Logistic–like Growth Model of \textit{Lactobacillus acidophilus}, \textit{Lactobacillus delbrueckii} subsp. \textit{bulgaricus} and \textit{Streptococcus thermophilus} in Palm Oil Santan

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Palm oil \textit{santan} is a healthier coconut milk replacement made from Palm oil. It was developed by the Malaysian Palm Oil Board (MPOB) in order to cater and to increase healthy food demand in Malaysia. In this study, we examined the growth and behavior of three yogurt starter cultures; \textit{Lactobacillus acidophilus}, \textit{Lactobacillus delbrueckii} subsp. \textit{bulgaricus} and \textit{Streptococcus thermophilus} when they were inoculated into palm oil \textit{santan}. All three starter cultures grew to 8–9 log(cfu/ml) in palm oil \textit{santan} and fitted with a logistic–like growth model. Interestingly, \textit{L. acidophilus} shows diauxic growth behaviour; an uncommon growth pattern usually found in substrates rich with sugar carbohydrates whereas palm oil in \textit{santan} is a lipid substrate.

\textbf{Keywords:} growth model, \textit{Lactobacillus acidophilus}, \textit{Lactobacillus delbrueckii} subsp. \textit{bulgaricus}, \textit{Streptococcus thermophilus}, palm oil \textit{santan}

\section{1. Introduction}

Dairy products are products which are universally consumed around the globe. However, many countries depend on dairy imports to satisfy their domestic demands. Malaysia is one such country \cite{1}. One method of alleviating this problem is to create similar foods from local renewable resources. Fortunately, Malaysia has local renewable resources such as palm oil which can be used to develop a non–dairy yogurt.

The idea of a non–dairy yogurt is not novel. Coconut yogurt and soy yogurt are some examples of non–dairy yogurt that have been developed, optimized and commercialized \cite{2-5}. Palm oil is another potential resource that should be considered for the development of new non–dairy yogurts \cite{6}.

The substrate to be used in the present study is called palm oil \textit{santan}. \textit{Santan} in the Malay language means plant milk, as in coconut milk. The palm oil \textit{santan} substrate is a reformulated product from palm oil. It was developed by the Malaysian Palm Oil Board (MPOB). The palm oil \textit{santan} is made with palm oil, emulsifier and coconut flavorings \cite{7}. Presently, palm oil \textit{santan} is marketed as a healthier coconut milk replacement. There is a need to evaluate yogurt starter culture growth in palm oil \textit{santan} for non–dairy yogurt production.

Bacterial growth analysis is an important step in food product quality control. There have been many research studies done to optimize the growth of bacteria in a controlled processing parameter. However, a majority of these research studies focused on dairy food products. The number of research studies on bacterial growth analysis in non–dairy products is very few and have mostly focused on soy yogurt \cite{2, 8}.

Bacterial growth analysis data can then be used to develop a growth equation model. A growth equation model is an equation that can predict a bacterial population at a certain period. This is a useful determination tool in food quality control agencies. In this research context, it is imperative to analyze the bacterial growth in palm oil \textit{santan}. By analyzing the bacterial growth, a growth equation model can be developed and used to further improve and optimize the quality of palm oil \textit{santan} yogurt.

The objective of the present study is to develop growth equation models of three common yogurt starter cultures, i.e. \textit{Lactobacillus acidophilus}, \textit{Lactobacillus delbrueckii} subsp. \textit{bulgaricus} and \textit{Streptococcus thermophilus}, in palm oil \textit{santan}.

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2. Materials and Methods

Palm oil santan yogurt was made using Khalis’s Palm Oil Santan (Khalis Santan, Selangor). Three beakers with 100 ml of palm oil santan were inoculated with single strain starter culture L. acidophilus, L. delbrueckii subsp. bulgaricus and S. thermophilus, respectively (Custom Probiotics, California USA). All yogurt samples were incubated at 37°C for 24 hours with Trio Yogurt Maker (Trio Sdn. Bhd., Selangor).

2.1 Proximate analysis

The protein content of the yogurt was analyzed according to the Kjeldahl method [9]. The protein content can be determined by multiplying the nitrogen content with the protein factor for dairy product 6.38. Lipid analysis was done according to the Rose–Gottlieb method [10]. Crude fiber analysis was conducted according to the AOCS Official method Ba 6-84 [11]. Ash content of the palm yogurt was determined by calcination [12]. All proximate analyses were analyzed after 24-hour incubation and conducted in triplicate. Analysis of variance was conducted using Minitab version 17 (Minitab Pty Ltd, Sydney) and the Tukey test was used as a multiple mean comparison test.

2.2 Bacterial growth enumeration

Growth analysis of the starter culture was done within 24 hours. Sampling for growth analysis was done every 3 hours. Sampling was conducted by obtaining 1 g of yogurt sample for serial dilution. Serial dilution was done using MRS Broth (CM0359, Oxoid) with a dilution factor $10^{-3} – 10^{-5}$ for the first sampling. The sampling dilution factor was increased throughout the 24–hour incubation. All yogurt samples were analyzed using the pour plated method with different agar depending on the starter culture. Yogurt samples inoculated with Lactobacillus species were poured onto MRS agar (CM0361, Oxoid), while the yogurt inoculated with Streptococcus were poured onto M17 agar (115108, Merck). Both types of agar were incubated at 37°C for 48–72 hour.

The starter culture colony counts were calculated according to ISO 6611 [13].

$$Total \ Colony \ (cfu/ml) = \frac{C}{V(N_i + 0.1N_2)D}$$ (1)

Herein, C is the colony count, V is the inoculation volume, $N_i$ is the number of petri dishes that contain the colony count on the first dilution, and $N_2$ is the number of petri dishes that contain the colony count on the sec-

2.3 Modelling of growth

The three starter culture growth data were fitted with a logistic–like growth model. The logistic–like growth model was chosen because the model was able to predict population growth in a limited supply environment (carrying capacity). The model formula is as follows.

$$\log P = \frac{\log L}{1+e^{(-kt)}}$$ (2)

Herein, $P$ is the predicted population, $L$ is the carrying capacity, $k$ is the steepness of growth curve, $t$ is the time and $e$ is Euler’s number (or the base of the natural logarithm). Note that the parameter $k$ is not the growth rate, although the initial growth rate at $t=0$ increases with the increase of $k$. Equation 2 shows a logistic–like growth curve. The inflection point of the curve is, however, fixed at $t=0$. Thus, another logistic–like growth curve was further derived.

$$\log P = \frac{\log L}{\left(1+e^{-Ckt}\right)^{m}}$$ (3)

The inflection point can be changed by introduction of a parameter $m$, although the parameter does not equal the inflection point. If the measured population count were identical to the predicted population count, then we can conclude that the model can be used for prediction purposes.

Regression analysis were done using Microsoft Excel 2013 (Microsoft Office, Washington) by correlating the predicted population with the measured population. The predicted population were obtained through modelling using bacterial growth enumeration in log(cfu/ml) as data input. The modelling was done using Statistical Analytical System (SAS) University Edition (SAS Institute, North Carolina).

3. Result and Discussion

There were no significant differences in the protein, lipid and ash content between the palm oil santan yogurts inoculated by the three starter cultures (Table 1). However, there were significant differences in crude fiber content between all three yogurts. Palm oil santan yogurts made from S. thermophilus have the highest crude fiber content, followed by yogurts made from L. delbrueckii subsp. bulgaricus and L. acidophilus. This is because Lactobacillus species have been known to digest
fiber ingredients. The digestion of fibers provides a beneficial effect in terms of yogurt acidification by the *Lactobacillus* species starter culture [14].

All three starter cultures were able to grow up to 8.00 log cfu/ml as shown in Figure 1. The total colony count is similar to the total colony count in another non-dairy yogurt which reached ~9 – 10 log cfu/g [2]. All three starter cultures show steady increments for the first 18 hours, but decline steadily after 18 hours. *L. acidophilus* shows a unique growth curve; the population of *L. acidophilus* increases up to the first 6 hours, but declines from the 9th hour (Figure 1a). Subsequently, the population grew again until the 18th hour. This type of growth is called diauxic growth. Usually, diauxic growth occurs due to the presence of several carbohydrate resources; the bacteria will digest one type of carbohydrate (which results in the first log phase) until it is depleted (forming the short lag phase). Afterwards, it will digest the second type of carbohydrate that is available (forming the second log phase) [15]. This can be seen through the crude fiber content of palm oil santan yogurt inoculated with *L. acidophilus* (Table 1). The crude fiber content of the yogurt inoculated with *L. acidophilus* is significantly less than other yogurts inoculated with *L. delbrueckii* subsp. *bulgaricus* or *S. thermophilus*, respectively. This could be due to the diauxic growth of *L. acidophilus* in

### Table 1  Proximate Analysis of the three palm oil santan yogurts.

| Inoculated lactic acid bacteria | Protein (g/100 g) | Lipid (g/100 g) | Crude Fiber (g/100 g) | Ash (g/100 g) |
|-------------------------------|-----------------|----------------|-----------------------|---------------|
| *L. acidophilus*              | 2.37±0.29<sup>a</sup> | 30.10±0.63<sup>a</sup> | 0.205±0.21<sup>a</sup> | 6.13±0.21<sup>a</sup> |
| *L. delbrueckii* subsp. *bulgaricus* | 2.35±0.23<sup>a</sup> | 31.62±0.46<sup>a</sup> | 0.264±0.03<sup>b</sup> | 5.93±0.15<sup>a</sup> |
| *S. thermophilus*            | 2.24±0.14<sup>a</sup> | 30.18±0.88<sup>a</sup> | 0.331±0.0003<sup>c</sup> | 6.23±0.21<sup>a</sup> |

<sup>a-c</sup> Different letters within column indicate significant difference (p ≤ 0.05) among the same analysis (n=3)

![Graph plot of measured and predicted population of all three starter cultures: (a) points in the graph where the diauxic growth of *L. acidophilus* occurs (L. acidophilus population decline at 9th hour, while L. delbrueckii subsp. bulgaricus and S. thermophilus population steadily increase).](image)

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palm oil *santan*.

From the graph, the predicted population of both *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* obtained through the model is almost the same as the measured population. However, the predicted population of *L. acidophilus* was different. This may be due to the absence of a diauxic growth pattern in the predicted population. Logistic growth model was unable to model the diauxic growth of bacteria [16].

Table 2 shows the modelling equation and regression coefficient for all three starter cultures. The regression coefficients for *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were 0.95 and 0.97, respectively. However, there were differences between the measured population of *L. acidophilus* and its predicted population. The regression coefficient for *L. acidophilus* is 0.84. Even though the starter culture *L. acidophilus* does not have a high regression coefficient value, the model equation can still be used for prediction purposes due to acceptable range of correlation value [17, 18].

According to the modelling parameters (Table 2), the model equation for all three starter culture are as follows.

\[
\log P (L. acidophilus) = \frac{8.39}{1+e^{(-0.222)}}^{0.69}
\]

(4)

\[
\log P (L. delbrueckii) = \frac{9.21}{1+e^{(-0.13)}}^{0.79}
\]

(5)

\[
\log P (S. thermophilus) = \frac{9.73}{1+e^{(-0.12)}}^{0.99}
\]

(6)

From these three equations (Eqs. 4–6), it is suggested that the population of the three starter culture is limited to 8.3–9.7 log(cfu/ml) at the current yogurt formulation. It is also suggested that the starter culture’s population will continue to grow at a rate of 4–8 hours (inverse of the growth rate). However, the growth will begin to slow after an hour at the inflection point.

Among the three starter cultures, *L. acidophilus* had the highest growth rate. However, the growth model of *L. acidophilus* is flawed due to its inability to model the diauxic growth. Therefore, the “true” highest growth rate was by *L. delbrueckii* subsp. *bulgaricus*, followed by *S. thermophilus*.

Based on this study, the equations of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* as starter cultures in developing palm oil *santan* yogurt formulations can be suggested as a reference to develop new palm oil *santan* yogurt formulations since they showed high regression coefficient.

### 4. Conclusions

All three starter cultures grew to 8–9 log(cfu/ml) in palm oil *santan* which enables the development of growth model equations on each starter culture utilized in the present study. However, only growth model equations for *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were developed with high confidence. The growth model equation for *L. acidophilus* was developed with lower confidence due to its inability to model the diauxic growth pattern as observed in the measured population. Therefore, only two equations (Eqs. (5) and (6)) developed in the present study can be used in developing future palm oil *santan* yogurt formulations using either *L. delbrueckii* subsp. *bulgaricus* or *S. thermophilus* starter culture at 37°C.

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