Agriculture and Natural Resources 50 (2016) 1–7

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original article

Survival and shelf life of *Lactobacillus lactis* 1464 in shrimp feed pellet after fluidized bed drying

Maneerat Wirunpan,[a] Wanticha Savedboworn,[b] Penkhae Wanchaitanawong[a,*]

[a] Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand
[b] Department of Agro-Industry Technology and Management, Faculty of Agro-Industry, King Mongkut's University of Technology North Bangkok, Prachinburi Campus, Prachinburi Province 25230, Thailand

ARTICLE INFO

Article history:
Received 10 June 2014
Accepted 5 January 2015
Available online 14 January 2016

Keywords:
Fluidized bed drying
Lactobacillus lactis
Prediction model
Probiotic
Protective agents

ABSTRACT

In the present study, *Lactobacillus lactis* 1464 was attempted to be incorporated in shrimp feed pellets. The fresh culture (25% volume per weight) with and without pH adjustment was mixed into feed ingredients prior to the pelleting process at ambient temperature. The wet pellets were dried using a fluidized bed dryer at 50 °C, 60 °C, 70 °C and 80 °C to achieve a moisture content below 11%. The results indicated that the strain viability depended on the drying temperature with a viable cell number of approximately 10^6–10^8 CFU/g and the pH of the culture was found to affect the strain viability during drying. At all drying temperatures, the strain survival after drying ranged from 75.94% to 92.28% at pH 3.8 and from 89.54% to 96.87% at pH 7.0. Moreover, the addition of protectants was found to enhance the strain survival during drying. In particular, milk powder and monosodium glutamate (MSG) exhibited significant ($p < 0.05$) protective effect on the viability at a high temperature of 80 °C. During storage at 30 °C, a high survival rate was found for the strain with MSG and acacia gum. Furthermore, the prediction model for long-term storage stability of the strain was found to validate only at a low temperature of 4 °C, in comparison to a high temperature of 30 °C.

Copyright © 2016, Kasetsart University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Nowadays, probiotics offer a promising alternative approach for controlling shrimp diseases and improving shrimp health through their potential to control pathogens, to stimulate the immune response, to improve water quality and to enhance nutrition through the production of digestive enzymes (Verschuere et al., 2000; Gullian et al., 2004; Wang, 2007). To achieve health benefits, probiotic bacteria must be viable and available at high concentration, typically 10^6–10^7 CFU/g of product (Kosin and Rakshit, 2006). Furthermore, incorporation into feed pellets is more effective in conveying probiotics into animals compared to direct application into rearing systems. It is also applicable for intensive aquaculture and requires no additional labor or shrimp handling (Gómez et al., 2007). The viability and stability of probiotics have been a technological challenge in feed manufacturing because probiotics, including *Lactobacillus, Bacillus* and yeast, are susceptible to the high temperature of the pelleting and drying process. According to Biourge et al. (1998), *Bacillus* CIP5832 spores in dog diet were found to have in excess of 99% loss after the extrusion, expansion and drying processes. Furthermore, the viable count of yeast in shrimp feed pellet decreased by 10^5 fold after extruding through a meat grinder at 72 °C for 31 s followed by drying at 65 °C for 6 h (Aguirre-Guzmán et al., 2002).

Fluidized bed drying is extensively used for drying wet particulate and granular materials. In a fluidized bed dryer, the probiotic cell suspension is mixed with a vibrating bed of absorbers or matrix molecules which helps to form capsules by adherence (Nag and Das, 2013). This process is comparatively economical. It involves low energy consumption, high throughput and imparts moderate heat stress to the bacterial cells (Beker and Rapoport, 1987; Nag and Das, 2013). Furthermore, this process was successfully used for the preparation of dried granules or powders containing lactic acid bacteria (Santivarangkna et al., 2007; Nag and Das, 2013). According to Nag and Das (2013), fluidized bed drying was able to retain viability of *Lactobacillus casei* CRL 431 of more than 7.7 log CFU/g during storage at 25 °C for 12 wk. Mille et al. (2004) revealed that the *Lactobacillus plantarum* viability in casein powder was up to 80% after fluidized bed drying at 35 °C for 30 min. Correspondingly, the

* Corresponding author.
E-mail address: fagipkw@ku.ac.th (P. Wanchaitanawong).

http://dx.doi.org/10.1016/j.anres.2016.01.001
2452-316X/Copyright © 2016, Kasetsart University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

survival of *Lactobacillus brevis* in fish feed was 99% (10^8–10^9 CFU/g) after fluidized bed drying at 40 °C for 40–60 min with a moisture content of 5% (Toledo et al., 2010). Several studies have reported that the viability of lactic acid bacteria during drying and storage was enhanced by the addition of protective agents such as trehalose, skim milk, whey protein, soy protein isolate, monosodium glutamate, sucrose, lactose, sorbitol and polymers such as carboxymethyl cellulose, dextran and acacia gum (Morgan et al., 2006; Santivarangkna et al., 2007; Golowczyc et al., 2011; Lapsiri et al., 2013). In the present study, an overnight culture of *Lactobacillus lactis* 1464 was incorporated into shrimp feed pellets prior to the pelleting process at ambient temperature and dried in a fluidized bed dryer to achieve a moisture content lower than 11%. The effect of the drying temperature, culture pH and protectants on the strain survival during drying was determined. Additionally, the storage stability of the strain in the pellets at 4 °C and 30 °C was also evaluated.

Materials and methods

Preparation of soymilk medium

Soymilk medium was prepared as described by Wang et al. (2002). Soybeans were washed and soaked overnight in distilled water. The soaked soybeans were blended with distilled water (soybean:water = 1:10 w/v) for 3 min and then filtered through a double-layer cheesecloth to obtain soymilk. 1% (w/v) glucose (Ajax Finechem; Taren Point, NSW, Australia) was added into the soymilk before sterilization at 121 °C for 15 min.

Microorganism

L. lactis 1464 isolated from the sediment of a Nile tilapia fish pond with antimicrobial activity against shrimp pathogen was obtained from the Department of Biotechnology, Kasetsart University, Thailand. After two successive transfers of the strain in de Man, Rogosa and Sharpe (MRS) broth (Merck; Darmstadt, Germany) at 37 °C for 24 h, the activated culture was again inoculated into MRS broth at 37 °C for 24 h which served as the inoculum (Lapsiri et al., 2011). The overnight cultures were conducted in 250 mL Erlenmeyer flasks containing 120 mL of sterile soymilk and inoculated with 5 mL of the inoculum (approximately 10^9 CFU/mL). The sample was incubated at 37 °C for 24 h.

Preparation of shrimp feed pellets

The formulated shrimp feed consisted of 40% fish meal, 8% shrimp head meal, 20% rice bran, 10% wheat flour, 5% sago flour, 10% horse tamarind leaves powder, 5% soybean oil and 2% premix by weight. The feed mixture was sterilized at 121 °C for 30 min and dried overnight in a hot air oven (Memmert GmbH; Memmert, Germany) at 55 °C. Gelatin (3% w/v) as a feed binder was mixed with the dried mixture in a stand mixer. The overnight culture (25% v/w) of *L. lactis* 1464 with and without pH adjustment to 7.0 with 5 M NaOH (Ajax Finechem; Taren Point, NSW, Australia) was then added. After mixing for 3 min, soybean oil was then added and mixed for 3 min again. The feed mixture was pressed into pellets at ambient temperature using a laboratory pellet mill with a 2 mm diameter (California Pellet Mill Co.; Crawfordsville, IN, USA). The wet pellets (250 g) were dried in a laboratory fluidized bed dryer (Sherwood Scientific; Cambridge, UK) with a 5 L stainless chamber at various air inlet temperatures—50 °C, 60 °C, 70 °C and 80 °C. The fluidizing air flow velocity was held constant at 3.10 m/s. Samples were collected during drying to determine the viability of *L. lactis* 1464 and the moisture content. The specific rate of degradation (k) of *L. lactis* 1464 during drying at constant temperature was calculated as described by first-order kinetics as shown in Equation (1) (Desmond et al., 1998).

$$\log N = \log N_0 - kt \quad (1)$$

where N_0 is the number of initial viable cells and N is the number of viable cells at any time both expressed in colony forming units (CFU) per gram, k is the specific rate of degradation per minute and t is the drying time in minutes. A plot of the term of log N versus time (t) yields the estimate of k from the slope.

Effect of protective agents on viability of L. lactis 1464 after fluidized bed drying

Each protective agent (5% w/v) including, monosodium glutamate (MSG) (Ajinomoto; Bangkok, Thailand), milk powder (Dumex; Samut Prakan, Thailand), acacia gum (MT Instrument; Bangkok, Thailand), maltodextrin (Du Zhi Xue, China) was added into the overnight cultures of *L. lactis* 1464 prior to mix with the feed mixture. The shrimp feed pellets were prepared by the same manner as described in the preparation of the shrimp feed pellets. The wet pellets with an initial moisture content of approximately 26.5% were dried at various temperatures (50 °C, 60 °C, 70 °C and 80 °C) until the moisture content was below 11%.

Storage of dried shrimp feed pellets

Dried shrimp feed pellets (5 g) were placed into plastic zip bags and kept at 4 °C and 30 °C for 6 mth. The viable cell counts were determined every month. Each treatment was duplicated.

Accelerated storage test

Dried shrimp feed pellets were incubated in a hot air oven at 50 °C, 60 °C, 70 °C and 80 °C. At 50 °C, samples were taken after 24 h, 48 h, 72 h, 96 h and 120 h of exposure; at 60 °C, after 3 h, 6 h, 9 h, 12 h, 15 h and 18 h; and at 70 °C and 80 °C after 1 h, 2 h, 3 h, 4 h, 5 h and 6 h to determine the residue viable counts. The specific rate of degradation (k) was calculated.

Enumeration viable counts

Viable counts were enumerated using a pour plate technique. The sample of feed pellets (5 g) was rehydrated with 45 mL of sterile 0.85% NaCl (Ajax Finechem; Taren Point, NSW, Australia) to obtain 1:10 dilution and mixed in a stomacher (Seward Laboratory Systems Inc; Davie, FL, USA) for 1 min. Serial dilutions were made for each sample and plated on MRS agar containing 0.03% bromocresol purple (Ajax Finechem; Taren Point, NSW, Australia). Plates were incubated at 37 °C for 24 h and enumerated for colony forming unit per gram (CFU/g). Each treatment was duplicated. The survival rates were calculated as: Survival rate (%) = $(\log N/\log N_0) \times 100$, where N_0 is the number of initial viable cells and N is the number of viable cells at any time both expressed in CFU per gram (Reddy et al., 2009).

Moisture content

The moisture content of the feed pellets was determined according to the relevant international standard 6496 (International Standard Organization, 1999).

Statistical analysis

All experiments were carried out in duplicate. The data were statistically analyzed for analysis of variance in a completely

randomized design. Significant divergences among mean values were established using Duncan's multiple range tests at the 95% confidence interval. All statistical analyses were performed using the SPSS Software version 12 (SPSS Inc., White Plains, NY, USA).

Results and discussion

Effect of temperatures and pH on the viability of L. lactis 1464 during fluidized bed drying

The fresh culture of *L. lactis* 1464 with and without pH adjustment was added into shrimp feed pellets and subjected to drying using a fluidized bed dryer to prevent deterioration over long term storage. The viability loss of the strain at pH 3.8 and pH 7.0 in shrimp feed pellets and the moisture content during the fluidized bed drying at various temperatures are shown in Fig. 1A and B. It was observed that the moisture content decreased gradually from approximately 26.5% to below 11% after drying at 50 °C for 15 min, 60 °C for 10 min, 70 °C for 5 min and 80 °C for 5 min. It was clear that higher temperatures resulted in a shorter time to reach the equilibrium moisture content. However, it affected the viability of the strain which decreased as the temperature increased. At pH 3.8, a cell reduction of only 0.57 log CFU/g and 0.94 log CFU/g with survival rates of 92.28% and 87.38%, respectively, (Table 1) were obtained during drying at 50 °C and 60 °C, respectively, while a high cell reduction of 1.63 log CFU/g and 1.80 log CFU/g with survival rates of 78.16% and 75.94%, respectively, were observed after drying at 70 °C and 80 °C, respectively. It is known that temperature is the important factor affecting the viability of probiotics during drying process (Chávez and Ledeboer, 2007). The viability loss was mainly due to the damage to the cell membrane and proteins (Ananta et al., 2005). Wang et al. (2004) reported that the numbers of bifidobacteria and other lactic acid bacteria decreased with increasing outlet air temperature in spray drying. A similar finding was reported by Bayrock and Ingledew (1997) for fluidized bed dried *Saccharomyces cerevisiae*. Additionally, the decrease in viable cell depends not only on the drying temperature but also on the time of exposure to heat (Chávez and Ledeboer, 2007). Hence, the drying time for biomaterial preservation should be as short as possible. In the current study, the wet pellets of probiotic shrimp feed were dried to achieve a moisture content below 11% and to maintain a high viable cell count (above 10^6 CFU/g) which was recommended for animal feed (Uppal et al., 2008). Therefore, drying times of 15 min, 10 min, 5 min and 5 min was sufficient for fluidized bed drying of shrimp feed pellets at 50 °C, 60 °C, 70 °C and 80 °C, respectively.

At pH 7.0 (Fig. 1B), a similar trend was also observed; however, the viability of the strain was more stable than at pH 3.8. The survival rate of the strain after drying ranged from 89.54% to 96.87% at all drying temperatures. As shown in Table 1, when heating at low pH, the reduction of the strain decreased faster than at high pH. The specific degradation rate (k) of the strain at pH 3.8 was higher than at pH 7.0 for every drying temperature. Many studies have reported that microorganisms have their maximum heat resistance at a pH value close to neutral (Ocio et al., 1994; López et al., 1996). Correspondingly, Juneja and Eblen (1999) found that the heat tolerance of bacteria decreased with decreasing pH and decreased even more when presented in high temperatures. Therefore, the strain with the pH adjustment to 7.0 was chosen for further study.

Effect of protective agents on survival of L. lactis 1464 in shrimp feed pellets

In order to enhance the viability of the strain during fluidized bed drying, four protective agents—MSG, acacia gum, milk powder and maltodextrin—were tested for their protective effect against various drying temperatures. As shown in Table 2, the survival rate of the strain was related to the type of protective agents. In all treatments, the strain exhibited greater high survival rates of approximately 84%–99% (viable cell number of 10^6–10^8 CFU/g) after the drying process. There was no significant difference in survival after drying at 50 °C. In contrast, a significant ($p < 0.05$) effect of protectants was observed during drying at 80 °C. Among the four protective agents, MSG showed the highest protective effect on the viability of the strain, followed by milk powder. This was consistent with the report of Sunny-Roberts and Knorr (2009), where the survival of spray-dried *Lactobacillus rhamnosus* increased approximately by 1 log cycle when MSG was added in the drying medium. MSG was able to stabilize the cell membrane via the reaction between its amino group and the carboxyl group of the microorganism proteins (Carvalho et al., 2003). The results also showed that milk powder exhibited a protective effect against high temperatures. This was probably because milk protein prevented cell injury by coating the cell wall proteins (Gharsallaoui et al., 2007; Ghandi et al., 2012) and stabilizing the cell membrane constituents (Reddy et al., 2009). Moreover, Silva et al. (2011) suggested that protein may create a structure which is easy to rehydrate after drying. The macromolecules of protein, such as sodium caseinate,

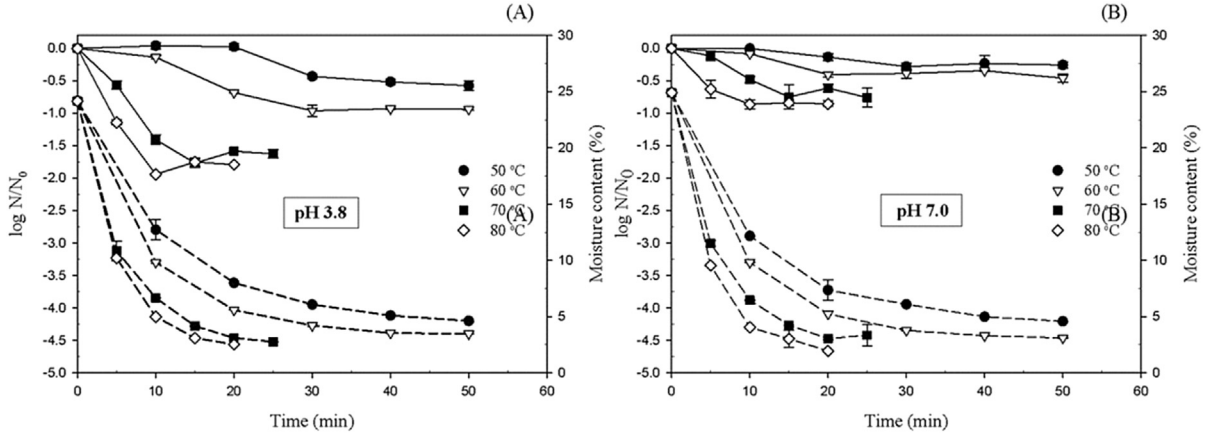

Fig. 1. Viability loss of *L. lactis* 1464 at (A) pH 3.8 and (B) pH 7.0 in shrimp feed pellets and moisture content during fluidized bed drying at various temperatures. Viability is shown with solid lines, while short dashed lines show the moisture content.

Table 1
Specific rate of degradation (k) and survival of *L. lactis* 1464 in shrimp feed pellets after fluidized bed drying at different temperatures.

Drying temperature (°C)	pH 3.8		pH 7.0	
	k^a (per min)	Survival (%)	k^a (per min)	Survival (%)
50	0.013	92.28 ± 0.92	0.005	96.87 ± 0.58
60	0.021	87.38 ± 0.2	0.006	94.38 ± 0.76
70	0.066	78.16 ± 0.8	0.025	90.73 ± 1.74
80	0.084	75.94 ± 0.18	0.034	89.54 ± 0.48

[a] k = Specific rate of degradation.

are not capable of passing through the structure of the peptidoglycan layer that covers the cell membrane of lactic acid bacteria (Ghandi et al., 2012). It is able to form non-interacting osmotically inactive bulking compounds causing spacing amongst cells and not allowing their cell walls to come closer and fuse (Oldenhof et al., 2005; Joshi and Thorat, 2011).

Viability of L. lactis 1464 in shrimp feed pellets during storage

In order to determine the shelf-life of *L. lactis* 1464 in shrimp feed pellets, the dried pellets were kept in plastic zip bags and stored at 4 °C and 30 °C for 6 mth. As shown in Fig. 2, the decline in viable cells is represented by the logarithmic value of the N/N_0 for different storage periods. After storage for 6 mth at 4 °C, there was no significant ($p > 0.05$) loss of initial viability of fluidized bed dried *L. lactis* 1464 either with or without protective agents. On the other hand, high mortality was observed in all treatments at the higher storage temperature of 30 °C. A similar finding was reported by Wang et al. (2004) where the viability of spray-dried *Streptococcus thermophilus* and *Bifidobacterium longum* decreased as the storage temperature increased. This was also consistent with Gardiner et al. (2002), who reported that the viability of a probiotic was maintained at 10^8 CFU/g during storage at 4 °C, while viable cells declined to 10^6 CFU/g after 49 d when stored at 30 °C.

In addition, it was also observed that the reduction rate of viable cells at 30 °C was markedly influenced by the drying temperature. The viability of the strain dried at higher temperature declined faster than at lower temperature. For example, the viability of the strain dried at 50 °C (Fig. 2A) slightly decreased approximately 2–4 log CFU/g (from approximately 10^8 CFU/g to approximately 10^3–10^5 CFU/g) after 6 mth. In contrast to the strain dried at 80 °C, viable cells reduced about 1.5–3 log CFU/g in 3 mth and decreased rapidly to an undetectable level after 4 mth (Fig. 2D). A similar trend was found in the pellets dried at 70 °C (Fig. 2C). Furthermore, the viability of the strain during storage was enhanced by the addition of protectants. All protectants showed a similar ability to protect cells in the first 3 mth. After 4 mth, acacia gum and MSG exhibited significant protective ability for the strain dried at 50 °C and 60 °C whereas no significant protective effect on the strain dried at 70 °C and 80 °C was observed. The viability loss during storage was mainly due to the lipid oxidation of the cell membrane (Teixeira et al., 1996; Santivarangkna et al., 2007). Acacia gum and MSG exhibited protective coating on the cell wall and had antioxidant properties which could prevent cell damage due to an oxidation reaction (Desmond et al., 2002; Sunny-Roberts and Knorr, 2009).

From the results, cell injury and inactivation occur not only during feed processing, but also during storage. The viability of probiotic bacteria during storage is inversely related to the storage temperature (Gardiner et al., 2000). It is clear that pellets stored at 4 °C showed the highest stability over 6 mth. However, refrigeration is inconvenient for the supplier and retailer due to its high cost. Thus, it is a major challenge to produce probiotic products that are stable at ambient temperature. In this study, the viability of the strain dried at 50 °C and 60 °C with the addition of acacia gum and MSG was maintained at 10^7 CFU/g over 3 mth which could still meet the requirements for the level of viable cells in probiotic products.

Prediction of the storage stability of L. lactis 1464 in shrimp feed pellets

In this study, the accelerated storage test was used to predict the storage stability of the strain in dried feed pellets. The changes of *L. lactis* 1464 viability in the 70°C-dried pellets with the addition of MSG under storage temperatures of 50 °C, 60 °C, 70 °C and 80 °C are shown in Fig. 3 and the slope of each line is equal to the specific degradation rate per hour (k) as shown in Table 3. The correlation between the temperature and the k value can be described by the Arrhenius equation as shown in Equation (2) (Lee, 1991).

$$k = k_0 e^{(-E_a/RT)} \quad (2)$$

where R is the universal gas constant (8.32 J/mol.K), E_a is the apparent activation energy in kilojoules per mol, T is the absolute temperature in degrees kelvin and k_0 is the pre-exponential constant. In Fig. 4, when the log k values were plotted against 1/T, the regression equation was obtained as log k = 28.048–9.4984 [(1/T) × 1000] which was used for determination of the k value at 4 °C and 30 °C

Table 2
Survival of *L. lactis* 1464 in shrimp feed pellets with various protective agents after fluidized bed drying at various temperatures, not adjusted pH in culture in shrimp feed pellets.

Protective agent	Survival* (%)			
	50 °C, 15 min	60 °C, 10 min	70 °C, 5 min	80 °C, 5 min
Control	98.42 ± 1.50^a	97.20 ± 0.16^{ab}	96.98 ± 0.08^{ab}	86.02 ± 0.23^d
MSG†	97.47 ± 0.13^a	98.21 ± 0.07^{ab}	99.36 ± 0.41^a	92.43 ± 0.14^a
Acacia gum	97.93 ± 0.40^a	98.78 ± 0.11^a	98.50 ± 0.88^a	85.31 ± 0.05^e
Milk powder	97.38 ± 0.59^a	97.37 ± 0.70^b	98.49 ± 1.08^a	91.25 ± 0.18^b
Maltodextrin	97.76 ± 0.37^a	96.09 ± 0.12^c	95.83 ± 0.06^b	87.50 ± 0.13^c

*Values in the same column with different superscripted lowercase letters (a–e) are significantly different using Duncan's multiple range test ($p < 0.05$).
†MSG = monosodium glutamate.

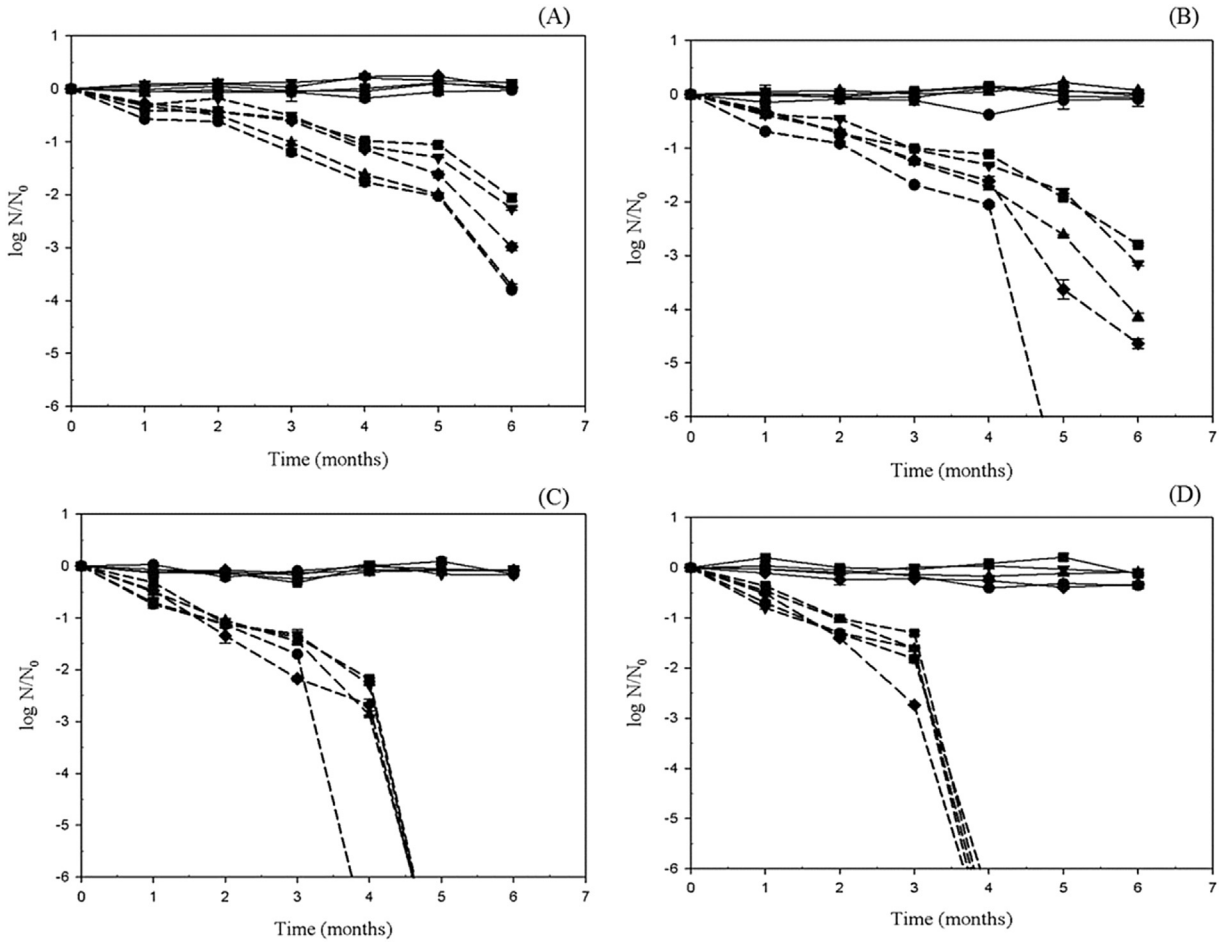

Fig. 2. Viability loss of *L. lactis* 1464 in feed pellet dried at: (A) 50 °C for 15 min; (B) 60 °C for 10 min; (C) 70 °C for 5 min; (D) 80 °C for 5 min during storage at 4 °C (solid lines) and 30 °C (short dash lines) for 6 mth with various protectants. (●; Control, ▼; MSG, ■; acacia gum, ◆; milk powder, ▲; maltodextrin). MSG = monosodium glutamate.

($k_4 = 6 \times 10^{-7}$/h and $k_{30} = 5 \times 10^{-4}$/h). Therefore, the prediction models of stability at 4 °C and 30 °C were obtained by replacing the value of N_0 and k_4 or k_{30} in Equation (1), as shown in Equations (3) and (4), respectively:

$$\log N = \log N_0 - 6 \times 10^{-7} t \quad (3)$$

$$\log N = \log N_0 - 5 \times 10^{-4} t \quad (4)$$

To validate Equations (3) and (4), the theoretical viability calculated from these equations and the experimental viability obtained from stability tests were compared. As shown in Fig. 5, there was no significant difference between the predicted and experimental viability during storage at 4 °C over 12 mth, confirming that the theoretical viability from Equation (3) can be used as a prediction model of stability of this strain in dried feed pellets. On the other hand, a great difference between the predicted experimental viability was found at 30 °C storage. The experimental viability followed the linear regression equation within

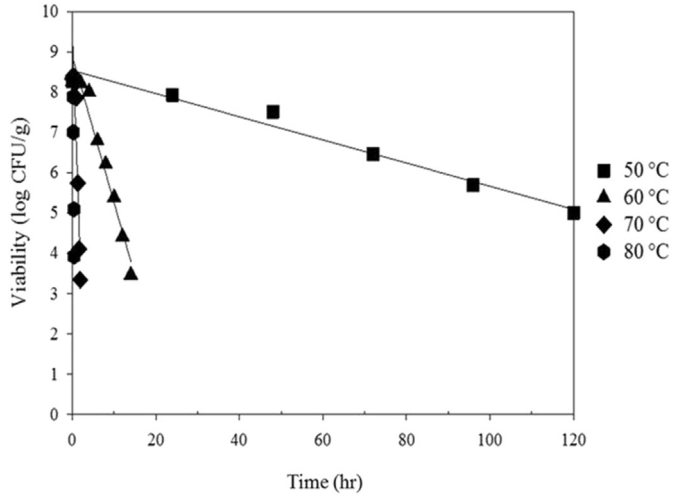

Fig. 3. Viability in the 70°C-dried pellets with the addition of monosodium glutamate stored at various temperatures.

Table 3
Specific degradation rate at 50 °C, 60 °C, 70 °C and 80 °C of *L. lactis* 1464 in pellets dried at 70 °C.

Storage temperature (°C)	Specific rate of degradation* (k, per h) (R^2)
50	0.0383^a (0.981)
60	0.3642^b (0.961)
70	2.9332^c (0.828)
80	11.2660^d (0.855)

*Values with different superscripted lowercase letters (a–d) are significantly different using Duncan's multiple range test ($p < 0.05$).

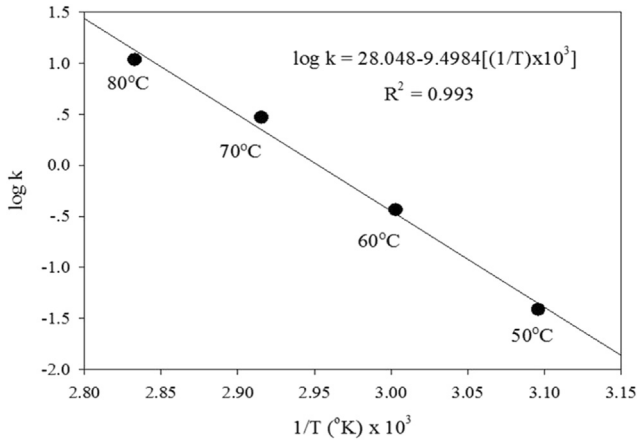

Fig. 4. Arrhenius plot for the inactivation of *L. lactis* 1464 in the pellets dried at 70 °C.

4 mth and exhibited a higher cell reduction than viability from the prediction model. It was found that the experimental *k* value was approximately 1.4 times higher than the predicted *k* value. This was probably due to a change in the physical state of the dried pellets during accelerated storage testing (Labuza and Riboh, 1982; Karmas et al., 1992). Another possible reason why the model overestimates is a possible contribution of nonenzymatic browning which is strongly temperature-dependent (Karmas et al., 1992; Kurtmann et al., 2009a, 2009b). The rate of browning is low below a critical temperature, above which the rate of the reaction increases substantially (Roos, 2001). Nonenzymatic browning is not always prevented in the glassy state. The reaction rates were much lower at temperatures below Tg compared with temperatures above Tg (Kawai et al., 2005). Few studies have been successful in predicting the viability of lactic acid bacteria during storage. Hamsupo et al. (2005) reported that there was no significant difference in viability between prediction and experimental survival rates of spray-dried *Lactobacillus reuteri* KUB-AC5 at 4 °C and 30 °C for 4 mth. This could indicate that the predicted model may vary according to the strain of microorganisms and also the capability of the protective agents used (Lapsiri et al., 2012).

In conclusion, this study indicated that it is possible to prepare shrimp feed pellet with high viable cell numbers of probiotic bacteria using fluidized bed drying. The viability of the strain during drying varied depending on the drying temperature, culture pH and type of protectants. However, the remaining viable cells in pellets were 10^6–10^8 CFU/g with a moisture content below 11% after drying at every drying temperature which met the level requirements for probiotic products. The study of probiotic product storage revealed that the drying and storage temperatures are critical factors for microbial survival. The addition of some protective agents enhanced viability during drying and storage. Moreover, the accelerated storage testing was a rapid and simple technique for estimating the long-term shelf life of this probiotic product but at a low specific temperature of 4 °C only in the model.

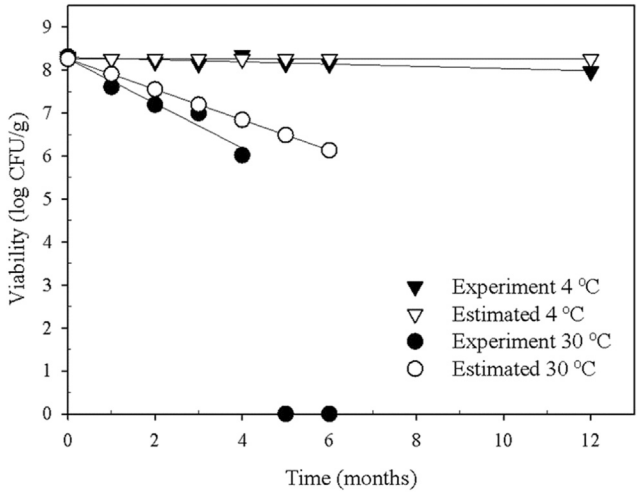

Fig. 5. Comparison of estimated and experimentally measured viability of *L. lactis* 1464 during storage at 4 °C and 30 °C.

Conflict of interest

There is no conflict of interest.

References

Aguirre-Guzmán, G., Ricque-Marie, D., Cruz-Suárez, L.E. 2002. Survival of agglomerated *Saccharomyces cerevisiae* in pelleted shrimp feeds. Aquaculture 208: 125–135.
Ananta, E., Volkert, M., Knorr, D. 2005. Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* GG. Int. Dairy J. 15: 399–409.
Bayrock, D., Ingledew, W.M. 1997. Mechanism of viability loss during fluidized bed drying of baker's yeast. Food Res. Int. 30: 417–425.
Beker, M., Rapoport, A. 1987. Conservation of yeasts by dehydration. Biotechnol. Meth. 35: 127–171.
Biourge, V., Vallet, C.L., Levesque, A., Sergheraert, R., Chevalier, S.P., Roberton, J.L. 1998. The use of probiotics in the diet of dogs. J. Nutr. 128: 2730S–2732S.
Carvalho, A.S., Silva, J., Ho, P., Teixeira, P., Malcata, F.X., Gibbs, P. 2003. Protective effect of sorbitol and monosodium glutamate during storage of freeze-dried lactic acid bacteria. Lait 83: 203–210.
Chávez, B.E., Ledeboer, A.M. 2007. Drying of probiotics: optimization of formulation and process to enhance storage survival. Dry. Technol. 25: 1193–1201.
Desmond, C., Ross, R.P., O'Callaghan, E., Fitzgerald, G., Stanton, C. 2002. Improved survival of *Lactobacillus paracasei* NFBC 338 in spray-dried powders containing gum acacia. J. Appl. Microbiol. 93: 1003–1011.
Desmond, S., Krhouz, H., Evrard, P., Thonart, P. 1998. Improvement of lactic cell production. Appl. Biochem. Biotechnol. 70–72: 513–526.
Gardiner, G.E., Bouchier, P., O'Sullivan, E., Kelly, J., Collins, J.K., Fitzgerald, G., Ross, R.P., Stanton, C. 2002. A spray-dried culture for probiotic Cheddar cheese manufacture. Int. Dairy J. 12: 749–756.
Gardiner, G.E., O'Sullivan, E., Kelly, J., Auty, M.A.E., Fitzgerald, G.F., Collins, J.K., Ross, R.P., Stanton, C. 2000. Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. Appl. Environ. Microbiol. 66: 2605–2612.
Ghandi, A., Powell, I.B., Chen, X.D., Adhikari, B. 2012. The effect of dryer inlet and outlet air temperatures and protectant solids on the survival of *Lactococcus lactis* during spray drying. Dry. Technol. 30: 1649–1657.
Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., Saurel, R. 2007. Applications of spray-drying in microencapsulation of food ingredients: an overview. Food Res. Int. 40: 1107–1121.
Golowczyc, M., Gerez, C., Silva, J., Abraham, A., De Antoni, G., Teixeira, P. 2011. Survival of spray-dried *Lactobacillus kefir* is affected by different protectants and storage conditions. Biotechnol. Lett. 33: 681–686.
Gómez, R.G.D., Balcázar, J.L., Shen, M. 2007. Probiotics as control agents in aquaculture. J. Ocean. Univ. China 6: 76–79.
Gullian, M., Thompson, F., Rodriguez, J. 2004. Selection of probiotic bacteria and study of their immunostimulatory effect in *Penaeus vannamei*. Aquaculture 233: 1–14.
Hamsupo, K., Sukyai, P., Loiseau, G., Nitisinprasert, S., Montet, D., Wanchaitanawong, P. 2005. Prediction on the stability of spray-dried *Lactobacillus reuteri* KUB-AC5 by Arrhenius Equation for long-term storage. J. Microbiol. Biotechnol. 15: 1178–1182.
International Standard Organization 1999. Animal Feeding Stuffs. Determination of Moisture and Other Volatile Matter Content. US ISO No. 6496.
Joshi, V.S., Thorat, B.N. 2011. Formulation and cost-effective drying of probiotic yeast. Dry. Technol. 29: 749–757.
Juneja, V.K., Eblen, B.S. 1999. Predictive thermal inactivation model for *Listeria monocytogenes* with temperature, pH, NaCl, and sodium pyrophosphate as controlling factors. J. Food Prot. 2: 986–993.
Karmas, R., Pilar, B.M., Karel, M. 1992. Effect of glass transition on rates of nonenzymic browning in food systems. J. Agr. Food Chem. 40: 873–879.
Kawai, K., Hagiwara, T., Takai, R., Suzuki, T. 2005. The rate of non-enzymatic browning reaction in model freeze-dried food system in the glassy state. Innov. Food Sci. Emerg. 6: 346–350.
Kosin, B., Rakshit, K.S. 2006. Microbial and processing criteria for production of probiotics: a review. Food Technol. Biotechnol. 44: 371–379.
Kurtmann, L., Carlsen, C.U., Skibsted, L.H., Risbo, J. 2009a. Water activity-temperature state diagrams of freeze-dried *Lactobacillus acidophilus* (La-5): influence of physical state on bacterial survival during storage. Biotechnol. Progr. 25: 265–270.

Kurtmann, L., Skibsted, L.H., Carlsen, C.U. 2009b. Browning of freeze-dried probiotic bacteria cultures in relation to loss of viability during storage. J. Agr. Food Chem. 57: 6736–6741.
Labuza, T.P., Riboh, D. 1982. Theory and application of Arrhenius kinetics to the prediction of nutrient losses in foods. Food Technol. 36: 66–74.
Lapsiri, W., Bhandari, B., Wanchaitanawong, P. 2013. Stability and probiotic properties of *Lactobacillus plantarum* spray-dried with protein and other protectants. Dry. Technol. 31: 1723–1733.
Lapsiri, W., Bhandari, B., Wanchaitanawong, P. 2012. Viability of *Lactobacillus plantarum* TISTR 2075 in different protectants during spray drying and storage. Dry. Technol. 30: 1407–1412.
Lapsiri, W., Nitisinprasert, S., Wanchaitanawong, P. 2011. *Lactobacillus plantarum* strains from fermented vegetables as potential probiotics. Kasetsart J. (Nat. Sci.) 45: 1071–1082.
Lee, J.M. 1991. Sterilization. Prentice Hall, Englewood Cliffs, NJ, USA.
López, M., González, I., Condón, S., Bernardo, A. 1996. Effect of pH heating medium on the thermal resistance of *Bacillus stearothermophilus* spores. Int. J. Food Microbiol. 28: 405–410.
Mille, Y., Obert, J.P., Beney, L., Gervais, P. 2004. New drying process for lactic bacteria based on their dehydration behavior in liquid medium. Biotechnol. Bioeng. 88: 71–76.
Morgan, C.A., Herman, N., White, P.A., Vesey, G. 2006. Preservation of micro-organisms by drying: a review. J. Microbiol. Meth. 66: 183–193.
Nag, A., Das, S. 2013. Improving ambient temperature stability of probiotics with stress adaptation and fluidized bed drying. J. Funct. Foods 5: 170–177.
Ocio, M.J., Sánchez, T., Fernandez, P.S., Rodrigo, M., Martínez, A. 1994. Thermal resistance characteristics of PA 3679 in the temperature range of 110–121 °C as affected by pH, type of acidulant and substrate. Int. J. Food Microbiol. 22: 239–247.
Oldenhof, H., Wolkers, W.F., Fonseca, F., Passot, S., Marin, M. 2005. Effect of sucrose and maltodextrin on the physical properties and survival of air-dried *Lactobacillus bulgaricus*: an in situ Fourier transform infrared spectroscopy study. Biotechnol. Progr. 21: 885–892.
Reddy, K.B.P.K., Madhu, A.N., Prapulla, S.G. 2009. Comparative survival and evaluation of functional probiotic properties of spray-dried lactic acid bacteria. Int. J. Dairy Technol. 62: 240–248.
Roos, Y.H. 2001. Water activity and plasticization. In: Michael Eskin, N.A., Robinson, D.S. (Eds.), Food Shelf Life Stability. Chemical, Biochemical and Microbiological Changes. CRC Press, London, UK.
Santivarangkna, C., Kulozik, U., Foerst, P. 2007. Alternative drying processes for the industrial preservation of lactic acid starter cultures. Biotechnol. Progr. 23: 302–315.
Silva, J., Freixo, R., Gibbs, P., Teixeira, P. 2011. Spray-drying for the production of dried cultures. Int. J. Dairy Technol. 64: 321–335.
Sunny-Roberts, E.O., Knorr, D. 2009. The protective effect of monosodium glutamate on survival of *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* E-97800 (E800) strains during spray-drying and storage in trehalose-containing powders. Int. Dairy J. 19: 209–214.
Teixeira, P., Castro, H., Kirby, R. 1996. Evidence of membrane lipid oxidation of spray-dried *Lactobacillus bulgaricus* during storage. Lett. Appl. Microbiol. 22: 34–38.
Toledo, N., Ferrer, J., Borquez, R. 2010. Drying and storage stability of a probiotic strain incorporated into a fish feed formulation. Dry. Technol. 28: 508–516.
Uppal, D.S., Ilyas, S.M., Silla, S.S. 2008. Quality and Safety of Animal Feed in India. United Nations Asian and Pacific Center for Agricultural Engineering and Machinery. Available from: http://www.unapcaem.org/activities%20files/a16/animal%20feed%20&%20quality.pdf [Sourced: 27 December 2008].
Verschuere, L., Rombaut, G., Sorgeloos, P., Verstraete, W. 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiol. Mol. Biol. Rev. 65: 655–671.
Wang, Y., Yu, R.C., Chou, C.C. 2004. Viability of lactic acid bacteria and bifidobacteria in fermented soymilk after drying, subsequent rehydration and storage. Int. J. Food Microbiol. 93: 209–217.
Wang, Y.B. 2007. Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. Aquac 269: 259–264.
Wang, Y.C., Yu, R.C., Chou, C.C. 2002. Growth and survival of bifidobacteria and lactic acid bacteria during the fermentation and storage of cultured soymilk drinks. Food Microbiol. 19: 501–508.