + A_o$
1 st order	$\frac{dA_t}{dt} = -k_1 A_t$	$\ln(A) = -k_1 t + \ln(A_o)$
2 nd order	$\frac{dA_t}{dt} = k_2 A_t^2$	$\frac{1}{A} = k_2 t + \frac{1}{A_o}$
3 rd order	$\frac{dA_t}{dt} = k_3 A_t^3$	$\frac{1}{A^2} = 2k_3 t + \frac{1}{A_o^2}$

Where:

k is the rate of the change of pH
A_o is the pH value before storage
A_t is pH value at the storage time t

The Arrhenius equation is shown in equation below:

$$k = k_o e^{-\frac{Ea}{RT}}$$

Where:

k is the constant pre-exponential or absolute rate
Ea is the activation energy (kJ/mol)
R is the gas constant (1.986 Cal/mol)
k is the reaction rate constant
T is the absolute reaction temperature (°K).

When we converted the natural logarithm of the above equation, the following equation was obtained:

$$\ln k = -\frac{Ea}{R} \frac{1}{T} + \ln k_o$$

Model evaluation

Root mean square error (RMSE) was used to evaluated how close the observed data points to the model's predicted values. The lower values of RMSE indicate the better model fit

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (X_{obs,i} - X_{model,i})^2}{n}}$$

Where

X_{obs,i} is the observation value
X_{model,i} is the predicted value
n is the total number of observations in a set of data.

Shelf-life estimation

The shelf-life of fermented fruit juice products in the current study was established based on the change of pH values

Table 2: Regression equations of pH against time under different storage temperatures (5°C, 15°C, 25°C and 35°C) using different models (zeroth-order, first-order, second-order and third-order)

Temperature	Models	Regression equations	Regression coefficient
35°C	0 th order	y = -0.1258x + 3.781	R ² = 0.7597
	1 st order	y = -0.0346x + 1.3296	R ² = 0.7763
	2 nd order	y = 0.0095x + 0.2647	R ² = 0.7927
	3 rd order	y = 0.0053x + 0.0701	R ² = 0.8086
25°C	0 th order	y = -0.0631x + 3.8137	R ² = 0.8355
	1 st order	y = -0.0173x + 1.3386	R ² = 0.8499
	2 nd order	y = 0.0047x + 0.2622	R ² = 0.8639
	3 rd order	y = 0.0026x + 0.0688	R ² = 0.8773
15°C	0 th order	y = -0.0277x + 3.8279	R ² = 0.8633
	1 st order	y = -0.0076x + 1.3424	R ² = 0.8761
	2 nd order	y = 0.0021x + 0.2612	R ² = 0.8882
	3 rd order	y = 0.0012x + 0.0682	R ² = 0.8996
5°C	0 th order	y = -0.0093x + 3.8504	R ² = 0.8954
	1 st order	y = -0.0026x + 1.3487	R ² = 0.904
	2 nd order	y = 0.0007x + 0.2594	R ² = 0.9115
	3 rd order	y = 0.0004x + 0.0672	R ² = 0.9178

Based on the coefficient of determination (R²), the third order model with the highest R² value (over 0.8) was in accordance with the pH kinetic. Therefore, the shelf-life of fermented fruit juice was predicted using third order model (equation 1).

$$\frac{1}{A^2} = 2k_3 t + \frac{1}{A_o^2} \Rightarrow t = \frac{\frac{1}{A^2} - \frac{1}{A_o^2}}{2k_3} \quad (1)$$

Based on the Arrhenius equation, the regression of rate constant against at temperatures was obtained (equation 2)

$$\ln k = -7317.2 \frac{1}{T} + 17.881 \text{ with } R^2 = 0.99 \quad (2)$$

References

- [1]. F. C. Prado, J. L. Parada, A. Pandey, and C. R. Soccol, "Trends in non-dairy probiotic beverages". *Food Research International*, 41, 111-123, 2008
[2]. Z. E. Mousavi, S. M. Mousavi, S. H. Razavi, M. Hadinejad, Z. Emam-Djomeh, and M. J. F. b. Mirzapour, "Effect of fermentation of pomegranate juice by *Lactobacillus plantarum* and *Lactobacillus acidophilus* on the antioxidant activity and metabolism of sugars, organic acids and phenolic compounds", vol. 27, no. 1, pp. 1-13, 2013

Model evaluation

In order to assess the model, a prediction of product' shelf-life at 30°C storage temperature was carried out. According to equation 2, k₃₀ = 0.001897 was obtained. The initial pH (A_o) of fermented fruit juice was 4.09, and the final pH (A) was 3.42. The shelf-life of the sample was predicted as t = 6.78 days (obtained from equation 1)

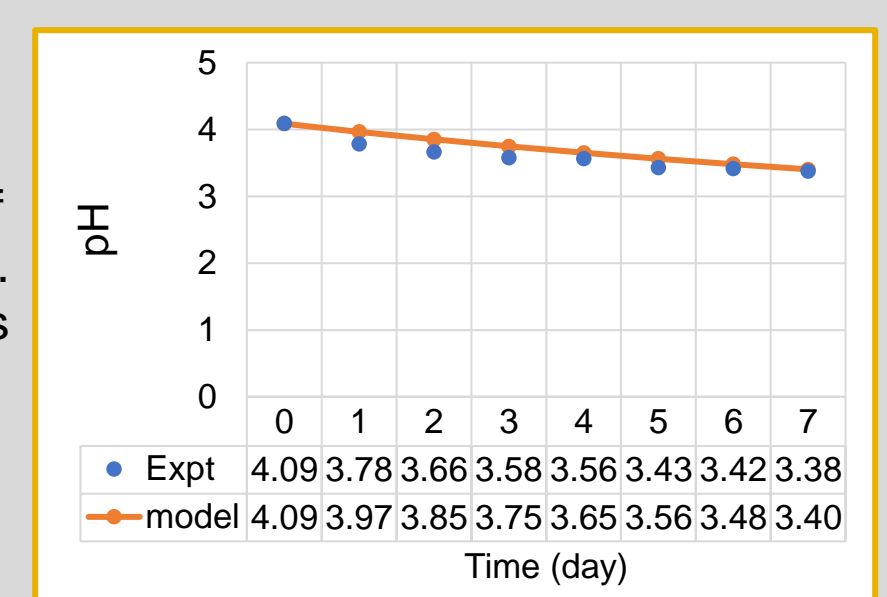


Figure 2: Plot of experimental vs. predicted values in a regression model

From these results, root mean square error (RMSE) was calculated. The value (0.1272) closes to zero, which reflects the ability of the model to predict the data accurately.

Conclusion

The microbial population and pH of products stored at the low temperature (5 and 15°C) were more stability than those at higher temperature (25 and 35°C). Moreover, this research highlighted the feasibility of the technique of modelling physicochemical change of probiotic fermented juice to determine the shelf-life and to improve the quality management of the probiotic fruit drink products.