Survival Rate of Probiotic Powder for Animal Feeding and Shelf Life Prediction

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Abstract

This study was conducted to determine the survival rate of microorganism in probiotic powder product of code 1 (*Lactobacillus plantarum* 7-40), code 2 (*Bacillus subtilis* E20) and code 5 (*Lactobacillus plantarum* 7-40, *Bacillus subtilis* E20 and *Saccharomyces cerevisiae* P13) and its storage stability at 4, 25, 35, 45 and 55 °C. The result showed that the storage temperature affected the specific death rate (k_d) of microorganism in all kinds of products. The increasing of temperature increased the k_d significantly. Moreover, the relation of *kd* and temperature was constructed as the linear regression. This model could be used to define the k_d of microorganism during storage in the warehouse. Then, the Taiwanese farmer could apply this simple model for predicting the shelf-life of probiotic product. In addition, it could be used as the information for management the stock of probiotic products and avoiding the using of an expired product in the animal.

Keywords: Probiotic powder product, Prediction model, *L. plantarum*, *B. subtilis*, *S. cerevisiae*, Arrhenius equation

1. Introduction

Probiotic is a Latin and Greek-derived word, meaning “for life” which was first used by Kollath (1953). Lilly and Stillwell (1965) were the first to propose a definition of probiotics as substances secreted by one microorganism that stimulate the growth of another. In 2002, an FAO/WHO joint panel defined probiotics as live microorganisms which when administered in adequate amounts confer a health benefit on the host. (Hossain *et al*., 2017) Most probiotics are bacteria Gram-positive, among which lactic acid bacteria (LAB) but a few molds and yeasts can also be used as probiotics (Oyetayo and Oyetayo, 2005) to increase food safety and consumer health by preventing and reducing pathogenic bacteria. Currently, probiotics are used in feeds animal to promoting good digestion, boosting immune function, include inhibiting the growth of pathogenic. Popularity use probiotic powder because ease of use, stability and flexibility for used. (Huang *et al*., 2017) for good performance of probiotic powder product is necessary to have high initial probiotics because through the digestive tract, probiotic may be reduce, resulting inadequate animal health promotion. Moreover, storage time, temperature including various factors in the production process. Therefore, this study will focus on studying the influence of temperature on the survival rate of probiotic powders and establish the equation for predict the shelf life of probiotic product to develop products and maximize efficiency before distribution to farmers.

2. Materials and Methods

2.1 Starter preparation and solid state fermentation

One millilitre of the freeze-dried of *Lactobacillus plantarum* 7-40 was reactivated in MRS broth 20 mL and incubated at 37 °C for 24 h. Subsequently, the culture to MRS and incubated at 37 °C for 24 h. The cultured medium was inoculate to 5 kg of SBM which added with 5 L of water and inoculated at 30 °C for 24 h

One millilitre of the freeze-dried of *Bacillus subtilis* E20 was reactivated in No.3 broth containing molasses 20 g, glucose 5 g, KH_2PO_4 0.2 g, $MgSO_4$ 0.2 g in 1 L distilled water for starter prepare 20 mL incubated at 37 °C, shaking at 150 rpm for 24 h subsequently, the culture to No.3 broth and incubated at 37 °C shaking at 150 rpm for 24 h. The cultured medium was inoculate to 5 kg of SBM which added with 5 L of hot water and inoculated at 35 °C for 24 h

One millilitre of the freeze-dried of *Saccharomyces cerevisiae* P13 was reactivated in YM broth 20 mL incubated at 30 °C, shaking at 150 rpm for 24 h subsequently, the culture to YM broth and incubated at 30 °C shaking at 150 rpm for 24 h. The cultured medium was inoculate to 5 kg of SBM which added with 5 L of 5% molasses and starter 500 mL incubated at 30 °C for 24 h

2.2 Storage stability

The fermented soy bean meal containing probiotic was dried at 40 °C for 24 h, before grinded to be probiotic powder. Probiotic powder products have 3 types contained, the code 1 and code 2 were contained single

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strain as *L. plantarum* 7-40 and *B. subtilis* E20, respectively. While codes 5 was contained with *L. plantarum* 7-40, *B. subtilis* E20 and *S. cerevisiae* P13. The final product was stored at 4, 25, 35, 45 and 55 °C and sample was collected during time interval for measuring the viable cell count.

2.3 Analytical method

Thirty grams of sample was mixed with 270 mL of 0.85% NaCl and distributed in stomacher for 2 min. Then, the solution was serial diluted and determined the viable cell number by pour plate technique

2.4 Shelf life prediction

Determination of specific death rate (k_d,day-^{1})

$$\ln(N_t/N_o) = -k_d t \qquad (1)$$

Where N_0 is viable cell number at initial time (log CFU/g), N_t is viable cell number at t (log CFU/g)
k_d is specific death rate (day^{-1}), t is storage time (day)

The relation of k_d and storage temperature was presented by Arrhenius equation (eq. (2))

$$\ln k_d = \ln A - (E_a/RT) \qquad (2)$$

Where A is Arrhenius constant, R is gas constant (8.314 J/K·mole), T is absolute temperature (°K), E is activated energy (J/mol)

3. Results and discussion

3.1 Effect of temperature on survival of microorganisms in probiotic powder product at various temperatures

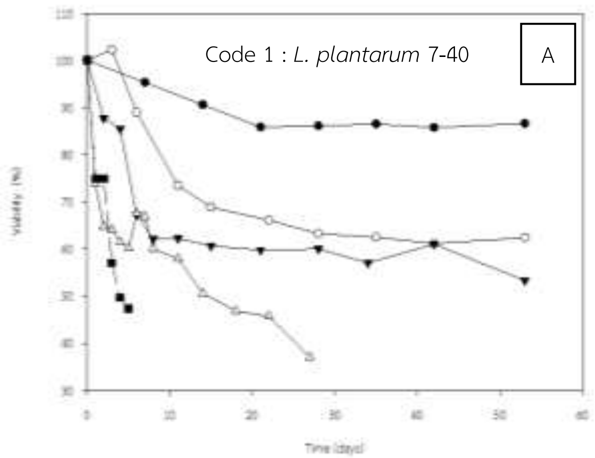
The result showed in Fig 1A. It was found that was decreased with increasing of storage temperature. The viable cell count of *L. plantarum* 7-40 was reduced from log 8.05 CFU/g to log 6.97 CFU/g, log 5.02 CFU/g, log 4.29 CFU/g, log 2.98 CFU/g and log 3.81 CFU/g at 4, 25, 35, 45 and 55 °C of storage temperature. Moreover the survival rate of *L. plantarum* 7-40 was decreased more than 50% within 5 and 27 day of storage at 45 and 55 °C, respectively. It was suggested that the *L. plantarum* 7-40 normally grow at 35-37 °C. By this rational, the *L. plantarum* 7-40 could not survive under high temperature of storage.

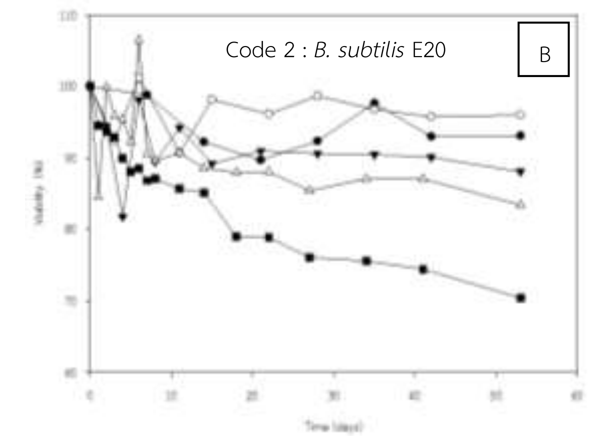
The result showed in Fig 1B. It was found that was decreased with increasing of storage temperature. The viable cell count of *B. subtilis* E20 was reduced from log 7.93 CFU/g to log 7.38 CFU/g, log 7.61 CFU/g, log 6.98 CFU/g, log 6.61 CFU/g and log 5.58 CFU/g at 4, 25, 35, 45 and 55 °C of storage temperature. Moreover the survival rate of *B. subtilis* E20 was not significantly. It was suggested that the 45 °C could survive under high temperature of storage.

The result showed in Fig 1C. It was found that was decreased with increasing of storage temperature. The viable cell count of *L. plantarum* 7-40 was reduced from log 6.61 CFU/g to log 6.20 CFU/g, log 6.21 CFU/g, log 5.75 CFU/g, log 3.84 to log 6.20 CFU/g, log 6.21 CFU/g, log 5.75 CFU/g, log 3.84 CFU/g and log 4.35 CFU/g at 4, 25, 35, 45 and 55 °C of storage temperature. Moreover the survival rate of *L. plantarum* 7-40 can tolerance to high temperature only 41 days.

The result showed in Fig 1D. It was found that was decreased with increasing of storage temperature. The viable cell count of *B. subtilis* E20 was reduced from log 6.20 CFU/g, log 6.21 CFU/g, log 5.75 CFU/g, log 3.84 CFU/g and log 4.35 CFU/g at 4, 25, 35, 45 and 55 °C of storage temperature. Moreover the survival rate of *B. subtilis* E20 was not significantly. It was suggested that the 45 °C

The result showed in Fig 1E. It was found that was decreased with increasing of storage temperature. The viable cell count of *S. cerevisiae* P13 was reduced from log 5.18 CFU/g to log 4.71 CFU/g, log 5.08 CFU/g, log 4.11 CFU/g, log 3.00 CFU/g and log 3.21 CFU/g at 4, 25, 35, 45 and 55 °C of storage temperature. Moreover the survival rate of *S. cerevisiae* P13 was decreased more than 50% within 18 and 3 day of storage at 45 and 55 °C, respectively. It was suggested that the *S. cerevisiae* P13 normally grow at 30 °C. By this rational, the *S. cerevisiae* P13 could not survive under high temperature of storage.





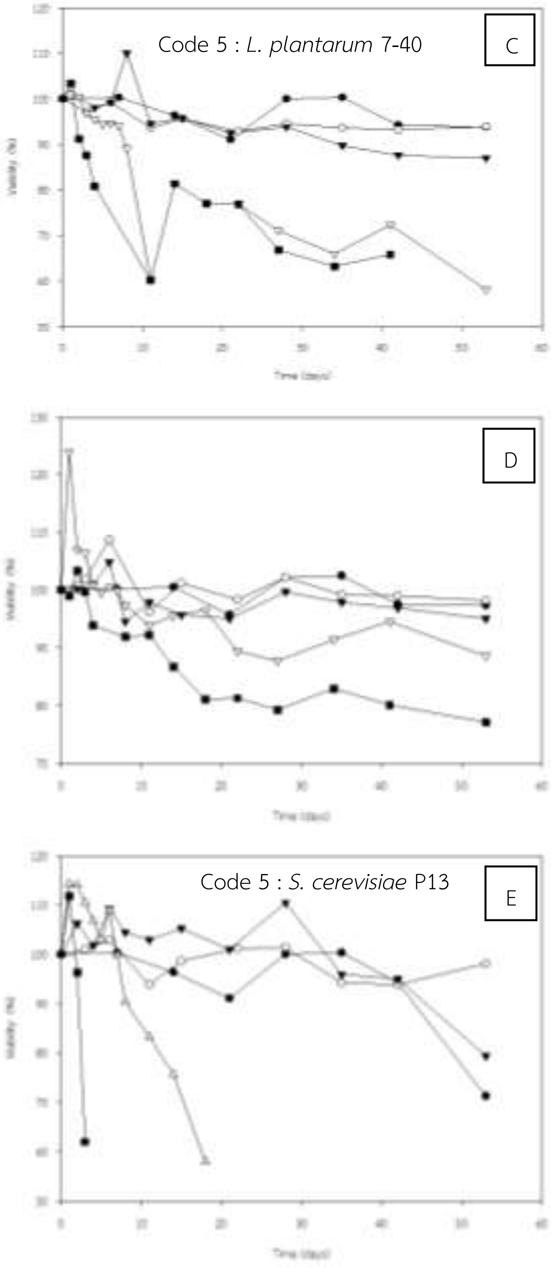


Fig. 1. Survival late of strains in probiotic powder product: (A) *L .plantarum* 7-40; code 1, (B) *B. subtilis* E20; code 2, (C) *L. plantarum* 7-40; code 5, (D) *B. subtilis* E20; code 5 and (E) *S. cerevisiae* P13; code 5 at various temperatures. (● is 4 °C, O is 25 °C, ▼ is 35 °C, ∆ is 45 °C, ■ is 55 °C)

3.2 Determination of specific date rate (k_d) at various temperatures

The k_d was expressed by using eq (1). The results demonstrated that the k_d of microorganism was effected by storage temperature. The increasing of storage temperature increased the k_d of all microorganism in all product. (Table 1)

Table 1

Specific date rate (k_d) at 4, 25, 35, 45 and 55 °C

Conditions	Strains	Temperature (°C)	K_d (day^{-1})
Code 1	7-40	4	0.0038
		25	0.0137
		35	00192
		45	0.0542
		55	0.1702
Code 2	E20	4	0.0040
		25	0.0050
		35	0.0055
		45	0.0060
		55	0.0090
Code 5	7-40	4	0.0035
		25	0.0050
		35	0.0060
		45	0.0100
		55	0.0129
	E20	4	0.0035
		25	0.0040
		35	0.0050
		45	0.0050
		55	0.0061
	P13	4	0.0026
		25	0.0050
		35	0.0060
		45	0.0190
		55	0.0300

3.3 Specific death rate of microorganism

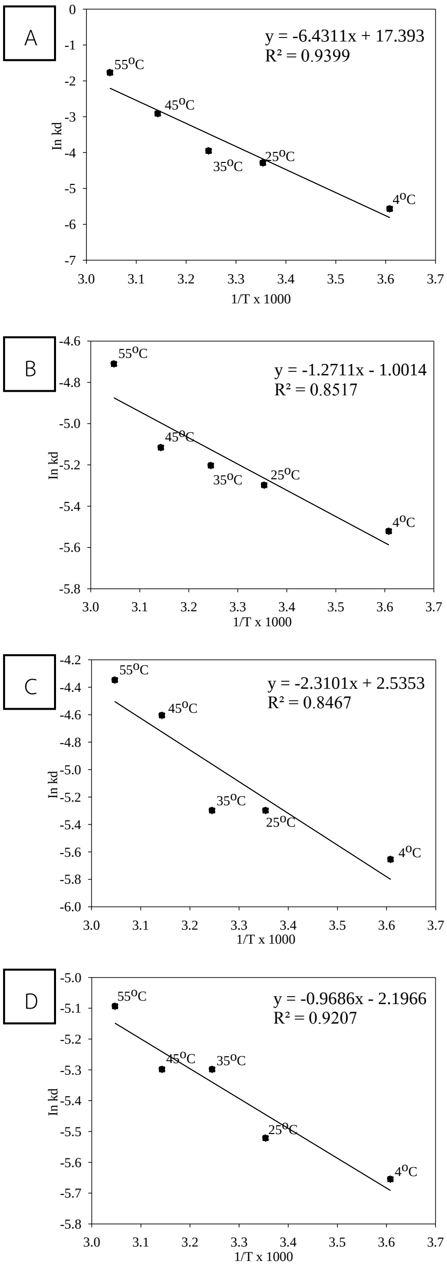
The k_d value from Table 1 is calculated as lnk_d and platted with temperature to was (kelvin,°K), in term of 1/T (Table 2 and Fig. 2). The results presented that the ln k_d was the function of 1/T as linear model.

Table 2

Specific date rate (k_d) at 4, 25, 35, 45 and 55 °C

code		Temp- (°K)	(1/T)*1000	lnk_d
Code1		277.15	3.608	-5.5677
		298.15	3.354	-4.2878
		308.15	3.245	-3.9525
		318.15	3.143	-2.9138
		328.15	3.047	-1.7707
Code 2		277.15	3.608	-5.5215
		298.15	3.354	-5.2983
		308.15	3.245	-5.2030
		318.15	3.143	-5.1160
		328.15	3.047	-4.7105
Code 5	7.40	277.15	3.608	-5.6550
		298.15	3.354	-5.2983
		308.15	3.245	-5.2983
		318.15	3.143	-4.6052
		328.15	3.047	-4.3477

	277.15	3.608	-5.6550
	298.15	3.354	-5.5215
E20	308.15	3.245	-5.2983
	318.15	3.143	-5.2983
	328.15	3.047	-5.0936
	277.15	3.608	-5.941
	298.15	3.354	-5.298
P13	308.15	3.245	-5.116
	318.15	3.143	-3.963
	328.15	3.047	-3.507



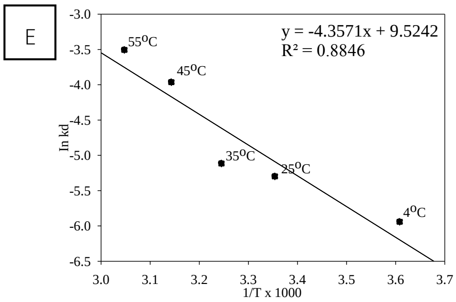


Fig. 2. Relationship between lnk_d and 1/T of (A) *L .plantarum* 7-40; code 1, (B) *B. subtilis* E20; code 2, (C) *L. plantarum* 7-40; code 5, (D) *B. subtilis* E20; code 5 and (E) *S. cerevisiae* P13; code 5 at 4, 25, 35, 45 and 55 °C.

Table 3

Arrhenius equation and R square

Code	Strains	Arrhenius equation	R^2
Code 1	7-40	lnk_d = -6.4311(1/T) + 17.393	0.9399
Code 2	E20	lnk_d = -1.2711(1/T) - 1.0014	0.8517
Code 5	7-40	lnk_d = -2.3101(1/T) + 2.5353	0.8467
	E20	lnk_d = -0.9686(1/T) - 0.9207	0.9207
	P13	lnk_d = -4.3571(1/T) + 9.5242	0.8846

3.4 Determination of survival rate by the Arenas equation

Prediction storage of probiotic powder product was descripted by Linear equation (Desmons *et al*., 1998), the k_d values which was obtained from the equation of the Arrhenius (Table 3), was used to construct the simple model for predict the viable cell during storage.

Table 4

Example of simple model for shelf life prediction of probiotic powder product at 4 and 55˚C of storage

Code	Strain	Temp-(°C)	Prediction model
Code 1	7-40	4	log N_t = log N_0 - (0.0030)t
		55	log N_t = log N_0 - (0.1105)t
Code 2	E20	4	log N_t = log N_0 - (0.0037)t
		55	log N_t = log N_0 - (0.0076)t
Code 5	7-40	4	log N_t = log N_0 - (0.0030)t
		55	log N_t = log N_0 - (0.011)t
	E20	4	log N_t = log N_0 - (0.0034)t
		55	log N_t = log N_0 - (0.0058)t
	P13	4	log N_t = log N_0 - (0.0020)t
		55	log N_t = log N_0 - (0.0234)t

4. Conclusion

The storage temperature effected the survival of microorganisms in all kind of probiotic product. The increasing of temperature would increase the specific death rate of bacteria and yeast probiotic. Thereby the Taiwanese farmer was suggested the probiotic product need to storage under low temperature for extending the shelf life of product. Moreover, this research also presented the simple shelf life prediction model for farmer. The farmer or buyer can apply this model to predict the cell viability at specific temperature. This result could help the farmer to manage the product in warehouse and avoid the opportunity of using expired product with animal.

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