microbiology research

MDPI

Article

Accelerated Storage Testing of Vacuum-Dried *Lactobacillus acidophilus TISTR 1338* for Feed

Apichet Muenaram [1], Bhundit Innawong [2], Imrana Niaz Sultan [1,3], Muhammad Waseem Khan [3], Hamza Ghilzai [3], Afrasiab Khan Tareen [1,3,*] and Pramuk Parakulsuksatid [1,*]

[1] Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand; api_m99@hotmail.com (A.M.); imrana.niaz@buitms.edu.pk (I.N.S.)
[2] Institute for Local Economic Foundation, Kanchanaburi 71000, Thailand; b_innawong@yahoo.com
[3] Department of Biotechnology, Faculty of Life Sciences, Balochistan University of Information Technology Engineering & Management Sciences, Quetta 87300, Pakistan; muhammad.waseem@buitms.edu.pk (M.W.K.); hamza.ghilzai1@gmail.com (H.G.)
* Correspondence: afrasiab.tareen@buitms.edu.pk (A.K.T.); fagipmp@ku.ac.th (P.P.); Tel.: +66-664-45945 (A.K.T. & P.P.)

Abstract: Lactic acid bacteria (LAB) are widely used to produce various food products, adding flavor, texture, and health benefits. The bacteria are commonly grown on expensive nutrients like glucose, sucrose, and yeast extracts, which makes them commercially unappealing. In the current study, *Lactobacillus acidophilus TISTR 1338* culture was studied using spent cell yeast as a nitrogen source and molasses as a carbon source. The drying process used to create starter cultures of *Lactobacillus acidophilus TISTR 1338* was vacuum drying. After vacuum drying, this bacterium had a survival rate of 8.08 log CFU/g. The dried strain survived for four months at 37 °C. With wasted cells at 0.5%, molasses concentration at 11% at 2.14 109 CFU/mL at 22 h, precise growth rate at 0.39 h^{-1}, and yield cell mass at 1.67 1011 CFU/g sugar, yeast produced the maximum cell mass. The lower viability of the tested strain was induced by a higher temperature during this prolonged storage. Meanwhile, dehydrated starter culture was subjected to accelerated storage testing at 50, 60, and 70 °C. To determine the vacuum-dried *Lactobacillus acidophilus TISTR 1338*'s long-term storage viability, a temperature-dependent prophecy model was created. Molasses and spent cell yeast serve as promising carbon and nitrogen sources when optimized conditions are employed. The study also suggests that vacuum drying is a promising method for producing dried cells suitable for non-refrigerated storage conditions.

Keywords: accelerated storage testing; Arrhenius theory; shelf life; vacuum drying; *Lactobacillus acidophilus TISTR 1338*

Academic Editor: Juan Ayala

Received: 1 December 2024
Revised: 26 December 2024
Accepted: 27 December 2024
Published: 29 December 2024

Citation: Muenaram, A.; Innawong, B.; Sultan, I.N.; Khan, M.W.; Ghilzai, H.; Tareen, A.K.; Parakulsuksatid, P. Accelerated Storage Testing of Vacuum-Dried *Lactobacillus acidophilus TISTR 1338* for Feed. *Microbiol. Res.* **2025**, *16*, 6. https://doi.org/10.3390/microbiolres16010006

1. Introduction

Lactic acid bacteria (LAB) are widely employed in the production of various food products. However, the media commonly used to support their growth are not cost-effective due to the high expense of nutrients like glucose and sucrose as carbon sources, as well as yeast extract and peptone as nitrogen sources. These costly ingredients make the media economically unappealing for large-scale applications [1]. Therefore, less expensive materials, such as molasses, are of interest as potential low-cost nutrient sources [2,3]. Spent cell yeast has also been utilized as a nutrient source for lactic acid production [4].

Lactobacillus acidophilus TISTR 1338 is a very strong probiotic strain that shows high acid adaptability and tolerance for both harsh and drying temperatures. Additionally, it

has shown promising results in health-promoting functional foods and nutritional supplements. Because of the major brand proteins involved in stress adaptation, DnaK and GrpE, *Lactobacillus acidophilus TISTR 1338* has the highest viability among the others (acid and bile condition). At the sub-lethal level, acid-adapted *L. acidophilus TISTR 1338* can be used for processing at both high and low temperatures [5].

By suppressing dangerous bacteria and maintaining pH stability, the strain also generates lactic acid and may synthesize antibiotics, both of which contribute to the balance of the gut microbiota [6]. When used therapeutically, it helps manage gastrointestinal issues and boosts immunological function [7]. Additionally, it is more suitable for a variety of food formulations because of its resistance to oxidative stress, moderate heat, and infections like *Escherichia coli* and Salmonella [6].

The majority of lactic acid bacteria commercial starter cultures are stored in lyophilized form, as this method ensures relatively high levels of activity and the maintenance of survival; however, since producing dried cultures through freeze drying is expensive, there have been numerous efforts to discover and develop more cost-effective drying techniques [8]. One of the effective methods for preserving lactic acid bacteria starter cultures is vacuum drying, which has been explored as a cost-efficient alternative to freeze drying while maintaining adequate levels of activity and survival [8]. Vacuum drying enables water removal at controlled low temperatures, preventing thermal inactivation of cells without freezing the final product, unlike lyophilization. Nevertheless, the low viability of cells during the drying process has hindered its commercial development despite its potential. This limitation necessitates further research into dehydration procedures to optimize vacuum drying. Additionally, many researchers opted for spray drying as an alternative to freeze drying. This method involves atomizing a LAB-containing suspension into a hot gas stream, where rapid evaporation occurs, leaving dry particles behind [9]. Conversely, spray drying has some advantages over freeze drying in terms of cost and continuous productivity [10], still, its primary disadvantages lie in the potential for oxidative and osmotic stress, as well as exposure to high temperature, which can limit its use in some cases [11]. Meanwhile, accelerated storage testing is widely used for extrapolating shelf life and predicting storage quality and stability [12].

The Arrhenius equation is the most widely accepted and reliable theory for explaining how temperature influences the rate of deterioration in various materials and processes [13]. Earlier studies have proposed a model to predict the viable cell counts of probiotics during storage in powdered form. Extensive research on freeze-dried cells has thoroughly documented the storage stability and shelf life of probiotics [14–17]. No similar studies have been reported on *Lactobacillus acidophilus*. In a recent study, accelerated storage testing was employed to predict the shelf life of both vacuum-dried and conventionally dried *L. acidophilus* without protectants, evaluating various drying processes during storage.

In this study, vacuum drying was used, which may offer a promising approach to address the limitations of traditional drying methods for lactic acid bacteria in terms of cost-effectiveness, product quality, storage testing, and shelf life. There is a significant research gap in optimizing the process to ensure optimal cell viability and other functional properties. Additionally, this research aimed to develop an economical growth medium for lactic acid bacteria using low-cost components and to investigate the impact of temperature on the viability of *Lactobacillus acidophilus TISTR 1338* during drying and storage. Furthermore, an Arrhenius equation-based predictive model was developed to estimate the storage shelf life of this strain in powdered form.

2. Materials and Methods

2.1. Materials

The *L. acidophilus TISTR 1338* culture was obtained from the Bangkok MIRCEN at the Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani 12,120, Thailand, and stored at −20 °C on de Man, Rogosa, and Sharpe (MRS) agar. The culture was grown in MRS broth at 37 °C, and the total working volume was maintained on stab MRS agar medium at 4 °C. Fresh stabs were prepared monthly using the working culture, and a new working culture was established every three months using the original reference culture [18].

2.2. Fermentation of Modified Media

The medium was prepared with the following components (g/L): molasses at 5%, 8%, and 11% *w*/*v*; spent cell yeast (brewer's yeast) at 0.5%, 1.5%, and 2.5% *w*/*v*; K_2HPO_4 at 2.00; Tween 80 at 1; $MgSO_4$ at 0.1; $MnSO_4$ at 0.05; and distilled water to make up to 1000 mL. The pH of the medium was adjusted to 6.5 using 1 M HCL before being sterilized at 121 °C for 15 min.

2.3. Preparation of L. acidophilus TISTR 1338

After two successive transfers of *L. acidophilus TISTR 1338* in MRS broth at 37 °C for 24 h, the culture was used as the inoculum. Fermentation experiments were conducted in 100 mL Erlenmeyer flasks containing 50 mL of sterile modified medium, which were inoculated with 2.5 mL of the 24 h culture (~10^9 CFU/mL). The samples were then incubated at 37 °C for 24 h [7].

2.4. Preparation of Culture

The filler, consisting of rice bran with hull, was sterilized at 121 °C for 30 min, and subsequently dried overnight at 55 °C in a forced-air oven. After drying, it was mixed with *L. acidophilus TISTR 1338* at a ratio of 50% *v*/*w*.

2.5. Vacuum Drying

The cultures were dried in a vacuum drier (EYELA VACUUM OVEN VOS 450SD) at 40 °C and 100 mbar to achieve a final moisture content below 11% [19]. After drying, the samples were rehydrated with a sterile 0.85% NaCl solution to obtain a 1:10 dilution, which was then homogenized in a Stomacher 80 for 1 min. Serial dilutions of each sample were prepared and plated on MRS agar containing bromocresol purple at 0.03%. The plates were incubated at 37 °C for 24 h, after which colony-forming units per milliliter (CFU/mL) were counted.

2.6. Viable Cell Count

The viable cell count of *Lactobacillus acidophilus TISTR 1338* was determined on MRS agar plates. A 1 g sample of vacuum-dried *L. acidophilus TISTR 1338* was rehydrated with 9 mL of 0.85% NaCl solution using a Stomacher for 1 min. The rehydrated sample was measured by serial dilution method in 0.85% NaCl solution, using a spread plate technique on 1.5% MRS agar in duplicates. The plates were incubated at 37 °C for 48 h. The survival rate of bacteria after vacuum drying was calculated as follows:

$$\text{Survival rate (\%)} = (N/N_o) \times 100$$

where N_o represents the initial viable cell count before vacuum drying (CFU/g of solids), **N** is the number of viable cells after vacuum drying (CFU/g of solids), and CFU/g of solids indicates the colony-forming units of viable cells per gram of vacuum-dried powder.

2.7. Moisture Content

Moisture content of the vacuum-dried powder was determined by following the Official Methods of Analysis [17]. Firstly, 1 g of vacuum-dried powder with three replicates was kept in an oven at 105 °C for 24 h. Then, the samples were taken from the drying oven and cooled in a desiccator and immediately weighed to obtain a constant weight.

2.8. Accelerated Storage Test

Vacuum-dried cell samples were incubated at 50 °C for durations ranging from 0 to 12 h, with 3 h intervals; at 60 °C for 0 to 2 h, with 0.5 h intervals; and at 70 °C for 0 to 1.5 h, with 0.25 h intervals [20]. At each time point, samples were collected to determine the residual viable cell count. The viable cell counts measured both before and after exposure to different temperatures were used to calculate the rate constant (k) values, based on a first-order reaction model [21].

$$k = 1/t\ (\log N_o - \log N)$$

where N represents the number of viable cells at any given time, N_o is the initial number of viable cells, and k is the rate constant (slope), expressed as the number of cells (in log10 units) per hour. The k values were calculated using the Arrhenius equation, with expected values at 4 °C and 30 °C.

$$\log k = \log k_o - (E_a/2.303R) * (1/T)$$

where k_o is the experimental constant, known as the frequency factor, R is the gas constant, T is the absolute temperature, and E_a is the activation energy [22–24].

2.9. Statistical Analysis

All statistical analyses were performed using SAS software (version 9.4, (SAS Institute, Inc., Cary, NC, USA). Results from duplicate trials were expressed as means and standard deviations. Data were analyzed using analysis of variance (ANOVA) with a significance level of $p < 0.05$. Significant differences among mean values were determined using Duncan's multiple-range test.

3. Results and Discussion

3.1. Influence of Molasses and Concentration of Spent Cell Yeast on the Growth of Lactic Acid Bacteria

To assess the impact of molasses and spent cell yeast concentration on lactic acid bacteria production, *Lactobacillus acidophilus TISTR 1338* was cultured in static flasks at 37 °C and pH 6.5, using 5%, 8%, and 11% *w*/*v* molasses and 0.5%, 1.5%, and 2.5% *w*/*v* spent cell yeast as carbon and nitrogen sources, respectively. As illustrated in Figure 1 [25], the supplementation of molasses with spent cell yeast significantly ($p < 0.05$) influenced lactic acid bacteria generation. A notable increase in viable cell count was observed as the molasses concentration was raised from 5% to 11% *w*/*v*. Conversely, the number of viable cells significantly decreased when the spent cell yeast concentration was increased from 0.5% to 2.5% *w*/*v*, likely due to substrate inhibition.

Compared to other conditions and media such as MRS, the combination of 11% molasses and 0.5% spent cell yeast resulted in the highest viable cell count. Additionally, during fermentation, a reduction in residual sugar concentration was observed. The residual sugar concentration increased significantly as the initial sugar concentration was raised from 5% to 11% *w*/*v*. This increase in residual sugar was attributed to the microorganism's inability to metabolize higher sugar concentrations, consistent with findings by [26,27],

who studied the optimization of lactic acid production from beet molasses using *Lactobacillus delbrueckii* NCIMB 8130. In that study, the residual sugar concentration increased significantly when the initial sugar concentration was raised from 80 to 120 g/L. Furthermore, the current study demonstrated that only a small amount of spent cell yeast was necessary as a nitrogen source for lactic acid bacteria development in molasses enriched with spent cell yeast, as the nitrogen content in molasses was sufficient to support bacterial growth. This finding aligns with the results of [28,29], which reported that in *Lactobacillus* growth in yeast extract-enriched cane molasses, only minimal yeast extract was required to supply nitrogen. Moreover, Suksawang et al. [30] utilized molasses and spent cell yeast to produce kefiran by *Lactobacillus kefiranofaciens*. Molasses at a concentration of 80 g/L gave the highest production of kefiran, at 235 ± 5.7 mg/L. Previous studies, such as [31], have investigated the combination of molasses and lactobacillus strains for processing corn silage, yielding high-quality corn silage. This finding indicates that molasses may serve as an optimal nutrient source to enhance the growth and activity of lactobacillus. In a similar study, the forage native grass before ensiling was treated with a combination of lactobacillus and molasses, which increased the nutritional quality of silage and inhibited the growth of unwanted bacteria, suggesting the high feed of lactobacillus on molasses [32]. Under the conditions of 0.5% spent cell yeast and 11% molasses, the maximum cell mass of *L. acidophilus* was achieved at 2.14 × 10^9 CFU/mL at 22 h, with a growth rate of 0.39 h^{-1} and a cell mass yield of 1.67 × 10^8 CFU/g sugar, as shown in Table 1. These conditions produced the highest viable cell count compared to other experimental conditions.

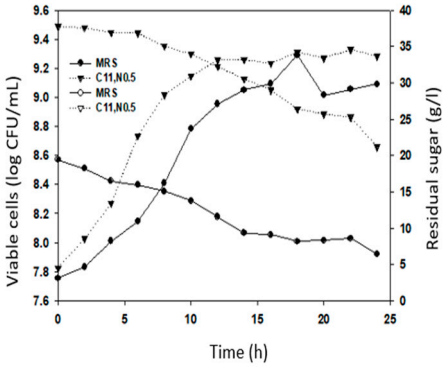

Figure 1. Growth outline of *L. acidophilus TISTR 1338* at different concentrations in the medium.

Table 1. Maximum concentration of cells, biomass yield, and specific growth rate and cell productivity at different concentrations on substrate of fermentation medium.

Medium	Maximum Cell Concentration (CFU/mL)	μ (h^{-1})	Yx/s (CFU/g Sugar)	Cell Productivity (CFU/mL/)
MRS	1.96 × 10^9	0.32	1.68 × 10^8	1.09 × 10^8
$C_{11}N_{0.5}$	2.14 × 10^9	0.39	1.67 × 10^8	9.73 × 10^7

Note: Mean values and the same letter in the same column are not statistically significant at 5% significance levels by the least significant difference.

3.2. Survival of L. acidophilus During Vacuum Drying

The survival rate of *Lactobacillus acidophilus* is summarized in Table 2. The moisture content of the vacuum-dried starter culture was found to be 9.89%. The results show that the viable cell count of the LAB strain cultures before drying was >1 × 10^9 CFU/g, while after drying, it decreased to >1 × 10^8 CFU/g. Temperature has been identified as a critical factor affecting the viability of probiotics during the drying process [33–35]. The loss of viability was primarily attributed to compromised cell membrane integrity and protein

denaturation [36]. Likewise, the reduction in viable cell count is not only influenced by the drying temperature but also by the duration of exposure to elevated temperatures [9,37–39].

Table 2. Viable (log CFU/mL) of *Lactobacillus acidophilus TISTR 1338* after vacuum drying.

Method	Viability (Log CFU)
Initial	9.18
Vacuum-drying	8.08

3.3. Survival of Vacuum-Dried L. acidophilus TISTR 1338 During Storage

The survivability of vacuum-dried *Lactobacillus acidophilus TISTR 1338* was evaluated over a storage period of 120 days at 4 °C and 30 °C, as shown in Figure 2. The results indicated that the highest viability of the strain was observed when stored at the lower temperature, highlighting the significant effect of storage temperature on the survival of vacuum-dried cells.

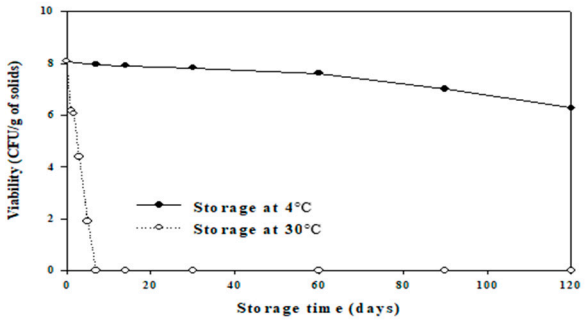

Figure 2. Viability of vacuum-dried *L. acidophilus TISTR 1338* during storage at 4 and 30 °C.

At 4 °C, the viability of *L. acidophilus TISTR 1338* remained stable at approximately 10^6 CFU/g for up to four months. In contrast, at the higher storage temperature of 30 °C, a significant decline in viability was observed, culminating in complete cell destruction within seven days. This aligns with findings from [37], where *L. paracasei* stored at 37 °C showed a decrease of seven log cycles in survival after 20 days with an aw of 0.33, whereas storage at 4 °C for three months resulted in no significant loss of viability. Additionally, similar results were observed by [39], where the vacuum-drying process showed significantly better storage stability of bovine serum albumin than the lyophilization process.

The degradation and inactivation of cells primarily occurred during processing and storage, demonstrating the inverse relationship between storage temperature and probiotic viability [39–43]. Furthermore, the rate of viable cell degradation was quantified using a specific degradation rate constant (k), which was found to increase with higher storage temperatures, as summarized in Table 3.

Table 3. The specific rate of degradation on *L. acidophilus TISTR 1338* during storage at 4 and 30 °C.

Drying Form	Experimental k Values (h^{-1})	
	k_4 (R^2)	k_{30} (R^2)
Vacuum-dried	0.0006 (0.94)	0.0501 (0.98)

3.4. Prediction on the Shelf Life of Vacuum-Dried L. acidophilus TISTR 1338 by Accelerated Storage Test

A prototype to assess the long-term preservation of lactic acid bacteria was developed using accelerated storage methods [39]. The specific degradation rate (k) of microorganisms can be calculated using Equation (1) [42–44].

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

where N_o represents the initial viable cells (CFU/g of solids), N denotes the viable cells at any given time (CFU/g of solids), k is the specific rate of degradation (h^{-1}), and t is the storage time.

The changes in the viability of vacuum-dried *Lactobacillus acidophilus TISTR 1338* under storage temperatures of 50 °C, 60 °C, and 70 °C are presented in Table 4. The slope of each linear regression corresponds to the specific rate of degradation (k, h^{-1}), as illustrated in Figure 3.

Table 4. Effect due to thermal death of Lactobacillus acidophilus on exposure to different temperatures TISTR 1338 after vacuum drying.

Temperature (°C)	Storage Time (h)	Log N_o − logN
		Vacuum-Dried
50	0	0
	3	2.33
	6	3.86
	9	5.29
	12	ND
60	0	0
	0.5	1.17
	1	2.67
	1.5	3.05
	2	5.53
70	0	0
	0.25	0.68
	0.5	1.00
	0.75	2.23
	1.0	3.49
	1.25	5.39

N_o: The initial number of viable cells; N: The number of viable cells after the time indicated. ND: No viable cell was detected.

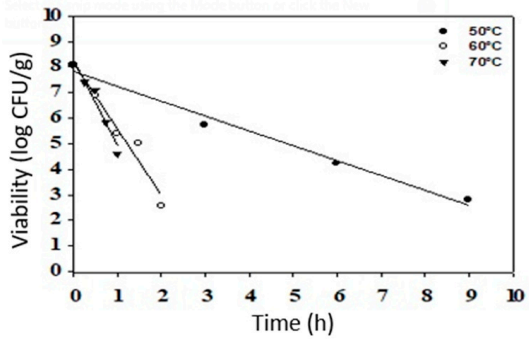

Figure 3. Viability of vacuum-dried *L. acidophilus* TISTR 1338 stored at 50 °C, 60 °C, and 70 °C as a function of time.

The relationship between the degradation rate constant (k) and temperature can be described using the Arrhenius equation, as shown in Equation (2).

$$K = Ae^{(-E_a/RT)} \quad (2)$$

where k is the specific rate of degradation (day^{-1}), R is the gas constant ($8.32\ J{\cdot}mol^{-1}{\cdot}K^{-1}$), $\mathbf{E_a}$ is the activation energy ($J{\cdot}mol^{-1}$), and T is the absolute temperature (K). Equation (3) is derived by taking the logarithm of both sides of Equation (2).

$$logk = logk_0 - \frac{E_a}{2.303\ R} \times \frac{1}{T} \quad (3)$$

From the Arrhenius equation, the constant value $\frac{Ea}{2.303R}$ as attained from a straight-line slope after the logarithms of determined k values, see Table 5, were charted against the multiplicative inverse of their absolute temperatures, as shown in Figure 4. As a result, the Arrhenius equation allowed for the determination of the energy of the activation constant, which led to the estimation of k_4 and k_{30}.

Table 5. The precise rate of degradation of vacuum-dried *L. acidophilus TISTR 1338*.

Storage Temperature (°C)	Precise Rate of Degradation. k (h^{-1}) (R^2)
50	0.5832 (0.9838)
60	2.1289 (0.9553)
70	5.1652 (0.9200)

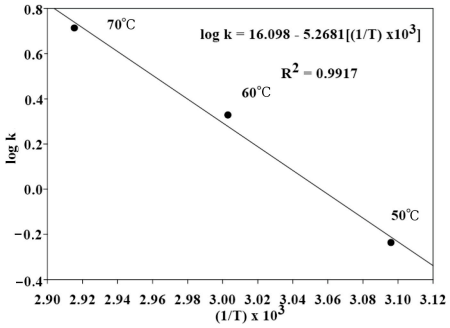

Figure 4. Arrhenius plot of precise rate of degradation (k) of vacuum-dried *L. acidophilus TISTR 1338* (log k versus 1/T × 1000).

The prediction equations of the strain viability for long-term preservation at 4 °C and 30 °C are shown in Table 6.

Table 6. Predicted specific rate of degradation of *L. acidophilus TISTR 1338* by vacuum drying during storage at 4 °C and 30 °C.

Drying Form	Expected Values k (h^{-1})	
	k_4	k_{30}
Vacuum-dried	0.0012	0.0515

Table 4 presents the decimal reduction values for the thermal inactivation of *Lactobacillus acidophilus TISTR 1338* following vacuum drying. Among the three temperatures evaluated (70 °C, 60 °C, and 50 °C), 70 °C exhibited the fastest thermal decline. The data also indicated that *L. acidophilus TISTR 1338* displayed a significantly lower decimal reduction at higher treatment temperatures (70 °C). A study by Poddar et al. [44] investigated

the mortality rates of freeze-dried and controlled low-temperature vacuum-dehydrated (CLTVD) *L. acidophilus*, as well as the protective effects of various protectants. They found that elevated temperatures resulted in a more pronounced thermal reduction.

The values of k (h^{-1}), representing the thermal reductions of *Lactobacillus acidophilus TISTR 1338* following vacuum drying, are shown in Table 5. These values range from 0.5832 to 5.1652 h^{-1}. The slope of the first-order microbial thermal reduction reaction and the thermal reduction rate of the cells are indicated by the k values. As observed in Table 5, higher temperatures corresponded to higher k values. These findings align with the study by [45], which reported that higher drying temperatures caused a faster decrease in the viability of *Saccharomyces cerevisiae* during the falling-rate drying period compared to lower drying temperatures.

The expected k values for vacuum-dried *Lactobacillus acidophilus TISTR 1338* at 4 °C and 30 °C are presented in Table 6. These results are consistent with the findings of [46,47].

The expected k values for vacuum-dried *Lactobacillus acidophilus TISTR 1338* were determined from the experimental k values. A comparison of the predicted and experimental survival rates of vacuum-dried *L. acidophilus TISTR 1338* at 4 °C and 30 °C is shown in Tables 3 and 6, with no significant differences observed over a 4-month storage period (Figure 5).

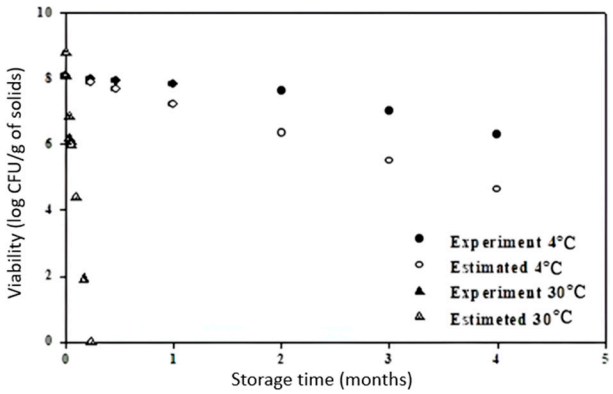

Figure 5. The estimated and empirically determined viabilities of vacuum-dried *L. acidophilus TISTR 1338* were compared during storage at 4 °C and 30 °C.

The results indicated that accelerated storage testing was a reliable tool for predicting the shelf life at 30 °C but was not an accurate predictor at 4 °C. The predicted k value at 4 °C was nearly twice the experimental k value. This discrepancy was likely caused by changes in the physical properties of the vacuum-dried powder during the accelerated storage tests, potentially altering the activation energy.

Other factors that could have contributed to the observed discrepancies include non-enzymatic reactions during storage. According to Barbosa et al. [48], several reasons can account for deviations from Arrhenius kinetics:

(i) Elevated temperatures may induce first-order phase transitions, such as the melting of solid fats, facilitating reactant flow in the liquid phase;
(ii) Water loss at high temperatures may alter reaction rates;
(iii) Reactions with different activation energies may dominate at various temperatures;
(iv) Increasing temperature and water activity could accelerate reaction rates;
(v) Protein denaturation could modify the reactivity of proteins at high temperatures;
(vi) Water release during amorphous sugar crystallization may change the proportion of reactants in the solute-water phase.

Although these anticipated models deviated from the Arrhenius theory, limited studies have shown success in predicting microbial survival during storage. This suggests that such models may vary depending on the specific strain and microorganism. Therefore, it was concluded that accelerated storage testing is a modest approach with limited accuracy and predictability. The study successfully demonstrated the cell viability of bacteria and the influence of molasses and spent cell yeast, as well as vacuum drying on bacterial growth. Additionally, predictions made by accelerated storage testing were too reliable and provided valuable insights into the long-term stability of dried bacteria. However, this study has certain limitations. The study was focused on a single strain of lactic acid bacteria, which limits the generalizability of the findings to other bacterial strains. Moreover, the accelerated storage testing did not provide accurate prediction on shelf life at 4 °C, indicating the pitfalls, and may not fully capture the effects of real-world storage conditions. Furthermore, analysis of the biochemical and functional properties of the dried bacteria could provide further insights into their potential applications.

4. Conclusions

All in all, the findings of this study indicate that molasses and spent cell yeast are viable carbon and nitrogen sources for the production of *Lactobacillus acidophilus TISTR 1338*. However, optimization of substrate concentrations is essential for maximizing cultivation efficiency. The study also suggests that vacuum drying is a promising method for producing dried cells suitable for non-refrigerated storage conditions. Temperature was identified as a critical factor influencing microbial survival during both vacuum drying and storage. While accelerated storage testing, based on the Arrhenius equation, provides limited predictability for long-term storage, it may be effectively employed to develop a model system for estimating the shelf life of vacuum-dried *L. acidophilus TISTR 1338*.

Author Contributions: Investigation and writing—original draft, A.M.; Investigation and methodology, B.I.; formal analysis, data curation, I.N.S.; software, data curation, M.W.K.; editing and reviewing the manuscript, H.G.; supervision, resources, project administration, P.P.; conceptualization and validation of data, A.K.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors gratefully acknowledge the support of the Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University for providing facilities.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Zhang, L.; García-Cano, I.; Jiménez-Flores, R. Effect of milk phospholipids on the growth and cryotolerance of lactic acid bacteria cultured and stored in acid whey-based media. *JDS Commun.* **2020**, *1*, 36–40. [CrossRef] [PubMed]
2. Ojo, A.O.; de Smidt, O. Lactic Acid: A Comprehensive Review of Production to Purification. *Processes* **2023**, *11*, 688. [CrossRef]
3. Vidra, A.; Tóth, A.J.; Németh, Á. Recovery, Lactic acid production from cane molasses. *Waste Treat. Recover.* **2017**, *2*, 13–16. [CrossRef]
4. Pejin, J.; Radosavljević, M.; Kocić-Tanackov, S.; Marković, R.; Djukić-Vuković, A.; Mojović, L. Use of spent brewer's yeast in L-(+) lactic acid fermentation. *J. Inst. Brew.* **2019**, *125*, 357–363. [CrossRef]
5. Tianwitawat, C.; Klaiprasitti, P. Rice bran as an encapsulating material to produce a healthy synbiotic product with improved gastrointestinal tolerance. *Arch. Microbiol.* **2023**, *205*, 265. [CrossRef]
6. Ardsiri, P.; Chaoruangrit, A.; Pongsetkul, J.; Bavisetty, S.C.B.; Sae-eaw, A.; Petsong, K. Survival of probiotics *Lactobacillus acidophilus* TISTR1338 in a synbiotic-supplemented plant-based protein powder under various pH and temperatures. *Asia-Pac. J. Sci. Technol.* **2024**, *29*, 29. [CrossRef]

7. Thaweesang, S.; Leenanon, B. Survival of Lactobacillus acidophilus TISTR1338 in bile salt stress conditions. *Asian J. Microbiol. Biotechnol. Environ. Sci.* **2016**, *18*, 569–579.
8. Sadat Naghavi, N.; Rezaeizadeh, G.; Sarami, F.; Khayam-Nekooii, M.S.; Khosravi-Darani, K. A Comparison between Different Methods for the Preservation of Lactic Acid Bacteria for Usage as a Starter Culture. *J. Microb. Biol.* **2022**, *11*, 77–94.
9. Huang, S.; Vignolles, M.-L.; Chen, X.D.; Le Loir, Y.; Jan, G.; Schuck, P.; Jeantet, R. Spray drying of probiotics and other food-grade bacteria: A review. *Trends Food Sci. Technol.* **2017**, *63*, 1–17. [CrossRef]
10. Lengyel, M.; Kállai-Szabó, N.; Antal, V.; Laki, A.J.; Antal, I. Microparticles, Microspheres, and Microcapsules for Advanced Drug Delivery. *Sci. Pharm.* **2019**, *87*, 20. [CrossRef]
11. Arepally, D.; Goswami, T.K. Effect of inlet air temperature and gum Arabic concentration on encapsulation of probiotics by spray drying. *LWT* **2019**, *99*, 583–593. [CrossRef]
12. Shalaev, E.; Ohtake, S.; Moussa, E.M.; Searles, J.; Nail, S.; Roberts, C.J. Accelerated Storage for Shelf-Life Prediction of Lyophiles: Temperature Dependence of Degradation of Amorphous Small Molecular Weight Drugs and Proteins. *J. Pharm. Sci.* **2023**, *112*, 1509–1522. [CrossRef] [PubMed]
13. Carvalho-Silva, V.H.; Coutinho, N.D.; Aquilanti, V. Temperature dependence of rate processes beyond Arrhenius and Eyring: Activation and Transitivity. *Front. Chem.* **2019**, *7*, 380. [CrossRef] [PubMed]
14. Chen, H.; Tian, M.; Chen, L.; Cui, X.; Meng, J.; Shu, G. Optimization of composite cryoprotectant for freeze-drying Bifidobacterium bifidum BB01 by response surface methodology. *Artif. Cells Nanomed. Biotechnol.* **2019**, *47*, 1559–1569. [CrossRef] [PubMed]
15. Jacobsen, N.M.Y.; Caglayan, I.; Caglayan, A.; Bar-Shalom, D.; Müllertz, A. Achieving delayed release of freeze-dried probiotic strains by extrusion, spheronization and fluid bed coating-evaluated using a three-step in vitro model. *Int. J. Pharm.* **2020**, *591*, 120022. [CrossRef] [PubMed]
16. Sornsenee, P.; Chimplee, S.; Saengsuwan, P.; Romyasamit, C. Characterization of probiotic properties and development of banana powder enriched with freeze-dried Lacticaseibacillus paracasei probiotics. *Heliyon* **2022**, *8*, e11063. [CrossRef] [PubMed]
17. AOAC. *Official Methods of Analysis*, 17th ed.; AOAC International: Gaithersburg, MD, USA, 2000.
18. Mulaw, G.; Sisay Tessema, T.; Muleta, D.; Tesfaye, A. In Vitro Evaluation of Probiotic Properties of Lactic Acid Bacteria Isolated from Some Traditionally Fermented Ethiopian Food Products. *Int. J. Microbiol.* **2019**, *2019*, 7179514. [CrossRef]
19. Santivarangkna, C.; Kulozik, U.; Foerst, P. Effect of carbohydrates on the survival of Lactobacillus helveticus during vacuum drying. *Lett. Appl. Microbiol.* **2006**, *42*, 271–276. [CrossRef]
20. Tsen, J.H.; Lin, Y.P.; Huang, H.Y.; King, V.A.E. Accelerated storage testing of freeze-dried immobilized *Lactobacillus acidophilus*-fermented banana media. *J. Food Process. Preserv.* **2007**, *31*, 688–701. [CrossRef]
21. Toledo, R.T.; Singh, R.K.; Kong, F. *Fundamentals of Food Process Engineering*; Springer: Berlin/Heidelberg, Germany, 2007; Volume 297.
22. King, V.A.-E.; Lin, H.-J.; Liu, C.-F. Accelerated storage testing of freeze-dried and controlled low-temperature vacuum dehydrated *Lactobacillus acidophilus*. *J. Gen. Appl. Microbiol.* **1998**, *44*, 161–165. [CrossRef]
23. Saguy, I.; Karel, M. Modeling of Quality Deterioration During Food Processing and Storage. *Agric. Food Sci* **1980**.
24. Van Boekel, M.A. Kinetic modeling of food quality: A critical review. *Compr. Rev. Food Sci. Food Saf.* **2008**, *7*, 144–158. [CrossRef]
25. Muenaram, A.; Leelawatcharamas, V.; Parakulsuksatid, P. Effect of molasses and spent cell yeast concentration on lactic acid bacteria production. In Proceedings of the 49th Kasetsart University Annual Conference, Bangkok, Thailand, 1–4 February 2011; Volume 7. Subject: Agro-Industry.
26. Abedi, E.; Hashemi, S.M.B. Lactic acid production–producing microorganisms and substrates sources-state of art. *Heliyon* **2020**, *6*, e04974. [CrossRef] [PubMed]
27. Ahmad, A.; Banat, F.; Taher, H. A review on the lactic acid fermentation from low-cost renewable materials: Recent developments and challenges. *Environ. Technol. Innov.* **2020**, *20*, 101138. [CrossRef]
28. Chen, W.; Hang, F. Lactic acid bacteria starter. In *Lactic Acid Bacteria: Bioengineering and Industrial Applications*; Springer: Singapore, 2019; pp. 93–143.
29. Hayek, S.A.; Gyawali, R.; Aljaloud, S.O.; Krastanov, A.; Ibrahim, S.A. Cultivation media for lactic acid bacteria used in dairy products. *J. Dairy Res.* **2019**, *86*, 490–502. [CrossRef]
30. Suksawang, S.; Cheirsilp, B.; Yeesang, J. Production of kefiran from molasses and spent yeast cells by lactobacillus kefiranofaciens JCM 6985. *Asia-Pac. J. Sci. Technol.* **2016**, *21*, 59–67.
31. Chen, S.; Chang, Y.; Huang, H.; Kuo, S.; Wang, H. Application of condensed molasses fermentation solubles and lactic acid bacteria in corn silage production. *J. Sci. Food Agric.* **2020**, *100*, 2722–2731. [CrossRef] [PubMed]
32. Li, Y.; Du, S.; Sun, L.; Cheng, Q.; Hao, J.; Lu, Q.; Ge, G.; Wang, Z.; Jia, Y. Effects of Lactic Acid Bacteria and Molasses Additives on Dynamic Fermentation Quality and Microbial Community of Native Grass Silage. *Front. Microbiol.* **2022**, *13*, 830121. [CrossRef] [PubMed]

33. De Cesare, A.; Sirri, F.; Manfreda, G.; Moniaci, P.; Giardini, A.; Zampiga, M.; Meluzzi, A. Effect of dietary supplementation with *Lactobacillus acidophilus* D2/CSL (CECT 4529) on caecum microbioma and productive performance in broiler chickens. *PLoS ONE* **2017**, *12*, e0176309. [CrossRef] [PubMed]
34. Murtaza, M.A.; Huma, N.; Shabbir, M.A.; Murtaza, M.S.; Anees-ur-Rehman, M. Survival of micro-organisms and organic acid profile of probiotic Cheddar cheese from buffalo milk during accelerated ripening. *Int. J. Dairy Technol.* **2017**, *70*, 562–571. [CrossRef]
35. Shim, Y.; Ingale, S.; Kim, J.; Kim, K.; Seo, D.; Lee, S.; Chae, B.; Kwon, I. A multi-microbe probiotic formulation processed at low and high drying temperatures: Effects on growth performance, nutrient retention and caecal microbiology of broilers. *Br. Poult. Sci.* **2012**, *53*, 482–490. [CrossRef] [PubMed]
36. Lapsiri, W.; Bhandari, B.; Wanchaitanawong, P. Viability of Lactobacillus plantarum TISTR 2075 in different protectants during spray drying and storage. *Dry. Technol.* **2012**, *30*, 1407–1412. [CrossRef]
37. de Almada, C.N.; Almada, C.N.; Martinez, R.C.; Sant'Ana, A.S. Paraprobiotics: Evidences on their ability to modify biological responses, inactivation methods and perspectives on their application in foods. *Trends Food Sci. Technol.* **2016**, *58*, 96–114. [CrossRef]
38. Peighambardoust, S.; Tafti, A.G.; Hesari, J. Application of spray drying for preservation of lactic acid starter cultures: A review. *Trends Food Sci. Technol.* **2011**, *22*, 215–224. [CrossRef]
39. Jangle, R.D.; Pisal, S.S. Vacuum foam drying: An alternative to lyophilization for biomolecule preservation. *Indian J Pharm Sci.* **2012**, *74*, 91–100. [CrossRef] [PubMed] [PubMed Central]
40. Foerst, P.; Kulozik, U.; Schmitt, M.; Bauer, S.; Santivarangkna, C. Storage stability of vacuum-dried probiotic bacterium Lactobacillus paracasei F19. *Food Bioprod. Process.* **2012**, *90*, 295–300. [CrossRef]
41. Santivarangkna, C.; Kulozik, U.; Foerst, P. Storing lactic acid bacteria: Current methodologies and physiological implications. In *Stress Responses of Lactic Acid Bacteria*; Springer: Boston, MA, USA, 2011; pp. 479–504.
42. Savedboworn, W.; Kerdwan, N.; Sakorn, A.; Charoen, R.; Tipkanon, S.; Pattayakorn, K. Role of protective agents on the viability of probiotic Lactobacillus plantarum during freeze drying and subsequent storage. *Int. Food Res. J.* **2017**, *24*, 787.
43. Wirunpan, M.; Savedboworn, W.; Wanchaitanawong, P.J.A.; Resources, N. Survival and shelf life of Lactobacillus lactis 1464 in shrimp feed pellet after fluidized bed drying. *Agric. Nat. Resour.* **2016**, *50*, 1–7. [CrossRef]
44. Poddar, D.; Nag, A.; Das, S.; Singh, H. 17 Stabilization of Probiotics for Industrial Application. 2016. Available online: https://www.researchgate.net/profile/Devastotra-Poddar/publication/284225314_Stabilization_of_Probiotics_for_Industrial_Application/links/5f5ed216299bf1d43c01c58a/Stabilization-of-Probiotics-for-Industrial-Application.pdf (accessed on 30 November 2024).
45. Soltani, B.; McClure, D.D.; Oveissi, F.; Langrish, T.A.; Kavanagh, J.M.J.F.; Processing, B. Experimental investigation and numerical modeling of pilot-scale fluidized-bed drying of yeast: Part A–Drying model development and validation. *Food Bioprod. Process.* **2020**, *119*, 246–256. [CrossRef]
46. Barbosa, J.; Borges, S.; Teixeira, P. Influence of sub-lethal stresses on the survival of lactic acid bacteria after spray-drying in orange juice. *Food Microbiol.* **2015**, *52*, 77–83. [CrossRef]
47. Savedboworn, W.; Noisumdang, C.; Arunyakanon, C.; Kongcharoen, P.; Phungamngoen, C.; Rittisak, S.; Charoen, R.; Phattayakorn, K. Potential of protein-prebiotic as protective matrices on the storage stability of vacuum-dried probiotic Lactobacillus casei. *LWT* **2020**, *131*, 109578. [CrossRef]
48. Calligaris, S.; Manzocco, L.; Anese, M.; Nicoli, M.C. Shelf-life assessment of food undergoing oxidation—A review. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 1903–1912. [CrossRef] [PubMed]